Mild Hypovolemic Stress Alters Autonomic Modulation of Heart Rate

John K. Triedman, Richard J. Cohen, and J. Philip Saul

In response to changes in central venous volume, changes in vagal efferent cardiac outflow have been demonstrated in animals but not in humans. In this study, frequency domain analysis was used to quantify modulation of heart rate by respiration and blood pressure in normal human adults undergoing mild central hypovolemic stress induced by blood donation and postural change. In supine subjects, blood donation caused no change in mean heart rate, pulse pressure, or in the variance of heart rate or blood pressure. There were small decreases in mean in systolic blood pressures. A significant decrease in vagal modulation of heart rate was seen in the 0.12-0.5-Hz frequency band, as measured by the change in the relation of lung volume to heart rate in this frequency band (−4.49 beats per minute [bpm] per liter [L], p<0.001). Comparison of supine and tilt positions revealed marked changes in heart rate and blood pressure means and variances consistent with more pronounced decreases in intracardiac filling pressures and unloading of the arterial baroreceptors. A further progressive decrease in the vagal modulation of heart rate by lung volume was observed in the 0.12-0.5-Hz band, with a near-linear response of magnitude of respiratory sinus arrhythmia over a range of estimated central venous volume. Transfer function analysis can detect changes in autonomic response to mild degrees of central hypovolemia, which are insufficient to cause changes in mean heart rate or heart rate variance. This represents evidence for modulation of heart rate control by cardiopulmonary baroreceptors. A near-linear relation between magnitude of respiratory sinus arrhythmia and central venous volume suggests that this may have clinical relevance in patient monitoring. (Hypertension 1995;21:236-247)

KEY WORDS • autonomic nervous system • pressoreceptors • hemorrhage • heart rate

Cardiopulmonary baroreceptor reflexes have been shown to control peripheral muscle sympathetic nerve activity and vascular resistance with physiological changes in central venous pressure in humans.1-3 Induction of mild decreases and increases in central venous volume by lower body negative pressure, mild hemorrhage, or volume loading leads to significant changes in vascular resistance without affecting heart rate (HR) or mean arterial blood pressure.1-4 Alterations in muscle sympathetic efferent output have been demonstrated to occur with changes in central venous volume.5 However, because of the absence of a HR response in these cases, it has been suggested that there is no change in the autonomic efferent outflow to the heart.2,3

The absence of a change in mean HR in this setting could result from 1) absence of cardiac sympathetic effect due to the heterogeneous nature of sympathetic neural response6-8; 2) offsetting changes in mean vagal and sympathetic cardiac activity; or 3) changes in sympathetic or vagal modulation, or both of HR without a change in mean autonomic cardiac tone. Animal studies by Bainbridge8,10 and Vatner et al11 have convincingly demonstrated reflex vagal changes in HR in response to both increases and decreases in central venous volume. Also, some reports have suggested that the sensitivity of the arterial HR baroreceptor reflex may be increased with decreases in central venous pressure.12 However, other studies have failed to demonstrate similar changes.5,12,14

Frequency domain analyses have been applied extensively over the last 10 years to the study of HR control. Studies that have used autonomic blockade and random interval breathing techniques in normal humans have resulted in a semiquantitative description of the sympathetic and vagal modulation of HR responsible for respiratory sinus arrhythmia.15,16 This approach offers advantages over traditional experimental techniques applied to human physiological research in that it can largely be conducted noninvasively, and it does not require the imposition of open-loop experimental conditions to perturb the cardiovascular system from the steady state.

To investigate the presence or absence of a cardiopulmonary reflex affecting HR control, we examined relations among lung volume (RESP), HR, and arterial blood pressure in the frequency domain in a group of

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healthy, young adults. To assess the usefulness of these techniques in detecting small changes in intravascular volume, subjects were studied before and after mild hemorrhage (450 ml whole blood donation) and in supine and upright postures.

**Methods**

**Subject Group**

Twenty-five healthy, young adults planning to undergo voluntary blood donation were recruited as the experimental group. None of the subjects was taking medication, nor had they smoked or consumed caffeinated beverages within 4 hours of the study. Two of the subjects were subsequently excluded from the analysis because of excessive ventricular bigeminy precluding off-line analysis of the recorded data. Of the remaining 23 subjects, 12 were women and 11 men (mean age, 29.0±4.4 years; mean weight, 72.6±14.3 kg). Using formulas for estimation of blood volume based on height and weight established by Nadler et al., a standard donation of 450 ml constituted 10.2±2.0% (range, 7.0–14.1%) of each subject's blood volume.

**Experimental Protocol**

The study protocol was approved by the institutional human studies committee, and written informed consent was obtained from all participants. In 21 subjects, surface electrocardiogram, instantaneous RESP, and continuous noninvasive arterial blood pressure were monitored; in two subjects, only electrocardiogram and RESP were monitored. RESP was recorded using a Respitrace two-beat impedance plethysmograph (Non-invasive Monitoring Systems, New York) that was calibrated by having subjects alternately inflate and deflate an 800-ml bag. Arterial blood pressure was recorded using a Finapres digital photoplethysmograph (Ohmeda, Englewood, Colo.). The Finapres was applied to the left second digit, which was fixed to the subject over the xiphoid process with a sling; a correction of 15 cm H2O was applied to blood pressures between the supine and tilt conditions because of a relative change in the position of the measured digit and the right atrium in accordance with the manufacturer's recommendations. During the course of an experiment, the electrocardiogram, arterial blood pressure, and RESP were monitored and recorded on a model 3968 eight-track FM tape recorder (Hewlett-Packard Co., Palo Alto, Calif.).

After the cardiovascular variables were observed to be stable, respiratory and arterial blood pressure calibrations were recorded. Then, with the subject in the supine position, the RESP, electrocardiogram, and arterial blood pressure signals were recorded during 15 minutes of controlled breathing, during which respiratory intervals were set with an auditory cue (beep). For the first 5 minutes, the cues were set at a constant 4-second interval (0.25 Hz, 15 per minute) to allow the subjects to become familiar and comfortable with the technique. For the next 10 minutes, the cues occurred with intervals between 1 and 15 seconds, randomly chosen from a Poisson distribution, with a mean interval of 4 seconds or 15 per minute. Tidal volume was not controlled. This technique spectrally "whitens" the respiratory input signal and has been described previously.15,18

Subsequent to this recording, subjects were placed in a 60° head-up tilt position and allowed a 10-minute period for equilibration. Instruments were recalibrated, and the process outlined above was repeated. After the subjects returned to the supine position, the procedure for obtaining a standard donation of 450 ml whole blood was performed. The subjects were allowed an additional 10 minutes in the supine position before all measurements were repeated in both the supine and 60° tilt positions. One patient failed to complete the final experimental condition due to hypotension and near-syncope, which resolved with return to the supine position and intake of fluids by mouth. No other patients became frankly hypotensive or reported symptoms more severe than mild light-headedness.

**Data Acquisition and Analysis**

Signals were analyzed off-line with a Sun 3/50 workstation. Data were digitized at 360 Hz after antialias filtering at 180 Hz. After R wave positions were detected digitally, the RR intervals were converted into a smoothed instantaneous HR time series constructed at 3 Hz, using an algorithm described previously.18 Blood pressure and RESP signals were digitally filtered and decimated to 3 Hz. The 3-Hz representation of the blood pressure signal is referred to as ABP. Systolic and diastolic pressures were identified from each beat of the nondecimated blood pressure signal, and the values obtained were splined and sampled at 3 Hz so that values of all the constructed time series occurred simultaneously. Pulse pressure was obtained by subtracting diastolic from systolic pressures. All signals were visually inspected for artifact. Approximately 6-minute (341 seconds=1,024 points) time series of HR, RESP, ABP, and systolic, diastolic, and pulse pressures were selected from each 10-minute period of random breathing for analysis. Mean and variance of the values for each experimental condition were calculated for each study.

**Frequency Domain Analyses**

Physiological signals have classically been represented and interpreted in the time domain, reflecting the manner in which they are acquired. Such signals are easily converted between the time domain and the frequency domain with Fourier techniques, which are broadly applied in the physical sciences. By using these techniques, the total variance of a signal can be broken down into its oscillatory components at individual frequencies. Alterations in HR and blood pressure variance, a coarse measure of frequency content, have been associated with cardiovascular pathology.19,20 Because many physiological processes relating to cardiovascular control have been shown to have distinctive signatures in the frequency domain, the more sophisticated techniques of power spectral analysis and transfer function analysis were applied to these data.

Power spectral analysis is a technique that parses the total variance in a signal into its frequency components. Conversely, the total power obtained by integrating the power spectrum over its frequency range is equal to the total variance of the signal. Power spectra were calculated for each time period using the Blackman-Tukey technique. Mean power spectral error were calculated for each experimental condition. Total power was calculated over the frequency band...
0.03–0.5 Hz, as well as in the subbands 0.03–0.12 Hz and 0.12–0.5 Hz for each study. Figure 1 presents representative time series and spectral data from a single experimental period, demonstrating the effect of random interval breathing on the respective physiological variables in both the time and frequency domains.

A transfer function estimates the relative power and timing of two signals over a range of frequencies. Thus, the transfer function of two signals defines their gain and phase relations at any given frequency and provides a statistical measure of reliability, called coherence, of the relation between the two signals. This technique has been simply but effectively used to study respiratory sinus arrhythmia by plotting the magnitude and phase relation of the HR response to a measured respiratory excursion over a number of discrete frequencies. Use of a random interval breathing technique allows efficient collection of data over a broader physiological spectrum. These principles can be extended to any physiologically coupled input and output signals, whether the coupling is thought to be mechanical or neural.

Transfer function analyses between the derived variables was performed using the cross-spectral technique (Appendix A). In a manner analogous to the power spectrum, transfer magnitude represents the relative amplitude, or gain, of the output signal for a given input signal at a given frequency. Note that the gain automatically corrects for changes in the amplitude of the input signal, e.g., variations of tidal volume and respiratory rate of the RESP signal. Transfer phase quantifies the degree of phase lead or lag between the two signals at a given frequency. A phase of 0° indicates that a positive change in the input signal coincides with a positive change in the output signal, e.g., the occurrence of cardiac acceleration simultaneously with inspiration. A phase of 180° indicates the opposite, e.g., cardiac deceleration with an increase in blood pressure. Phase values intermediate between these values may indicate either a delay between the two signals or a mixed response of the output signal to the input signal. The coherence estimate, which varies from 0 to 1, does not have direct physiological significance but serves as a statistical measure of the reliability of the transfer function estimate and of the linearity of the input/output relation. In this study, coherence was used to weight individual values of transfer magnitude and transfer phase in the calculation of group-averaged
FIGURE 2. Tracings show time series and transfer function analysis from one subject before blood donation and during random interval breathing, in the supine and 60° tilt positions. Mean heart rate (HR) increased, as did the variability of both lung volume (RESP) and HR in the tilted posture. This is reflected in an increase in the magnitude of the power spectra of both variables, with RESP power increasing more than HR power at all frequencies less than 0.5 Hz. Transfer function magnitude, analogous to ratio between the two power spectra, shows a corresponding decrease in the RESP-HR relation across the frequency range studied, with a maximum value in the supine condition of between 15 and 20 beats per minute (bpm) per liter at a frequency of 0.1–0.15 Hz. Note that the transfer function has normalized the effect of increased respiratory variability on HR. In this patient, transfer phase is nearly flat and 0° at frequencies greater than 0.1 Hz. This indicates the nearly instantaneous and unopposed effect of vagal activity in this frequency band, with cardiac acceleration occurring simultaneously with inspiration. Coherence spectra demonstrate adequate reliability of the transfer function estimation (coherence >0.5) over most of the frequency band examined.

Statistical Techniques

All values were examined for normal distribution. Mean values of HR, ABP, and systolic, diastolic, and pulse pressures were normally distributed. Comparisons for these parameters between experimental conditions were performed using Student’s paired t test with Bonferroni correction and analysis of variance. Mean values of ABP and systolic and diastolic pressures were not compared between supine and tilt conditions because it was necessary to apply a correction for movement of the subjects’ upper extremities when they were shifted from a supine to an upright position. Pulse pressure was compared between these conditions since it was unaffected by the correction. The variance of HR, ABP, and systolic, diastolic, and pulse pressures, and all values obtained in the frequency domain, was skewed by outlier values and was compared using the Wilcoxon signed-rank test and the Kruskal-Wallis one-way analysis for multiple values. Differences between before- and after-donation values of mean HR, indexes of blood pressure, and RESP>HR transfer magnitudes were related to estimated percentage of blood donation using a linear regression. A value of p<0.05 was considered to be statistically significant.

Results

Response of Means and Variances

Mean and variance of HR, ABP, and systolic, diastolic, and pulse pressures are presented in Table 1. The variance of ABP is not presented because...
TABLE 1. Means and Variances of Heart Rate and Blood Pressures

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>66.9±9.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Tilt</td>
<td>80.7±10.2</td>
<td>NS</td>
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<tr>
<td>After donation</td>
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<tr>
<td>Supine</td>
<td>67.5±9.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Tilt</td>
<td>93.8±14.2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>72.1±10.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Tilt</td>
<td>73.9±11.3</td>
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</tr>
<tr>
<td>After donation</td>
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<td></td>
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<tr>
<td>Supine</td>
<td>66.6±10.4</td>
<td></td>
</tr>
<tr>
<td>Tilt</td>
<td>68.9±12.4</td>
<td></td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
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<tr>
<td>Before donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>54.1±9.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Tilt</td>
<td>58.0±11.2</td>
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<tr>
<td>After donation</td>
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<td></td>
</tr>
<tr>
<td>Supine</td>
<td>50.0±9.6</td>
<td></td>
</tr>
<tr>
<td>Tilt</td>
<td>55.0±11.1</td>
<td></td>
</tr>
<tr>
<td><strong>Pulse pressures (mm Hg)</strong></td>
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<td></td>
</tr>
<tr>
<td>Before donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>54.8±8.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Tilt</td>
<td>50.4±9.5</td>
<td></td>
</tr>
<tr>
<td>After donation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>53.7±10.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Tilt</td>
<td>46.6±10.9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

bpm, Beats per minute; ABP, 3-Hz representation of the blood pressure signal. Mean values were compared with paired Student's t test, variances with Wilcoxon signed-rank test. All multiple comparisons were corrected with Bonferroni technique. All bracketed probability values refer to the mean values in each column. ABP variance excluded secondary to sampling issues (see text). All values are expressed as mean±SD.

Comparison of the before- and after-donation states in the supine position revealed no change in mean HR (Δ mean HR=+0.6 bpm, p=0.51) or HR variance (Δσ²HR=-4.2 bpm, p=0.17). A small but significant decrease was noted in ABP (Δ mean ABP=−5.5 mm Hg, p<0.01) and systolic pressure (Δ mean systolic=−5.2 mm Hg, p<0.05). No significant changes were noted in the mean values of diastolic and pulse pressures or in the variances of any of the blood pressure indexes. No significant correlation was found between the change in mean HR and the change in any index of blood pressure. Similarly, no correlation was noted between percentage of blood volume donated and the change in mean HR or the change in any index of blood pressure.

Comparison of supine and tilted positions revealed marked increases in mean HR and HR variance in both the before- and after-donation states. Mean pulse pressure decreased with tilt, and this decrease was more marked in the after-donation state (Δ mean pulse before donation=−4.4 mm Hg, p<0.05; Δ mean pulse after donation=−7.1 mm Hg, p<0.01). The variances of systolic, diastolic, and pulse pressures all increased significantly with the change from supine to tilt.

Response of Power Spectra

Integrated power spectral densities for RESP, HR, and ABP signals are given for the High and Low frequency bands (Table 2). These values represent the
signal variance found within the Low and High frequency bands. Integrating over the range from 0 to 1.5 Hz (Nyquist frequency for 3-Hz sampling) yields the total variances reported in Table 1.

In the supine position, no significant changes were noted in any of the signals in the Low frequency band between the before- and after-donation conditions. In the High frequency band, a significant decrease in HR power was seen after blood donation (Δ power HR<sub>High</sub> = -4.03 bpm<sup>2</sup>, p < 0.05). With change in position from supine to head-up tilt, marked increases in the power of the ABP signal were seen (A power ABP<sub>High</sub> = +1.79 mm Hg<sup>2</sup>, p < 0.05). In the before- and after-donation conditions (before donation: A power ABP<sub>Low</sub> = +0.009 mm Hg<sup>2</sup>, p = 0.05; after donation: A power ABP<sub>Low</sub> = +0.014 mm Hg<sup>2</sup>, p < 0.01).

### Response of Transfer Function: Respiratory Sinus Arrhythmia (RESP>HR)

The coherence-weighted, averaged transfer magnitude and phase spectra and the averaged coherence functions for the RESP to HR (RESP>HR) relation are presented in Figure 3. Mean transfer magnitudes for Low and High frequency bands are given in Table 3. No significant changes were found in the Low band in transfer magnitude, whereas a highly significant decrease was observed in the High band (p < 0.0001 by Kruskal-Wallis). Changes in the Low band of transfer phase show a progressive decrease in phase slope, with a value at 0 Hz (DC value) approaching 270° in the tilt position. As outlined in Table 3, these changes are significant within pairwise comparisons between supine and tilt conditions and between before- and after-donation conditions. Pooling of data into supine and tilt groups emphasizes the difference between Low and High frequency bands in each experimental condition.
Figure 3. Tracings show group-averaged transfer function analysis of lung volume to heart rate (RESP>HR), the relation between RESP and HR. Low and High frequency bands from which band-averaged magnitudes were calculated are graphically indicated. No significant difference in Low frequency transfer magnitude is noted among the four experimental conditions. In contrast, a steady and statistically significant decrease is noted in High frequency transfer magnitude, with highest magnitudes noted in the supine/before (pre) donation period, followed by supine/after (post) donation, tilt/before donation, and tilt/after donation. Transfer phase is nearly flat from 0.03 to 0.5 Hz in both of the supine conditions, whereas the Low frequency phase in both tilted conditions approaches 180° and manifests a steep roll-off, as described previously in states of sympathetic activation. Note that coherence below 0.05 Hz is <<0.5, making interpretation of extremely low frequency phenomena unreliable. bpm, Beats per minute.

Discussion

Autonomic Modulation of Respiratory Sinus Arrhythmia

In our subjects, there was no significant change in mean HR or HR variance with blood donation in the supine position. At the same time, a highly significant change was observed in the beat-to-beat control of HR. This was manifested by a decrease in the transfer magnitude of RESP>HR in the High frequency band without significant change in the Low frequency band. Changes were also observed in the Low frequency transfer phase, with a trend toward a more negative phase slope and a DC value changing from -180° before donation to 0° after donation. The magnitude changes noted were more pronounced with a change in position from supine to 60° tilt.

Prior studies have demonstrated that the transfer magnitude and phase characteristics of autonomic modulation of HR by respiration can be efficiently quantified with a broadband respiratory input. In the present study, the transfer function of respiration to HR, which quantifies respiratory sinus arrhythmia in the frequency domain, was comparable to both fre-
TABLE 3. Group Average Transfer Magnitudes

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Low frequency (0.03–0.12 Hz)</th>
<th>High frequency (0.12–0.5 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before donation</td>
<td>After donation</td>
</tr>
<tr>
<td></td>
<td>Supine</td>
<td>Tilt</td>
</tr>
<tr>
<td></td>
<td>19.67±14.73</td>
<td>19.63±12.81</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>17.48±9.91</td>
<td>9.39±3.98</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>12.99±6.51</td>
<td>7.03±3.49</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>18.67±13.75</td>
<td>18.67±13.75</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supine</td>
<td>Tilt</td>
</tr>
<tr>
<td></td>
<td>18.53±13.50</td>
<td>15.21±8.58</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>8.23±3.87</td>
<td></td>
</tr>
</tbody>
</table>

bpm, Beats per minute; ABP, 3-Hz representation of the blood pressure signal. Comparisons were made with Wilcoxon signed-rank test, with multiple comparisons corrected with Bonferroni technique. All bracketed probability values refer to the mean values in each column. All values are expressed as mean±SD of band-averaged values in Low and High frequency bands in each experimental condition and are grouped into supine and tilt positions.

frequency domain and time domain analyses of this relation reported in prior studies.24 Vagal HR control is characterized by significant transfer magnitude at both High and Low frequencies, with a peak magnitude of about 15 bpm/l at a frequency of 0.12–0.15 Hz. A slight phase lead of HR to respiration indicates that increases in HR slightly precede inspiration, as measured by our technique, and is consistent with a central effect of respiration on vagal efferent activity.15 In contrast, sympathetic HR control is characterized by reduced magnitude at Low frequencies and virtually none at High frequencies, with a phase delay between inspiration and subsequent decreases in HR. Thus, the findings from the present study indicate a steady decrease in vagal modulation of HR in the High frequency band as estimated central venous volume decreased. Because decreased vagal modulation of HR would also have been expected to produce some decrease in the Low frequency band, the lack of an observed decrease in the Low frequency transfer magnitude for this relation suggests reciprocal increases in sympathetic modulation of HR. This hypothesis is supported by the observed changes in the Low frequency transfer phase with central venous volume reduction (Figure 3). Of note, the absence of a change in mean HR with blood donation suggests that the observed changes in vagal and sympathetic modulation of HR variability may be independent of changes in mean cardiac vagal or sympathetic neural activity. Because efferent cardiac autonomic nervous activity was not directly measured in this

![Figure 4](http://hyper.ahajournals.org/)

**FIGURE 4.** Scatterplots show estimated percentage of blood volume donated versus change in transfer magnitude of lung volume to heart rate (RESP>HR). A weak linear positive relation is noted between percentage of blood volume donated and the decrease in RESP>HR High frequency transfer magnitude associated with blood donation. Relation is noted in both the supine and tilt positions; both slopes are significantly greater than zero (p<0.05) but not significantly different from one another. RH, lung volume or respiration to heart rate; bpm, beats per minute.
ABP to Heart Rate (ABP>HR)

Transfer Magnitude (bpm/mmHg)

Transfer Phase (degrees)

Coherence

Figure 5. Tracings show group-averaged transfer function analysis of 3-Hz representation of blood pressure signal to heart rate (ABP>HR), the relation between ABP and HR. Low and High frequency bands from which band-averaged magnitudes were calculated are graphically indicated. In contrast to lung volume to heart rate (RESP>HR) relation, significant decreases are noted in Low frequency transfer magnitude among the four experimental conditions, and coherence values are relatively high in this range. Transfer phase is approximately 90° in all four conditions, indicating a mixed signal response (see text).

Frequency (Hz)

Study, we are unable to state whether changes in mean cardiac autonomic activity were present or what the sensitivity of the mean HR is in humans to changes in mean sympathetic and vagal activity. However, since mean HR was unchanged, mean cardiac vagal and sympathetic firing rates must have either changed very little or undergone offsetting changes, with both increasing or both decreasing.

We also found a weak, linear relation between estimated percentage of blood volume donated and change in RESP>HR transfer magnitude in the High frequency band in both the supine and 60° tilt positions. No similar correlations were found between percentage of blood volume donated and either HR or indexes of blood pressure. The change in absolute transfer magnitude of respiration to HR was greater in the supine than 60° tilt position, resulting in a decreased slope for this relation in the tilted position (Figure 4). Because the correlation of this relation was not improved by examining the percentage change in the respiration to HR transfer magnitude, these findings suggest that the gain of the response is smaller during tilt. In the upright position, central venous volume is decreased by both pooling of blood in the dependent extremities and transudation of approximately 10% of plasma volume into the interstitium; the total effect of posture on central venous volume in animal studies has been estimated to be approximately 20% of central blood volume.27 The relation between the magnitude of respiratory sinus arrhythmia and blood volume that we observed may represent a linear response or the linear portion of a sigmoidal response of respiratory sinus arrhythmia to decrease in blood volume (Figure 6). Although this proposed relation must be verified by direct measurement of central venous pressure and blood volume, our data indicate that a relatively simple calibrated measure of respiratory sinus arrhythmia may provide a noninvasive means of monitoring small changes in blood volume in a clinical setting.

Why did vagal modulation of HR decrease? Respiratory modulation of HR may result from a number of mechanisms, which include 1) direct coupling between respiratory activity and efferent cardiac vagal activity in the brain stem; 2) central respiratory modulation of arterial baroreceptor reflex sensitivity; 3) mechanical modulation of atrial size leading to changes in cardiopulmonary baroreceptor activity; 4) mechanical atrial stretch effects on sinoatrial node function;
5) mechanical modulation of ventricular preload and afterload, resulting in arterial pressure changes and subsequent arterial baroreceptor reflex modulation of HR;22 and 6) central respiratory modulation of peripheral or central chemoreceptors, or both.33 Because a decrease in central venous volume decreases atrial size, deactivates cardiopulmonary baroreceptors, possibly changes their sensitivity, and decreases ventricular preload, eventually decreasing arterial pressure, changes in any of the first five mechanisms listed above might explain our findings of decreased respiratory-related vagal modulation to mild central hypovolemia.

With the exception of mechanical effects on the sinoatrial node, the effects of central volume listed above are all mediated by changes in modulation of cardiac vagal and sympathetic efferent activity. However, the frequency response characteristics of the sinoatrial node to vagal and sympathetic stimulation have been shown to vary at different mean rates of vagal and sympathetic stimulation, with a relative increase in low frequency gain at lower mean rates of nerve stimulation.22 In the 60° tilt position, our subjects had increased mean HRs and were likely to have been experiencing changes in mean vagal and sympathetic cardiac autonomic activity. It is thus possible that part of the change in shape of the RESP>HR transfer spectra, with a relative increase in the Low frequency band compared with the High frequency band in the 60° tilt position, is due to such changes in mean autonomic activity. However, this mechanism seems less likely to account for such changes in the supine position, when there was no change in mean HR and either no change or simultaneous increase or decrease of both mean cardiac vagal and sympathetic activity.

Studies in dogs have more convincingly demonstrated a HR reflex effect of central venous volume. In 1915, Bainbridge described an immediate reflex tachycardia in response to rapid volume infusion in dogs, as well as a return to normal HRs with subsequent hemorrhage. He subsequently proposed that respiratory sinus arrhythmia was caused by reflex changes in HR secondary to atrial size.9 Vatner et al confirmed this findings and documented decreased arterial HR baroreceptor reflex sensitivity with volume loading. No such reflex tachycardia has been convincingly demonstrated in humans,1,2,3 and until this study, a role for atrial reflexes in HR control in humans had not been identified.

Although the current experimental conditions do not allow us to resolve the mechanism of decreased vagal HR modulation, the slight phase lead of changes in HR to changes in respiration that we observed in the High frequency band after blood donation in the supine position indicates that decreases in vagal efferent activity and increases in HR actually anticipate active inspiration. This is concordant with direct evidence of vagal deactivation before onset of phrenic nerve activity26 and effectively rules out a primary role for direct or indirect mechanical effects of respiration on atrial size. By the same reasoning, a centrally mediated effect incorporating outflow from the respiratory control centers in the brain stem seems likely to play a role in mediating the observed changes. Although a significant decrease in blood pressure was noted in the supine posture with blood donation, this decrease was small in magnitude and not associated with any significant change in mean HR, HR variance, or pulse pressure. These findings suggest that the changes in the RESP>HR transfer function magnitude that we observed in the supine position are primarily due to unloading of the cardiopulmonary baroreceptors in the supine position by hemorrhage and are not due to significant unloading of arterial baroreceptors. Therefore, the results of the present study strongly suggest that a cardiopulmonary baroreceptor reflex controlling centrally mediated vagal respiratory modulation of HR is active in normal humans.

**Relation of Arterial Blood Pressure to Heart Rate**

A closed-loop relation exists between HR and arterial blood pressure. This loop includes 1) feedback effects of the arterial baroreceptor reflex, which may affect both HR and peripheral vascular resistance, and 2) several feedforward effects of circulatory mechanics, a complex property that describes the effects of changes in HR on blood pressure and includes vascular compliance and impedance and the effect of HR on stroke volume. Because the Fourier techniques used in the present study to estimate transfer functions cannot attribute causality, it is not possible to discriminate the feedforward and feedback effects between HR and blood pressure in the transfer function ABP>HR. The transfer magnitude function in this closed-loop setting constitutes the complex sum of both feedforward and feedback transfer functions and does not exactly repre-
sent the true value of either limb of the relation or the algebraic sum of the two magnitudes. The isolated, open-loop effect of the arterial baroreceptor reflex is to produce a decrease in HR in response to a step increase in blood pressure when changes in arterial blood pressure are slow compared with the response of the baroreceptor reflex. This describes a phase relation that approaches 180° at 0 Hz. Under similar conditions, properties determining feedforward from HR to blood pressure are more complex but might be expected to have a phase relation of 0°. The observed phase of our ABP>HR transfer function in Figure 6 is located near 90°, which suggests that both processes contribute to its genesis.

Although these problems limit our ability to identify open-loop system responses during closed-loop operation, certain observations can be made. The steady decrease of transfer magnitudes noted are consistent with either a decrease in baroreceptor reflex (feedback) gain, an increase in circulatory mechanical (feedforward) gain, or both. In light of prior experimental findings with respect to the effect of unloading of the cardiopulmonary baroreceptors on the arterial baroreceptor reflex, a decrease in sensitivity of the arterial baroreceptor reflex seems unlikely.1214 Peripheral vascular resistance is known to increase with unloading of the cardiopulmonary baroreceptors13,6,14; an increase in peripheral vascular resistance or vascular impedance would cause an increase in circulatory mechanical gain and therefore could account for our findings. The effects of HR on stroke volume are mediated by filling time, atrial pressure, and ventricular compliance; although no change in mean HR (and therefore in filling time) occurred in the supine positions, changes in mean atrial pressure or ventricular compliance could alter the relation between HR and stroke volume changes. These feedback and feedforward effects are not exclusive; it is possible that large increases in peripheral vascular resistance could mask smaller increases in baroreceptor reflex gain.

In conclusion, using transfer function analysis in combination with random interval breathing, we investigated noninvasively the effect of mild hemodynamic stress on healthy, young adults. Our findings concerning the transfer function between respiration and HR indicate: 1) mild degrees of hypovolemia induced by hemorrhage that are not manifested by significant changes in mean HR or HR variance produce significant changes in vagal and sympathetic modulation of HR by respiration; 2) these changes in autonomic activity appear to be secondary to unloading of cardiopulmonary baroreceptors; and 3) the degree of vagal withdrawal measured by this technique is linearly related to the percentage of blood volume removed in blood donation. Thus, a quantitative measure of respiratory sinus arrhythmia using the transfer function between respiration and HR may serve as a clinical indicator of central volume status during mild-to-moderate central hypovolemia.

With respect to the closed-loop relation between blood pressure and HR, our findings indicate that 1) a decrease in central venous volume is associated with decreased arterial baroreceptor reflex gain (feedback), increased circulatory mechanical gain (feedforward), or both, causing a decrease in the blood pressure to HR transfer magnitude at High frequencies; and 2) although increases in peripheral vascular resistance could account for the observed changes, the noncausal analytic approach used in this study cannot separate the feedforward and feedback effects.

Appendix A

Calculation of Transfer Function Estimates

Transfer functions were calculated according to the cross-spectral technique:

\[ H(f) = \frac{S_{xy}(f)}{S_{xx}(f)} \]  

where \( H(f) \) represents the complex transfer function and \( S_{xy} \) and \( S_{xx} \) represent the autospectrum and the cross-spectrum of the input and output signals, \( x \) and \( y \). Cross-spectral and autospectral estimates were computed using the Blackman-Tukey method, with a four-point (0.006 Hz) Gaussian window for smoothing in the frequency domain. The real and imaginary components of \( H(f) \) \( \{H_a(f) \text{ and } H_i(f)\} \) were used to compute the transfer magnitude or gain \( |H(f)| \), transfer phase \( \Theta(f) \), and coherence \( \text{Coh}^2(f) \) (equations 2–4) of the relation between the input and output as a function of frequency.

\[ |H(f)| = \left( |H_a(f)|^2 + |H_i(f)|^2 \right)^{1/2} \]  

\[ \Theta(f) = \tan^{-1} \left( \frac{H_i(f)}{H_a(f)} \right) \]  

\[ \text{Coh}^2(f) = \frac{|S_{xy}(f)|^2}{S_{xx}(f)S_{yy}(f)} \]  

Appendix B

Band-Average Transfer Magnitude Estimates

Measurement variance is associated with the transfer function computation at each frequency, as reflected by a coherence less than one. In addition, a population variance is present secondary to differences between individuals. It has been previously shown that the measurement variance \( \pi_{\text{mag}}^2(f) \) associated with the \( i \)th individual transfer magnitude estimate is

\[ \pi_{\text{mag}}^2(f) = K |H_i(f)|^2 \left[ 1 - \text{Coh}^2(f) \right] \]  

where \( H_i(f) \) and \( \text{Coh}^2(f) \) are the \( i \)th individual transfer magnitude and squared coherence functions, and \( K \) is a constant related to the degree of spectral smoothing.25 The population variance \( \pi_{\text{mag}}^2(f) \) is independent of this variable and is subsequently calculated by standard statistical techniques. The estimator variances \( \pi_{\text{mag}}^2(f) \) can be used as weights in calculating the band-average transfer magnitude estimate \( H(\text{band}) \)

\[ |H(\text{band})| = \frac{\sum_{i=1}^{N} \left| H_i(f) \right|^{1/\pi_{\text{mag}}^2(f)}}{\sum_{i=1}^{N} \left[ 1/\pi_{\text{mag}}^2(f) \right]} \]
where $N$ is the number of individual estimates in each frequency band of interest.

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