Cardiac vagal withdrawal when moving from supine to an upright posture may be independent of respiratory sinus arrhythmia. Further, ventilatory efficiency of an upright lung may improve with clustering of heart beats during inhalation. We studied healthy human subjects (n=8, 6 male) during supine rest (SUP) and 80° head-up tilt (HUT). ECG and expired breath were sampled continuously to determine heart rate, mean and end-tidal (ET) fractional content (F) of O₂ and CO₂, tidal volume (VT) and breathing frequency (Bf). HUT increased heart rate (47±3 vs. 59±9 beats min⁻¹, p<0.01), decreased the high frequency component of heart rate variability (8.76± vs. 7.07±1.12, p<0.05), and increased the ratio of low to high frequency components in the heart rate (0.62±0.6 vs. 1.79±2.07, p<0.05). HUT did not change VT, Bf, or minute ventilation (VE), but decreased F CO₂ (4.90±0.48 vs. 4.56±0.42 %, p<0.05) and F ETCO₂ (6.64±0.24 vs. 6.30±0.27 %, p<0.01). HUT increased the CO₂ ventilatory equivalent (24.88±2.50 vs. 26.74±2.61, p<0.01). Mean heart rate during inhalation increased with HUT (26±3 vs. 34±6 beats min⁻¹), with no change during exhalation. Increased clustering of heart beats during inhalation independent of a decrease in HF cardiac variability may partly offset decreases in ventilatory efficiency of an upright lung.

Key words: Heart rate variability, cardiac autonomic control, ventilatory equivalents.

INTRODUCTION

An increase in heart rate and a small decrease in end-tidal partial pressure of CO₂ (P ETCO₂) occur in humans when moving from supine to an upright posture (1-5). Head up tilt also affects beat-to-beat irregularities in the resting heart rate, whereby variability at respiratory frequencies has consistently been shown to decrease in healthy humans (2, 6-8). However, directional changes in the low
frequency (LF) component of heart rate variability (HRV) with head-up tilt appear equivocal, where no changes (2, 6, 9) or an increase (7, 8, 10) has been reported for healthy humans. Minute ventilation ($V'_E$) is closely linked to CO$_2$ output ($V'_CO_2$), such that the efficiency of pulmonary gas exchange may be determined by how much the lung needs to be ventilated in order to remove a given quantity of CO$_2$ - this is commonly referred to as the ventilatory equivalent ($V'_E / V'_CO_2$). Efficiency may be improved by matching alveolar ventilation with pulmonary perfusion throughout the ventilatory cycle - this synchronization of heart rate with ventilation is known as respiratory sinus arrhythmia (11-14). The high frequency (HF) component of HRV, which normally include respiratory frequencies, has been used to quantify respiratory sinus arrhythmia (15-18), and has also been used as an indirect measure of cardiac vagal tone (14, 19, 20). The decrease in the HF component of HRV with head-up tilt may indicate a decrease in respiratory sinus arrhythmia, although given that respiratory sinus arrhythmia describes a clustering of heart beats during inhalation in order to improve gas exchange efficiency, this is inconsistent with the reduction in $P_{ETCO_2}$ with head-up tilt.

A change in respiratory sinus arrhythmia with head-up tilt has not previously been quantified by measuring the heart rates during the inhalatory and exhalatory phases of breathing. Also, there are limited data on simultaneous measures of tilt-induced changes in respiratory sinus arrhythmia, indices of HRV, and ventilatory efficiency. Therefore, the purpose of this study was to quantify respiratory sinus arrhythmia when supine and in the upright position, with simultaneous measures of HRV and respiratory variables. It was hypothesized that a decrease in the HF component of HRV (indicative of vagal withdrawal) would be independent of an increase in respiratory sinus arrhythmia, as measured by the clustering of heart beats during inhalation. It was further hypothesized that the reduction in $P_{ETCO_2}$ would indicate an increase in ventilatory efficiency, in part attributable to an increased clustering of heart beats during inhalation.

**MATERIAL AND METHODS**

Eight adult non-smokers (age range 20 - 42 years, 6 males), with no known cardiovascular, respiratory, or medical abnormalities were studied at rest. Subjects were at least 4hr post-prandial, and refrained from caffeine containing drinks in the preceding 4 hrs. All subjects undertook regular physical exercise of duration greater than 1 hour, at least three times per week. The $V'_{O_2PEAK}$ of subjects was previously measured using an incremental treadmill running protocol, and/or an incremental cycle ergometer test. For all subjects, values were greater than 60ml Kg$^{-1}$ min$^{-1}$, placing them in the 'Excellent' category for aerobic fitness according to the American College of Sports Medicine guidelines for exercise testing and prescription. All experimentation was carried out in a large, well ventilated fit-for-purpose human performance laboratory, at an ambient temperature of between 19 and 21°C. All procedures were approved by the local Ethics Committee.

Subject's lay supine (SUP) on a purpose built tilting table for 11 min, followed immediately by 11 min passive head-up tilt at 80° (HUT). Moving from SUP to HUT took approximately 3 s.
Subjects breathed through a mouthpiece connected to a pneumotach (MLT1000L Respiratory Flow Head, AD Instruments, Australia) - the mouthpiece and pneumotach added approximately 100 ml to the total ventilatory dead space of each subject. The fractional content of $O_2$ and $CO_2$ in the air flowing through the pneumotach was dried (MLA6024 AD Instruments, Australia) and continuously sampled (response time approximately 100ms) throughout inhalation and exhalation using an "on-line" gas analyser (ML206 AD Instruments, Australia). The $O_2$ transducer used absorption spectroscopy at $\lambda$ 760nm and the $CO_2$ transducer used an infrared sensor. The analyzer was calibrated with samples of known $O_2$ and $CO_2$ gas mixtures prior to testing each subject. The length of the sampling lines from the pneumotach to analyzer were minimized to reduce the signal offset - in subsequent data analyses the chart recorder $O_2$ and $CO_2$ data streams were time shifted such that the initial changes from typical room air coincided with the start of exhalation, as defined by a continuous positive deflection in ventilatory flow.

Ventilatory flow was integrated to determine volume, and this signal was calibrated using a 3L calibration syringe (Hans Rudolph, USA). Before each 11min recording, the pneumotach signal was reset to zero when disconnected from the subject. Values for tidal volume ($V_T$) and breathing frequency ($Bf$) were used to determine minute ventilation ($V_{ET}$) in L min$^{-1}$ BTPS. Values of $V_{ET}$ were converted to L min$^{-1}$ BTPS using appropriate formulae (21). The mixed expired fractional content of $O_2$ and $CO_2$ ($%F_{O2}$ and $%F_{CO2}$ respectively) and the expired end-tidal fractional content of $O_2$ and $CO_2$ ($%F_{ETO2}$ and $%F_{ETCO2}$ respectively) were recorded. Oxygen uptake ($V'O_2$) and carbon dioxide output ($V'CO_2$) were calculated from $V'_{ET}$ (STPD) x $F_{O2}$ or $F_{CO2}$ respectively. An estimate of physiological dead space ($V_D$) was obtained using $V_D = V_T (1- F_{CO2}/ F_{ETCO2})$, and this was used to calculate the $V_D/V_T$ ratio. The ventilatory equivalents for $CO_2$ and $O_2$ are reported as the quotients of $V'_{ET}$/ $V'_{CO2}$ and $V'_{ET}$/ $V'_{O2}$ respectively, where $V'_{ET}$ is in L min$^{-1}$ (BTPS) and both $V'_{CO2}$ and $V'_{O2}$ are in L min$^{-1}$ (STPD).

Simultaneous recordings of electrocardiogram (ECG limb lead 2, band pass filtered between 10Hz and 200Hz) and ventilatory flow (Spirometer ML141, AD Instruments, Australia) were sampled at 1 KHz and collected using a multi-channel analogue-to-digital data acquisition system with appropriate software (PowerLab 4/25T and Chart v5.4 Pro, AD Instruments, Australia). Data were recorded for 11 min for both SUP and HUT conditions.

ECG recordings were used to assess heart rate and HRV using commercially available software (HRV Module for Chart 5, AD Instruments, Australia). Heart rate was calculated by expressing R-R intervals as beats per minute (beats min$^{-1}$). Intervals between adjacent R waves were detected using a threshold detection of between 0.5 and 1.0 mV, and classified as artifact (< 5 ms and > 2000 ms), ectopic (5 ms to 600 ms, and 1800 ms to 2000 ms), and normal (400 ms to 1500 ms). HRV data were analysed in the time domain using the mean R-R interval, and the standard deviation of the normal mean R-R interval, and in the frequency domain using a Fourier analysis and a Welch averaged periodogram method, and banded as low frequency (LF: 0.04 - 0.15 Hz), and high frequency (HF: 0.15 - 0.4 Hz). Total power for each spectrum was defined as the area under the spectrum from 0 to 0.5 Hz, and normalised units for the LF and HF components (which take into account any changes in total spectrum power) were used to calculate the low frequency : high frequency ratio (LF/HF).

Values of flow were negative for inhalation and positive for exhalation. For all ECG R waves recorded throughout each 11 min period, the corresponding value of the ventilatory flow signal was recorded and sorted according to value. For each subject under each condition, the total number of all positive, and of all negative values, was used to determine the heart rates (in beats min$^{-1}$) during exhalation and inhalation respectively.

The initial 2 min of the 11 min recording was excluded from analyses due to the potential short-term adjustments to instrumentation of the subject and to any residual movement artefact from attaining the HUT position. Thus, nine minute periods were subsequently analysed for all subjects.
under both conditions. Paired data (SUP vs. HUT) were compared with Student t-tests, where significance was set at P<0.05. A repeated measures analysis of variance was used to determine differences in heart rate during inhalation and exhalation across the two conditions, with post-hoc comparisons made using Student t-tests (Bonferroni corrected significance: P<0.03). Linear regression was used to define the relation between \( \Delta \) in heart rate and the \( \Delta \) in the normalised HF component of HRV with HUT. Throughout, values are cited as mean ± 1 standard deviation (SD).

RESULTS

Cardiovascular measures during SUP and HUT are reported in Table 1. Compared to SUP, tilting increased heart rate from 47 (3) to 59 (9) beats min\(^{-1}\), and increased the difference between the heart rates measured during inhalation and exhalation.

Table 1. Measures of heart rate variability in time and frequency domains during supine (SUP) rest and 80° head up tilt (HUT). Values are mean (sd) for 8 subjects. LF (nu) and HF (nu) are low frequency and high frequency respectively, with data normalized (nu) for total spectrum power.

<table>
<thead>
<tr>
<th></th>
<th>Total Power (0 - 0.5 Hz)</th>
<th>VLF (0 - 0.04 Hz)</th>
<th>LF (0.04 - 0.15 Hz)</th>
<th>HF (0.15 - 0.4 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUP</td>
<td>Mean R-R ((ms^2))</td>
<td>SD R-R ((ms^2))</td>
<td>Median R-R ((ms^2))</td>
<td>Heart Rate ((beats min^{-1}))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1286</td>
<td>117</td>
<td>1289</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>(95)</td>
<td>(25)</td>
<td>(92)</td>
<td>(3)</td>
</tr>
<tr>
<td>HUT</td>
<td>1041**</td>
<td>78*</td>
<td>1042**</td>
<td>59**</td>
</tr>
<tr>
<td></td>
<td>(155)</td>
<td>(29)</td>
<td>(156)</td>
<td>(9)</td>
</tr>
</tbody>
</table>

*P=0.05, **P=0.01, paired sample Student's t-test.

Fig. 1. Mean (+ SD) measures of heart rate variability in the frequency domain during supine (shaded) and head up tilt (clear) rest. VLF: very low frequency; LF: low frequency; HF: high frequency. * P<0.05, paired sample Student's t-test.
Mean R-R interval, SD R-R, and median R-R interval all decreased with HUT when compared to SUP. Values for the low and high frequency components of HRV, when expressed as relative contribution to the total frequency spectrum between 0 Hz and 0.5 Hz, and the LF / HF ratio are shown in Table 1. Measures of HRV in the frequency domain are reported in Fig. 1. Significant reductions in total power and power at high frequency occurred with

![Graph](image-url)

**Fig. 2.** Change (Δ) in heart rate (HR) plotted as a function of the change (Δ) in the normalized high frequency component (HF n.u.) of heart rate variability. For both variables, change has been calculated by 80° head-up-tilt minus supine rest, from 8 subjects. A linear regression equation has been fitted to the data, where \( R^2 = 0.59 \).

**Table 2.** Ventilatory and respiratory measures during supine (SUP) rest and 80° head up tilt (HUT). Values are mean (sd) for 8 subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SUP</th>
<th>HUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT (L)</td>
<td>13.89</td>
<td>14.46</td>
</tr>
<tr>
<td>BF (B min⁻¹)</td>
<td>13.71</td>
<td>14.67</td>
</tr>
<tr>
<td>VE (L min⁻¹)</td>
<td>4.90</td>
<td>5.03</td>
</tr>
<tr>
<td>FCO₂ %</td>
<td>6.64</td>
<td>6.30**</td>
</tr>
<tr>
<td>FO₂ %</td>
<td>15.48</td>
<td>15.91</td>
</tr>
<tr>
<td>FET CO₂ %</td>
<td>0.549</td>
<td>0.548</td>
</tr>
<tr>
<td>FET O₂ %</td>
<td>0.591</td>
<td>0.599</td>
</tr>
<tr>
<td>VCO₂ (L min⁻¹)</td>
<td>26.304</td>
<td>27.654</td>
</tr>
<tr>
<td>VO₂ (L min⁻¹)</td>
<td>24.88</td>
<td>26.74**</td>
</tr>
<tr>
<td>V / VT</td>
<td>23.19</td>
<td>24.50</td>
</tr>
</tbody>
</table>

*p = 0.05, **p = 0.01, paired sample Student's t-test.

VT: Tidal volume; BF: breathing frequency; VE: Minute ventilation; FCO₂: Fractional content of CO₂ in expired volume; FO₂: Fractional content of O₂ in expired volume; FETCO₂: Expired end tidal fractional content of CO₂; FETO₂: Expired end tidal fractional content of O₂; VCO₂: Carbon dioxide output; VO₂: Oxygen uptake; V / VT: Dead space / tidal volume ratio; VE / VCO₂: Ventilatory equivalent for CO₂; VE / VO₂: Ventilatory equivalent for O₂.
HUT. Fig. 2 describes the relation between the change in heart rate and the change in the HF component of HRV induced by HUT.

Ventilatory and respiratory measures are reported in Table 2. Significant decreases in \( F_{CO2} \), \( F_{ETCO2} \) and \( V'_E / V'_CO2 \) were recorded with HUT. Tidal volume, breathing frequency, minute ventilation, and the dead space / tidal volume ratio were not affected by HUT. Measures of \( F_{O2} \), \( F_{ETO2} \) and \( V'_E / V'_O2 \) were not affected by HUT. Oxygen uptake and carbon dioxide output were not affected by HUT.

**DISCUSSION**

This study uniquely reports an increased clustering of heart beats during inhalation when a HUT position is compared to a supine position in healthy subjects. This increase in respiratory sinus arrhythmia occurred despite a decrease in the HF component of HRV. This study also reported an increased \( V'_E / V'_CO2 \) (i.e. decreased ventilatory efficiency) with HUT, despite a reduction in \( F_{ETCO2} \) and no change in \( V'_E \), estimated \( V'_A \), or estimated \( V_D/V_T \). We speculate that the further matching of perfusion to ventilation in the upright lung with respiratory sinus arrhythmia is a potential mechanism to attenuate the decrease in ventilatory efficiency that occurs with HUT.

An upright position compared to a supine position has previously been shown to increase \( V'_E / V'_CO2 \) in healthy subjects (22, 23), and it has been suggested that this may reflect the HUT-induced increase in \( V_D/V_T \) (24, 25), \( V'_E \) (22), or \( V'_A \) (23). In the current study, and consistent with the findings of others (23), the estimated
$\frac{V_D}{V_T}$ did not change, however, the use of Bohr's equation with $F_{ETCO2}$ substituted for a measure of arterial $CO_2$ content may be inappropriate (21).

A wide range of values for $\frac{V'E}{V'CO_2}$ have been reported previously. For example, resting $\frac{V'E}{V'CO_2}$ values between 50 and 58 (26) and between 38 and 40 (22, 23) have been reported for healthy subjects. Normal values for middle aged sedentary men during sub-maximal exercise ranged from 23 to 29 (21, 27). The lower values for resting $\frac{V'E}{V'CO_2}$ reported in the current study (range 18.8 to 26.3) are in part due to the athletic conditioning of the subjects used in this study (all subjects undertook regular exercise), and the lower estimated $\frac{V_D}{V_T}$ compared to those reported by others (21, 23). Subjects in the current study also had lower resting heart rates than their typical untrained counterparts, again, indicative of their athletic conditioning. Previous studies (22, 23, 28-30) have reported an increase in $V'E$ and $V_T$ with HUT. In the current study, no increases in $V'E$, $V'A$ or $V_T$ were recorded with HUT, and since there was no difference in $V'CO_2$ between SUP and HUT, this suggested that pulmonary ventilation was matched to $CO_2$ output in both conditions. However, in the current study, a lower $F_{ETCO2}$ occurred with HUT despite a decrease in resting ventilatory efficiency and no change in dead space. An increased mixing of alveolar gas with air from regions of the lung with reduced perfusion may occur in the HUT position thereby lowering $F_{ETCO2}$, however this did not significantly increase measures of $F_{ETO2}$.

The increase in heart rate with HUT is well known (2). In the current study it has been shown that the magnitude of increase in heart rate was associated with a proportional decrease in the HF component of HRV (shown in Fig. 2), consistent with vagal withdrawal occurring during head-up tilt (2). This suggested a reciprocal change in R-R interval and the HF component of HRV during HUT, a relation which may not be consistent for other methodologies. For example, HF cardiac variability may be abolished with administration of atropine while mean R-R interval remained unchanged (31), and HF cardiac variability increased markedly with a respiratory acidosis with minimal or no change in R-R interval (32). However, the current study uniquely reported a greater difference in the heart rate during inhalation compared to exhalation in the HUT position compared to SUP, despite the reduced HF component of HRV. The increased clustering of heart beats during inhalation with HUT potentially matched pulmonary blood flow with ventilation in an attempt to increase ventilatory efficiency (12, 13, 18). However, when hypercapnia was used to increase pulmonary ventilation in healthy humans (17, 32, 33), no increase in the clustering of heart beats during inhalation was observed despite a significant increase in the HF component of HRV. This was reported to be evidence of the dissociation of cardiac vagal tone and respiratory sinus arrhythmia. Data from the present study further indicate the independence of respiratory sinus arrhythmia and cardiac vagal tone.
The predominant increase in heart rate occurring with head-up tilt was a further clustering of heart beats during inhalation, with relatively small changes occurring to heart rate during exhalation (see Table 1). There was a positive association between the changes in heart rate during inhalation and the changes in ventilatory efficiency (\(V_E / V^*_{CO_2}\)), as shown in Fig. 3. Although speculative, this suggested that a further clustering of heart beats during inhalation (traditionally described as an increase in respiratory sinus arrhythmia) occurs as the lung loses efficiency. This adds empirical support to the notion that clustering of heart beats through inhalation in an upright lung is a physiological mechanism by which the decrease in ventilatory efficiency that occurs with HUT may be attenuated.

In summary, the original hypothesis that a decrease in the HF component of HRV would be independent on an increase in respiratory sinus arrhythmia could be supported by the current study. The hypothesis that a reduced \(F_{ETCO_2}\) with HUT would indicate an increase in ventilatory efficiency could not be supported. However, the possibility remains that the increased clustering of heart beats during inhalation in the HUT position may in part offset a decrease in pulmonary efficiency which occurs in the upright lung.

Conflicts of interest statement: None declared.

REFERENCES


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Author’s address: Address for correspondence: Dr. Stephen Brown, IFNHH, Massey University, Private Bag 102-904, Auckland, New Zealand; Phone: 64 9 414 0800 ext 41101; Fax: 64 9 443 9640; e-mail: s.j.brown@massey.ac.nz