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Association of cardiac autonomic function measures with severity of sleep-disordered breathing in a community-based sample

Wenli Wang¹, Suradej Tretriluxana¹, Susan Redline², Susan Surovec², Daniel J. Gottlieb³, and Michael C. K. Khoo¹

¹Biomedical Engineering Department, University of Southern California, Los Angeles, CA

²Division of Clinical Epidemiology, Department of, Pediatrics, Case Western Reserve University, Cleveland, OH

³Department of Medicine, Boston University and the VA Boston Healthcare, System, Boston, MA, USA

SUMMARY

The goal of this study was to test the hypothesis that spectral indices of heart rate variability, such as high-frequency power (HFP), low-to-high frequency power (LHR), and their respiration-adjusted counterparts (HFPra, LHRra) are correlated with severity of sleep-disordered breathing (SDB), as quantified by the respiratory disturbance index (RDI). A total of 436 subjects, non-smoking, normotensive, and free of cardiovascular disease and diabetes were selected from the Sleep Heart Health Study (SHHS). Of these, 288 records with sufficiently high quality electrocardiogram signals were selected for further analysis [males / females: 221 / 67; age: 46.1 to 74.9 years; body mass index (BMI): 21.5 to 46.4 kg m⁻²; 0.3 < RDI < 85.0⁻¹]. From each polysomnogram, the respiration channels (thoracic and abdominal) and R-R interval (RRI) derived from the electrocardiogram were subjected to spectral analysis and autoregressive moving average modeling in consecutive 5-min segments. After adjusting for age and BMI, mean RRI was found to be negatively correlated with RDI in men in all sleep-wake states (all $P < 0.001$). HFP and HFPra were negatively correlated with RDI in men only during wakefulness (all $P < 0.01$). In women, LHR and LHRra were not correlated with RDI during wakefulness, but were positively correlated during non-rapid eye movement Stage 1 and 2 sleep (all $P < 0.01$). These findings suggest that the indices of cardiac autonomic control are correlated with SDB severity, but gender and state affect the nature of these correlations. In both genders, however, vagal modulation of heart rate increases while sympathetic modulation decreases from wakefulness to sleep.

Keywords

autonomic function; heart rate variability; obstructive sleep apnea; power spectral analysis

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Correspondence: Michael C. K. Khoo, PhD, Biomedical Engineering, Department, University of Southern California, 1042 Downey Way, Los Angeles, CA 90089-0260, USA. Tel.: 213-740-0347; fax: 213-740-0343; khoo@bmsr.usc.edu.

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INTRODUCTION

It is now well recognized that sleep-disordered breathing (SDB) can lead to adverse effects on the heart and vasculature, resulting in cardiovascular diseases that include pulmonary and systemic hypertension, ischemic heart disease and myocardial infarction (Leung and Bradley, 2001; Parish and Shepard, 1990). The evidence for this view in humans has come primarily from large epidemiologic studies. For instance, the Wisconsin Sleep Cohort Study with over 700 subjects demonstrated that the adjusted odds ratio of incident systemic hypertension in SDB subjects with respiratory disturbance index (RDI) >15 was 2.89 compared with subjects without SDB (Peppard *et al.*, 2000). Shahar *et al.*, (2001) found in the Sleep Heart Health Study (SHHS) cohort of 6132 subjects that SDB is strongly correlated with prevalent coronary heart disease, heart failure, and stroke. In another report from the Sleep Heart Health Study Research Group, the odds ratio of hypertension was found to increase with severity of SDB, as quantified by RDI, even after adjustment of possible confounders (Nieto *et al.*, 2000).

A key factor in the causal link between SDB with cardiovascular disease may be abnormal autonomic control (Somers *et al.*, 1995). Evidence of sympathetic overactivity has been demonstrated in the form of elevated plasma and urinary catecholamine levels in patients with SDB (Fletcher *et al.*, 1987) as well as in direct measurements of sympathetic activity from peroneal microneurography (Somers *et al.*, 1995). Treatment of SDB by nocturnal application of continuous positive airway pressure over several months has been found to reduce muscle sympathetic nerve activity and plasma catecholamine levels (Hedner *et al.*, 1995; Narkiewicz *et al.*, 1999). Assuming that SDB leads to autonomic nervous system (ANS) dysfunction which, in turn, predisposes to cardiovascular disease, we hypothesize that after discounting for confounding variables, measures of ANS dysfunction should be correlated with severity of SDB.

Although there are more direct measures of autonomic activity, heart rate variability (HRV) has become a widely employed tool because of its non-invasiveness and ease of application. In addition to the mean R-R interval (mRRI) derived from the electrocardiogram (ECG), the power spectrum of HRV time-series provides information about parasympathetic (vagal) and sympathetic modulation of heart rate (Task Force, 1996). High-frequency power (HFP) (0.15–0.40 Hz) is believed to be parasympathetic in origin, while low-frequency power (LFP) (0.04–0.15 Hz) reflects a combination of both sympathetic and parasympathetic activities. As such, the ratio of low-to-high frequency power (LHR = LFP / HFP) has been widely used to represent the balance between sympathetic and vagal activities ('sympath-ovagal balance') (Task Force, 1996). However, caution needs to be exercised when changes in these HRV spectral indices are interpreted as representing changes in vagal and sympathetic activities (Eckberg, 1998). For instance, an increase in LHR that occurs concomitantly with a reduction in HFP could be because of a simultaneous increase in sympathetic activity and decrease in vagal activity, or simply a decrease in vagal activity. On the other hand, an increase in LHR that occurs without a corresponding reduction in HFP would suggest an increase in cardiac sympathetic activity.

High-frequency power is closely associated with the respiratory modulation of heart rate, commonly known as respiratory sinus arrhythmia (RSA). The fact that both tidal volume and breathing frequency can be highly variable within a given individual at different times introduces some complications into the interpretation of HFP as a measure of parasympathetic activity. The traditional means by which researchers have sought to circumvent the confounding influence of respiration on RSA magnitude has been to control the breathing pattern (Grossman *et al.*, 1991). Typically, breathing is voluntarily controlled at a given rate and / or depth, while the responses in RSA to various pharmacologic or posture-related interventions which affect sympathovagal balance are measured. However, this allows changes in RSA to be determined only at the controlled respiratory frequency and volume. Moreover,

this kind of voluntary control of the breathing pattern is not possible during sleep when the level of ventilation and breathing pattern can change considerably with various sleep stages. In subjects with SDB, ventilatory pattern can go through even larger temporal changes, as breathing alternates between regular tidal respiration, and episodes of apnea and hyperpnea during night. We have demonstrated previously (Khoo *et al.*, 1999) that these non-uniform ventilatory patterns can substantially influence LFP and HFP, and also introduce substantial power in the very-low frequency (VLF, <0.04 Hz) region. These effects can seriously complicate the interpretation of the traditional spectral measures of HFP and LHR as indices of autonomic regulation. To be able to extract useful information about the ANS from indices of HRV, it is thus important for the effects of respiration to be discounted when these measures are being computed.

To test our hypothesis that autonomic control, as measured from HRV, is affected by severity of SDB, we computed five ANS power spectral indices (mRRI, LHR, HFP, LHRa, HFPra) from a large number of overnight polysomnographic studies and tested each of these indices for possible correlation with RDI. The latter two indices were employed to adjust for the effects of differences in respiratory pattern and ventilation within and between subjects.

METHODS

Subject pool and measurements

A total of 436 participants in the second SHHS examination (1999–2004) who were free of known chronic cardiopulmonary disorders and were representative of the full range of SDB severity, as quantified by RDI (the number of hypopneic or apneic events occurring per hour overnight) were identified. Exclusionary criteria included history of lung disease (chronic obstructive pulmonary disease, asthma), cardiovascular disease, diabetes, hypertension (systolic blood pressure > 140 or diastolic pressure > 90 mmHg; subjects taking anti-hypertensive medications were also excluded) and current smoking. In addition, subjects were selected to achieve a sample of an equal number of subjects in each of four RDI categories (< 5, 5–15, 15–30, > 30 h⁻¹), who also were of generally comparable age (± 1 years), and roughly similar BMI (± 2 kg m⁻²). After detailed examination of the polysomnograms using an automated algorithm with manual checks, studies with long segments of extensive ectopic-beats [more than 6 b min⁻¹(ectopic) overnight was considered pathological] (Dubin, 1998) and excessive artifact in the ECG signals (with RRI > 3 SDs after interpolation) were excluded from further analysis. If the majority of overnight 5-min ECG segments met our criteria (more than ¾ of overnight data length), the whole night data was used to avoid selection bias from using only the ‘clean’ segments. Overall, the analysis procedures that are described below were applied to only 288 subjects (males / females: 221 / 67, age: 62.6 $\pm$ 6.7 years; BMI: 30.3 $\pm$ 4.3 kg m⁻²; 0.3 < RDI < 85.0). The characteristics of these 288 subjects are summarized in Table 1. Although there was an attempt in the subject selection process to limit the variation of BMI across the different RDI groups, the male subjects with RDI < 5 h⁻¹ were significantly lower in BMI compared with the other three RDI categories (Table 1). A comparison of the subject characteristics displayed in Table 1 with a corresponding table derived using all 436 subjects showed no significant differences. For instance, the distribution of the 288 subjects among the four RDI groups was relatively uniform, similar to the uniform distribution in the original sample of 436 subjects. Thus, the exclusion of bad-quality recordings did not lead to a selection bias in the final sample that was analyzed.

The technical details of the instrumentation and data collection techniques employed in the Sleep Heart Health Study Research Group, 1996 have been described elsewhere (Quan *et al.*, 1997; Redline *et al.*, 1998), but a brief summary of the main features is as follows: physiological data (e.g., ECG) of participants in SHHS were collected in single night, unattended polysomnography at home accompanied with body weight and blood pressure measurement;

other anthropometric and medical history information was obtained from a parent study database (e.g., height) or by a standard questionnaire (e.g., smoking status). Apneas were scored by SHHS if complete or almost complete cessation ($> 75\%$ decrease from baseline amplitude) of airflow measured by a nasal-oral thermal sensor was observed and hypopneas were scored if the amplitude signals from the thoracic and respiratory inductance plethysmography signals or thermistries decreased by 25% or more. All events were required to last longer than 10 s and also associated with oxyhemoglobin desaturation of 4% or greater as compared with the baseline. All channels of selected polysomnograms were manually checked to be of reasonably high signal-to-noise ratio.

Signal processing and spectral analysis

QRS detection from the ECG was achieved by employing an automatic derivative-based algorithm with the threshold set at 0.3 times the maximum magnitude of the of the 5 min ECG segment (Kohler *et al.*, 2002). Validation of correct processing by the automatic algorithm was performed using visual inspection of the ECG segment with superimposed R-wave markers. In the noisier recordings, the noisy segments were rejected altogether and only the shorter segments of sufficiently good quality were employed in subsequent analyses. RRI was computed on a beat-to-beat basis, but the resulting time-series was resampled at 2 Hz using the Berger algorithm (Berger *et al.*, 1986) for subsequent spectral analysis. The abdominal and thoracic signals were also resampled at the same frequency to enable synchronization with the RRI time-series.

Calculation of 'respiration-adjusted' HRV spectral indices

The algorithm employed to produce these indices has been summarized schematically in Fig. 1. To every consecutive 5-min segment of each polysomnogram, the modeling technique introduced by Khoo *et al.* (1999) was applied to establish the temporal relationship (shown in Fig. 1 as 'HRV model') between respiration and fluctuations in RRI during the segment of time in question. This relationship (or 'transfer function', in engineering parlance) enabled the delineation of the respiratory-correlated component of HRV from the respiratory-uncorrelated component. The transfer function was also used to predict what the time-course of the RRI fluctuations would have been if the ventilatory pattern had remained the same as what was measured during the period of wakefulness prior to sleep onset, i.e., the 'respiration-adjusted RRI' time-series. A detailed account of this algorithm is given in the Appendix.

The spectra of the original RRI time-series and the 'respiration adjusted' RRI time-series were computed using the Welch method with Hanning windowing (Tompkins, 1993). The respective areas in the appropriate frequency bands (low-frequency: 0.04–0.15 Hz; high-frequency: 0.15–0.4 Hz) under each RRI spectrum were calculated to yield the HRV spectral indices, LFP, and HFP. Subsequently, LFP was divided by HFP to yield LHR. Analogous calculations were performed to arrive at the corresponding 'respiration adjusted' indices: LFPra, HFPra, and LHRra.

Statistical analyses

Using the aforementioned methods, HRV spectral indices were computed based on consecutive 5-min segments of ECG and respiration. As pointed out by the 1996 Task Force report on HRV measurement (Task Force., 1996), a duration of 5 min is probably the longest period over which stationarity in the ECG time-series can be assumed and standard spectral analysis algorithms applied. Moreover, as spectral analysis was applied to data segments that were only 5 min long, estimation of the power of the VLF < 0.04 Hz component was likely to be inaccurate (Task Force., 1996). Because of this and other reasons (see Discussion), estimates of VLF power are not reported here.

Scoring of sleep-wake state was carried out in consecutive segments of 30 s (epochs). To deduce the median HRV spectral index for each sleep state, the algorithm illustrated in Fig. 2 was employed. As each 5-minute segment of data was used to produce one value of each HRV spectral index, the segment was divided up into equal-length (30 s) sub-segments carrying the same HRV spectral index. The median for each parameter at a given sleep-wake state was deduced from all 30-s epochs in the overnight study. Figure 2 displays an example of the computations used to determine the median value of LHR representative of each sleep-wake state in each individual subject. Similarly, each of the other HRV spectral indices was represented by the median of all the overnight 5-min segment values for each sleep state. Medians were adopted instead of means to exclude outlier values that were produced primarily by ectopic events. Although there were periods after the start of each overnight study that were classified as 'wake', we employed only the last 10 min of quiet wakefulness prior to sleep onset to represent 'true wakefulness'. In other words, the wakefulness stage in this study represents only the 10-min period before sleep onset; none of indices computed from periods classified as 'wake' after sleep onset were taken into consideration.

Preliminary analysis of the data indicated that there were significant gender differences (for LHR, LHRra, mRRI, $P < 0.0001$) based on three-way repeated measures ANOVA on RDI levels, gender, and sleep-wake state. Thus, our subsequent analyses considered male and female subjects separately. Our preliminary tests (Wang *et al.*, 2006) also suggested significant confounding effects from age and BMI. Thus, multiple linear regression was applied with BMI and age as continuous independent variables and the log-transformed value of each HRV index as the dependent variable. Following linear adjustment for the effects of age and BMI, the HRV indices were subjected to two-way repeated measures ANOVA, with sleep-wake state [Wake, rapid eye movement (REM), non-rapid eye movement (NREM) Stage 1, NREM Stage 2, NREM Stage 3–4] being the repeated factor and RDI category (Normal: $RDI < 5$, Mild SDB: $5 < RDI < 15$, Moderate SDB: $15 < RDI < 30$, Severe SDB: $RDI > 30 \text{ h}^{-1}$) as the unreplicated factor. *Post hoc* multiple pair-wise comparisons (Holm–Sidak test) were carried out if statistical significance was indicated.

To determine whether there were significant correlations between each HRV index and RDI in the different sleep-wake states, we subsequently extended the multiple linear regression model to include RDI, BMI, and age as continuous independent variables, following stratification of the data according to gender and sleep-wake state.

All statistical analyses were conducted using SIGMASTAT[®]3.11 (Systat Software Inc., San Jose, CA, USA). A significance level of 0.05 was assumed.

RESULTS

Figure 3 illustrates examples of the time-series for HFP, HFPr, LHR, and LHRra, along with corresponding hypnograms for two male subjects: a subject without evident SDB ($RDI = 4.7 \text{ h}^{-1}$) and a subject with SDB ($RDI = 55.1 \text{ h}^{-1}$). Both are of similar age (53 year) and BMI (29 kg m^{-2}). Although the time courses of the respiration-adjusted spectral indices (HFPr, LHRra) are quite similar in form to the corresponding 'raw' spectral indices (HFP, LHR), there are noticeable differences in *magnitude* between the plots, particularly between LHR and LHRra in the subject with high RDI. These differences reflect the effect of non-uniform ventilatory patterns (e.g., alternating episodes of apnea and hyperpnea) on HRV during sleep, and thus, they are expected to be larger as RDI increases. In the comparison of this particular pair of individuals, it turned out that mRRI was generally higher in the SDB subject versus the subject without SDB. However, as demonstrated in the results to follow, we found the opposite to be true when statistical analysis was applied to all datasets.

Figure 4 summarizes the results computed from all 288 studies for the 'raw' and respiration-adjusted HRV spectral indices as well as mRRI, following adjustment for age and BMI. Because of significant differences in trends between the male and female subjects, the findings for each gender have been displayed separately. For the same sleep-wake state and RDI group, the female subjects tended to have lower mRRI (or equivalently, higher heart rate), LHR, and LHRra than their male counterparts. There were no gender differences in HFP and HFPr.

As expected, in both gender groups, LHR and LHRra tended to decrease as sleep-wake state changed from wakefulness to REM and to NREM Stages 1–3 / 4. HFP and HFPr also tended to increase from wakefulness to REM to NREM sleep, although without further increasing as the depth of NREM sleep increased from Stage 1 to Stage 3 / 4. This strong dependence on sleep-wake state is confirmed by the results of the two-way repeated measures ANOVA performed on the autonomic indices following adjustment for age and BMI (Table 2). In addition, mRRI was lower (i.e., average heart rate was higher) in wakefulness compared with all sleep states. The male subjects showed a significant negative relationship between RDI and mRRI ($P < 0.001$) independent of sleep-wake state: as severity of SDB increased from normal or mild (apnea-hypopnea index, RDI: 0–15) to moderate and severe (RDI > 15), mRRI decreased. Men also displayed a significant interaction of HFPr between RDI and sleep / wake state (Table 2, $P = 0.018$), which could be readily recognized by comparing the trend of HFPr as RDI level changes between wakefulness and other sleep states. During wakefulness, HFPr was lower in those with RDI ≥ 15 than in those with RDI < 15 . During sleep, however, the relation of HFPr with RDI was not monotonic, being highest in those with RDI ≥ 30 but lowest in those with RDI of 15–30. In women, there was some tendency for mRRI to be higher in the subjects with mild and moderate SDB relative to the normals and those subjects with severe SDB, but these differences were not significant.

Careful inspection of Fig. 4 shows that in wakefulness, HFP and HFPr were noticeably lower in the subjects with moderate-to-severe RDI, relative to the normals and subjects with mild SDB. As well, in females during the light stages of NREM sleep, LHR and LHRra tended to increase with severity of SDB. Stratifying for sleep-wake state, the multiple regression analysis with RDI as one of the continuous independent variables provided further clarification of this issue. Table 3 shows the linear regression coefficients linking each log-transformed index to RDI for each sleep-wake state, following adjustment for the effects of age and BMI. Consistent with the findings displayed in Fig. 4, mRRI decreased with increasing RDI in men across all sleep stages (all $P < 0.001$). However, no significant association between RDI and mRRI among women was identified. Significant negative correlations between HFP and RDI ($P = 0.004$) and HFPr and RDI ($P = 0.007$) were also identified during wakefulness in men, but not in women. On the contrary, significant trends for LHR and LHRra to increase with RDI were identified during NREM Stages 1 and 2 ($P < 0.01$ in all cases) in women, but not in men.

DISCUSSION

Adjustment for non-uniform ventilatory patterns in SDB

The premise that spectral indices of HRV can provide useful information about autonomic control of the heart to a large extent is based on the original study of Katona and Jih (1975), which showed in an animal preparation that respiratory-related fluctuations in RRI were linearly related to cardiac vagal firing rates. In that study and the subsequent validation studies on humans that followed (Berntson *et al.*, 1997; Grossman *et al.*, 1991), respiration was either controlled or changed little, while vagal tone was altered using pharmacologic or physiologic interventions. Although some studies have underscored the important effect of respiratory rate and tidal volume on the HRV spectrum (Brown *et al.*, 1993; Grossman *et al.*, 1991), this confounding influence of respiration has been largely ignored in the HRV literature. Brown *et al.* (1993) showed that depending on breathing frequency, changes in respiration within a

given individual can substantially alter estimates of *both* HFP and LFP of the RRI spectrum. We have previously demonstrated that the highly non-uniform ventilatory patterns associated with SDB during sleep can profoundly exaggerate this confounding influence of respiration on estimates of HRV spectral power (Khoo *et al.*, 1999). For instance, the emergence of hyperpneic breaths following the termination of apnea can increase estimates of HFP, leading to the erroneous suggestion of an increase in vagal tone during these episodes. In this and our previous study (Khoo *et al.*, 1999), we found that there was significant power in the VLF region, consistent with the ventilatory periodicities that ranged from 30 s to 1.5 min in SDB in the HRV spectra of data segments with episodic apnea or hypopnea. Respiratory entrainment of heart rate at such low frequencies has been demonstrated in experiments by Lorenzi-Filho *et al.* (1999), in which normal subjects were able to generate VLF oscillations in the HRV spectrum by simulating periodic breathing through voluntary control of the ventilatory pattern.

In the present study, we introduced the approach of ‘computationally correcting’ these respiratory-related distortions of the HRV spectrum in each dataset by first estimating the transfer function between respiration and HRV, and subsequently convolving this transfer function with the respiratory signal measured during wakefulness in the same subject to derive the ‘respiration-adjusted’ HRV spectral indices (i.e., HFPr_a and LHRr_a). This procedure also largely eliminated the HRV oscillations in the VLF region. Thus, analogous to the well-accepted statistical technique of ‘adjusting’ for confounding variables, our ‘respiration adjustment’ approach permits a more valid comparison of HRV spectral indices computed from different times during wakefulness and sleep within a given individual, as well as across individuals.

Differences from previous studies

Previous studies employing spectral analysis of HRV have generally reported significantly reduced HFP and enhanced LHR in *awake* SDB subjects relative to normal controls (Khoo *et al.*, 1999; Narkiewicz *et al.*, 1998; Wiklund *et al.*, 2000). Similar findings on frequency-domain indices of HRV have appeared in studies of subjects with SDB conducted during sleep (Shiomi *et al.*, 1996; Vanninen *et al.*, 1996) or through Holter monitoring over 24 h (Aydin *et al.*, 2004; Gula *et al.*, 2003). However, the major limitations in all these studies were that sample size was relatively small and that the effects of potential confounding factors, such as subject gender, BMI, and differences in respiration were not carefully accounted for. Wiklund’s study (Wiklund *et al.*, 2000) produced findings from a relatively large group of 117 subjects, but these were based on short periods of measurement (several minutes) conducted only during wakefulness. In contrast, the present study was based on data collected from close to 300 normotensive subjects drawn from a community-based cohort. Another important difference is that, in our study, the HRV spectral indices representing the various sleep-wake states in each of the subjects were determined from the *entire* overnight poly-somnographic recording and not from selected short segments of data. As well, close attention was paid in minimizing the effects of potential confounding variables or co-morbidities through subject selection or by computationally adjusting these effects during analysis.

Dependence of HRV indices on sleep/wake state

One important finding from this study that stood out among the others was the clear dependence of mRRI and the other indices of HRV on sleep-wake state among all subject groups. LHR and LHRr_a were generally highest in wakefulness but decreased with NREM sleep depth. mRRI, HFP, and HFPr_a tended to be lowest in wakefulness but increased during sleep. These findings are consistent with well-accepted observations in normals where sympathetic activity decreases and parasympathetic activity increases during sleep (Mancia, 1993; Zemaityte *et al.*, 1984). However, the indices of parasympathetic activity (mRRI, HFP, and HFPr_a) did not increase with increasing depth of NREM sleep. It should also be noted that our methodology

is limited by the underlying assumption that all sleep states of the same stage (e.g., NREM Stage 2) were the same and that there were no time of night or circadian effects. As well, another limitation is that each 5-min segment would contribute the same HRV index to all the sleep stages that appeared within that duration, in effect averaging out the influences of rapid changes in state. One alternative could have been to compute the HRV spectra over consecutive epochs of 30 s segments, each of which would be scored for sleep stage; however, this would have produced inaccurate estimates of LFP and even HFP. In the final analysis, it is not possible to completely dissociate the changes in ANS activity from the accompanying changes in physiology (i.e., changes in state and respiration) that occur during sleep in SDB as all these variables are by nature highly interconnected.

Gender differences

Consistent with previous studies (Antelmi *et al.*, 2004; Evans *et al.*, 2001; Kuo *et al.*, 1999; Liao *et al.*, 1995; Ryan *et al.*, 1994) on normal subjects, we found significant gender differences in LHR: men had higher LHR values than women. Also consistent with previous studies (Evans *et al.*, 2001; Liao *et al.*, 1995; Ryan *et al.*, 1994), HFP was generally not different between genders. However, there is a key finding from this study that is relatively new: the substantial difference between men and women in the relationship between SDB severity and the HRV indices. Sympathetic modulation (as reflected by LHR, LHRra) and mRRI in women were consistently lower than corresponding levels in men across all RDI and sleep levels. Compared with men, parasympathetic modulation in women during sleep was higher in the groups with mild and moderate SDB severity. However, these gender differences were not apparent in wakefulness. In men, mRRI was significantly and negatively associated with RDI in both wakefulness and sleep. As well, during wakefulness, HFP and HFPr were each negatively correlated with RDI, suggesting that as SDB severity increases, waking parasympathetic modulation of heart rate becomes progressively reduced. In contrast, in women during wakefulness, we found no relationship between RDI and high-frequency modulation of HRV. Instead, in sleep Stages 1 and 2, both indices of sympathovagal balance (LHR and LHRra) were positively correlated with RDI. These findings suggest that in men, SDB is associated with impaired cardiac autonomic control primarily through a reduction in vagal modulation of heart rate, whereas in women, the association appears to be different, with sympathetic modulation becoming more important with increased SDB severity during the light stages of NREM sleep. It appears that these effects tend to be quite subtle in otherwise healthy individuals, as they are easily obscured by the presence of other confounding factors. The impact becomes most visible only at the lowest levels of vagal tone (i.e., during wakefulness) in men or in states of low sympathetic tone (i.e., during NREM sleep) in women. However, the aforementioned findings should include the caveat that the statistical power of our computations for male subjects is probably substantially higher than that for the female subjects because of the smaller sample size of the latter.

Elimination of confounding influences

Although initial efforts were taken to limit the effects of confounding variables, precise matching for age and BMI across RDI groups was problematic. As it is known that autonomic function can be affected by obesity (Grassi *et al.*, 1995) and aging (Brandenberger *et al.*, 2003), we employed multiple linear regression to statistically adjust for the effects of these influences on the HRV indices. It was only after adjustment for age and BMI that the correlation between SDB severity and the HRV indices in certain sleep-wake states became visible.

CONCLUSIONS

In summary, we conducted a statistical analysis of the HRV spectral indices derived from 288 polysomnograms of middle-to-old aged subjects drawn from the SHHS cohort, in which

computational adjustments were made to attenuate the confounding influences from differences in respiration, BMI, and age. Consistent with previous findings from others, we found that changes in state from wakefulness to sleep led to increased parasympathetic modulation and decreased sympathetic modulation of heart rate. Our findings point to an association between SDB severity and cardiac autonomic control, but the nature of the association appears to be gender-dependent. In men, mRRI and the indices representing HFP of HRV decrease with increasing RDI during wakefulness, suggesting a progressive reduction in vagal modulation of heart rate as SDB becomes more severe. In women, however, mRRI and the indices representing high-frequency HRV power are *not* correlated with RDI during wakefulness; instead, both LHR and LHRa are positively correlated with RDI during the light stages of NREM sleep. These findings suggest that there are subtle but significant gender differences in the way in which SDB severity and changes in sleep-wake state affect cardiac autonomic control. These newly unravelled gender differences are as yet unexplained and require further confirmation using other tools for assessing ANS activity.

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APPENDIX: DETAILED METHODOLOGY FOR COMPUTING RESPIRATION-ADJUSTED HRV SPECTRAL INDICES

To adjust for temporal variations in the respiratory contribution to the HRV spectral indices in each subject, we employed an extension of ARX input modeling approach published previously (Khoo *et al.*, 1999). In Khoo's original method, esophageal pressure (Khoo *et al.*, 1999) or respiratory airflow (Khoo *et al.*, 2001) was used as the 'input' to the model while RRI was treated as the 'output'. In the SHHS studies, however, respiration was monitored in the form of uncalibrated thoracic and abdominal inductance plethysmography. Therefore, two data channels were used as inputs to the model: the thoracic component and the abdominal component of the respiration signal.

Thus, the ARX model relating (uncalibrated) changes in lung volume, $x(n)$, to changes in RRI, $y(n)$, at time point n was:

$$y(n) = - \sum_{i=1}^p a_i y(n-i) + \sum_{k=0}^q b_k x(n-k) + e(n) \quad n=1, \dots, N \quad (1)$$

where N represents the total number of data points and $e(n)$ is the difference between the actual and predicted RRI. The uncalibrated lung volume change was assumed to be the weighted sum of the abdominal $[x_a(n)]$ and thoracic $[x_t(n)]$ excursions:

$$x(n) = x_a(n) + \gamma x_t(n) \quad (2)$$

with an unknown coefficient γ , representing the relative contribution of the thoracic excursion to overall respiratory signal. γ and the other unknown parameters in the ARX model $\{p, q, a_i (1 \leq i \leq p) \text{ and } b_k (0 \leq k \leq q)\}$ were estimated for each successive 5-min segment of data by least-squares optimization. The 'optimal' model was selected by searching for a model candidate (with maximum model order 10) that produced the lowest value of the criterion known as the 'minimum description length' (Rissanen, 1982).

Equation 1 allows $y(n)$ to be partitioned into a component that is correlated with respiration, $y_{rc}(n)$, and a respiration-uncorrelated component, $y_{ru}(n)$:

$$y(n) = y_{rc}(n) + y_{ru}(n) \quad (3)$$

where

$$y_{rc}(n) = \frac{\sum_{k=0}^q b_k q^{-k}}{1 - \sum_{i=1}^p a_i q^{-i}} x(n) \quad (4)$$

and

$$y_{ru}(n) = \frac{1}{1 - \sum_{i=1}^p a_i q^{-i}} e(n) \quad (5)$$

where q^{-m} represents a backward shift of m time-steps.

In Equation 4, the respiration-correlated component of HRV can change considerably even in the same subject if the magnitude and pattern of respiration, $x(n)$, undergo significant change, such as what occurs during different sleep / wake stages. To enable comparisons of autonomic activity to be made across different times during the night in a given subject, we computed the ‘respiration-adjusted’ Δ RRI time-series (y_{ra}) for each consecutive data segment. The ‘respiration-adjusted’ Δ RRI time-series is defined as:

$$y_{ra}(n) = \frac{1}{1 - \sum_{i=1}^p a_i q^{-i}} e(n) + \frac{\sum_{k=0}^q b_k q^{-k}}{1 - \sum_{i=1}^p a_i q^{-i}} x_0(n) \quad (6)$$

where $x_0(n)$ is the respiration time-series during the wakefulness period prior to sleep onset. Thus, for each given data segment during sleep, the respiration-correlated component was first dissociated from the respiration-uncorrelated component. From the respiration-correlated component, the transfer function between respiration and RRI was estimated. This transfer function was next used to predict what the respiration-correlated component of RRI would be if the respiration time-series during that interval of time were to take the same form as the respiration time-series measured during the short period of wakefulness immediately prior to the start of the sleep study. The predicted respiration-correlated component was subsequently added to the respiration-uncorrelated component to generate the ‘respiration-adjusted’ RRI time-series.

The spectra of the original RRI time-series and the ‘respiration adjusted’ RRI time-series were computed using the Welch method with Hanning windowing (Tompkins, 1993). The respective areas in the appropriate frequency bands (low-frequency: 0.04–0.15 Hz; high-frequency: 0.15–0.4 Hz) under each RRI spectrum were calculated to yield the HRV spectral indices, LFP, and

HFP. Subsequently, LFP was divided by HFP to yield LHR. Analogous calculations were performed to arrive at the corresponding ‘respiration adjusted’ indices: LFPra, HFPr, and LHRra.

Figure 1.
Schematic representation of analysis procedures.

Figure 3.
 Examples of overnight time courses of heart rate variability indices and hypnograms: normal versus sleep-disordered breathing subject.

Figure 4. Median heart rate variability spectral indices across different levels of respiratory disturbance index and sleep-wake state, after adjusting for age and body mass index.

Table 1

Demographics (statistically significant postulated relationships in bold)

	Overall	RDI categories (h ⁻¹)			P-value *
		0-5	5-15	15-30	
Men	221	57	53	60	51
RDI (h ⁻¹)	19.1 (17.0)	2.9 (1.4)	9.4 (2.8)	21.3 (4.1)	44.5 (13.1)
Age (years)	62.2 (6.9)	61.0 (7.2)	62.8 (7.2)	62.0 (6.7)	63.1 (6.4)
BMI (kg m ⁻²)	29.5 (3.6)	26.6 (2.5)	30.4 (3.4)	30.6 (3.5)	30.4 (3.3)
SBP (mmHg)	121.1 (10.3)	120.1 (10.6)	124.0 (8.7)	123.0 (10.4)	121.3 (11.0)
DBP (mmHg)	74.0 (7.0)	72.5 (7.9)	74.7 (7.1)	73.2 (7.9)	75.8 (7.5)
Women	67	17	14	18	18
RDI (h ⁻¹)	20.0 (16.4)	2.8 (1.5)	10.5 (3.5)	20.6 (3.6)	43.0 (9.5)
Age (years)	64.0 (5.9)	62.8 (6.2)	63.4 (6.0)	65.7 (4.7)	63.7 (6.7)
BMI (kg m ⁻²)	33.1 (5.3)	33.2 (3.9)	32.4 (6.0)	31.5 (4.6)	35.1 (6.0)
SBP (mmHg)	119.8 (10.3)	118.6 (10.8)	117.1 (8.8)	123.8 (11.0)	119.0 (10.0)
DBP (mmHg)	71.0 (6.4)	74.3 (8.2)	69.1 (6.4)	70.7 (4.1)	69.6 (5.7)

RDI, respiratory disturbance index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. Mean (SD) for continuous variables and counts for gender.

* P-values are from one-way ANOVA, and if it is significant, all pair-wise comparisons to control are significant (Holm-Sidak).

P-values from two-way repeated measures ANOVA on HRV indices after adjusting for age and BMI (statistically significant postulated relationships in bold)

Indices	Men (n = 221)		Women (n = 67)		
	RDI	SSInteraction	RDI	SS	Interaction
LHR	0.910	< 0.0010.322	0.636	< 0.001	0.126
LHRra	0.823	< 0.0010.466	0.503	< 0.001	0.244
HFP	0.098	< 0.0010.039	0.577	< 0.001	0.397
HFPra	0.070	0.0050.018	0.647	< 0.001	0.465
mRRI	< 0.001	< 0.0010.198	0.317	< 0.001	0.958

HRV, heart rate variability; RDI, respiratory disturbance index; HFP, high-frequency power; mRRI, mean R-R interval; LHR, low-to-high frequency ratio; SS, sleep-wake state; HFPra, HFP respiration-adjusted counterparts; LHRra LFP respiration-adjusted counterparts; R, REM; W, wakefulness; BMI, body mass index. There are four levels (0–5, 5–15, 15–30, 30+) of RDI and five repeated levels (R, W, S1, S2, S3) of SS.

Table 3

Linear regression analysis of HRV indices on RDI over REM, wakefulness and sleep Stage 1–3 after adjusting for age and BMI (statistically significant postulated relationships in bold)

		RDI					
		Men (n = 221)		Women (n = 67)			
Sleep state	Indices (log-transformed)	β^*	(SE [*])	P-value	β^* (SE [*]) P-value		
R	LHR	-0.18	(0.14)	0.190	0.06	(0.27)	0.237
	LHRra	-0.25	(0.14)	0.069	0.10	(0.25)	0.702
	HFP	0.26	(0.20)	0.200	0.76	(0.47)	0.109
	HFPra	0.31	(0.20)	0.128	0.73	(0.47)	0.124
W	mRRI	-0.09	(0.02)	<0.001	-0.06	(0.04)	0.147
	LHR	0.21	(0.17)	0.212	0.03	(0.27)	0.927
	LHRra	0.16	(0.16)	0.318	0.13	(0.27)	0.626
	HFP	-0.67	(0.23)	0.004	-0.20	(0.40)	0.627
S1	HFPra	-0.62	(0.23)	0.007	-0.22	(0.41)	0.583
	mRRI	-0.10	(0.03)	<0.001	-0.07	(0.04)	0.128
	LHR	0.03	(0.13)	0.825	0.74	(0.26)	0.005
	LHRra	-0.04	(0.12)	0.733	0.75	(0.25)	0.004
S2	HFP	-0.15	(0.19)	0.419	-0.18	(0.42)	0.660
	HFPra	-0.11	(0.19)	0.553	-0.17	(0.42)	0.688
	mRRI	-0.09	(0.02)	<0.001	-0.06	(0.04)	0.166
	LHR	0.05	(0.14)	0.703	0.66	(0.24)	0.007
S3	LHRra	-0.02	(0.13)	0.899	0.67	(0.22)	0.004
	HFP	0.01	(0.19)	0.953	0.10	(0.43)	0.812
	HFPra	0.06	(0.19)	0.755	0.09	(0.42)	0.834
	mRRI	-0.10	(0.02)	<0.001	-0.05	(0.04)	0.288
	LHR	-0.08	(0.16)	0.615	0.39	(0.28)	0.164
	LHRra	-0.08	(0.16)	0.633	0.49	(0.28)	0.086
	HFP	-0.07	(0.21)	0.752	0.45	(0.44)	0.310
	HFPra	-0.02	(0.21)	0.913	0.27	(0.43)	0.534
	mRRI	-0.11	(0.02)	<0.001	-0.03	(0.04)	0.529

BMI, body mass index; HRV, heart rate variability; RDI, respiratory disturbance index; HFP, high-frequency power; mRRI, mean R-R interval; LHR, low-to-high frequency ratio; HFPra, HFP respiration-adjusted counterparts; LHRra LFP respiration-adjusted counterparts; R, REM; W, wakefulness.

* Values are scaled to 10^{-2} .