Heart Rate Variability During Waking and Sleep in Healthy Males and Females

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Study Objectives: The study goal was to investigate autonomic activity with heart rate variability analysis during different sleep stages in males and females.
Design: The study utilized a 2 Groups (males, females) X 4 States (waking, stage 2 sleep, stage 4 sleep, rapid-eye movement sleep) mixed design with one repeated, within-subjects factor (i.e., state).
Setting: The study was carried out in the sleep laboratory of the Thomas N. Lynn Institute for Healthcare Research.
Participants: Twenty-four healthy adults (fourteen females and ten males).
Interventions: NA
Measurements and Results: All participants underwent polysomnographic monitoring and electrocardiogram recordings during pre-sleep waking and one night of sleep. Fifteen-minute segments of beat-to-beat heart rate intervals during waking, stage 2 sleep, stage 4 sleep, and REM sleep were subjected to spectral analysis. Compared to NREM sleep, REM sleep was associated with decreased high frequency (HF) band power, and significantly increased low frequency (LF) to (HF) ratio. Compared to females, males showed significantly elevated LF/HF ratio during REM sleep. Males also demonstrated significantly decreased HF band power during waking when compared to females. No significant sleep- or gender-related changes in LF band power were found.
Conclusions: The results confirmed changes in autonomic activity from waking to sleep, with marked differences between NREM and REM sleep. These changes were primarily due to stage-related alterations in vagal tone. REM sleep was characterized by increased sympathetic dominance, secondary to vagal withdrawal. The data also suggested gender differences in autonomic functioning during waking and sleep, with decreased vagal tone during waking and increased sympathetic dominance during REM sleep in the males.
Key words: Sleep; Heart rate variability; autonomic nervous system; rapid eye movement sleep; vagus; sex; electrocardiography; heart rate; gender differences

INTRODUCTION

IT HAS BEEN WELL-ESTABLISHED THAT SPECTRAL ANALYSIS OF HEART RATE VARIABILITY (HRV) is a non-invasive method that provides a quantitative evaluation of the sympathovagal interaction modulating cardiac function.[1-4] Parameters commonly derived from HRV analysis include the power in the high frequency (HF) band (0.15-0.4 Hz), which is associated with vagal tone as it regulates respiratory sinus arrhythmia. The power in the low frequency (LF) band (0.05-0.15 Hz) reflects mainly sympathetic outflow associated with vasomotor waves, although several investigators have also demonstrated an influence of vagal activity in the LF frequency range.[3-5]

Previous studies in healthy individuals have documented that traditional autonomic measures, such as mean heart rate and blood pressure, demonstrate characteristic changes during sleep, and have established differences between non-rapid eye movement (NREM) and rapid eye movement (REM) sleep.[6-8] More recently, several studies utilizing HRV analysis have confirmed differences between NREM and REM sleep in sympathovagal activity.[9-13] There appears to be consensus that compared to waking, NREM sleep is characterized by a decreased LF/HF ratio suggesting vagal predominance, while REM sleep is associated with an increased LF/HF ratio indicating sympathetic dominance.[9-13] Sleep-related changes in the individual LF and HF bands generating the described alterations in LF/HF ratio have been less consistent. For example, Vaughn et al. found an increase in the LF component during REM sleep, while others have instead reported decreased HF band power as the main cause of the

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increased LF/HF ratio during REM.[9,13]

Several studies have investigated the factors influencing autonomic nervous system(ANS) activity in healthy humans, and have suggested a role of age, race, and sex.[14-16] However, to our knowledge, these studies have exclusively assessed HRV in the waking state, and results concerning gender differences during wakefulness have been inconsistent. For example, data collected as part of the Atherosclerosis Risk in Communities (ARIC) study revealed that women had lower sympathetic activity, as reflected by the LF band power, but higher LF/HF ratio.[15] Conversely, Huikuri et al. reported that women demonstrated a lower LF/HF ratio when compared to men, which was the result of both lower LF band power and increased HF band power.[14]

In conclusion, there is abundant evidence that there are marked changes in ANS activity as measured by HRV during sleep. However, little is known about gender differences in HRV during sleep, and controversy remains regarding the contributions of the parasympathetic and sympathetic branches to the observed differences in sympathovagal balance during NREM and REM sleep in healthy human subjects. The aim of this study was to compare the autonomic regulation of the heart rate as assessed by spectral analysis of HRV data during waking, NREM, and REM sleep in healthy males and females.

METHODS

Subjects

Twenty-four healthy volunteers (14 females; 10 males) participated in the study. Subjects were between 22 and 55 years old, with a mean age of 36.2±2.3 years (35.1±2.9 years for females; 34.8±3.3 years for males). All participants were of normal weight with a mean body mass index (BMI) of 23±0.8. None of the subjects had a history of cardiovascular disease or sleep disorders, and none were currently using any medications. The study protocol was approved by the Institutional Review Board of the Integris Baptist Medical Center of Oklahoma. All participants gave informed written consent prior to entering the study and were paid for their participation.

Data Acquisition

Standard polysomnographic recordings were used for determination of sleep stages, and were carried out using an Alice 3 Polysomnographic System (Healthdyne, Marietta, GA), which consists of an integrated system of amplifiers and computerized data collection. Four channels of EEG (central channels C3 & C4, occipital channels O1 & O2), two channels of electrooculogram (EOG), one channel of chin electromyogram (EMG), and a single channel of electrocardiogram (ECG) were collected at a sampling rate of 100 Hz and stored on magneto-optical disk. Placement of ECG electrodes included two chest electrodes below the left and right clavicles, respectively, and one electrode on the side below the subject's lowest left rib.

Experimental Protocol

Polysomnography and ECG recordings were performed during one night in the sleep laboratory, and included a one-hour pre-sleep wake recording, and approximately seven hours of sleep recording. All subjects refrained from any over-the-counter medication for a minimum of 24-hours prior to the study day, and from caffeine for at least six hours prior to reporting to the lab. They consumed dinner prior to 17:00, after which time they restrained from all food and drink. Four subjects reported to the laboratory at 20:30 p.m. Upon reporting to the lab, electrodes were applied for polysomnographic recordings. The wake recording lasted from 22:00 to 23:00. During this period, subjects remained in the supine position and watched television. Subjects were monitored via EEG to ensure wakefulness. The sleep-recording was started at 23:00, and subjects were allowed to sleep spontaneously until 06:00.

Data Analysis

Polysomnographic recordings were scored according to internationally accepted sleep staging criteria using 30 second epochs.[17] Upon visual inspection of the ECG signal, no subjects showed any evidence of abnormal cardiac functioning. Fifteen-minute segments of ECG data during waking, NREM sleep (i.e., stage 2 and stage 4 sleep) and REM sleep were selected for analysis. These segments were chosen based on a number of polysomnographic criteria, which included that they were not interrupted by any stage shift, movement, or movement arousal, and that the ECG tracing was artifact-free.

HRV data were derived from the ECG signal: The beat-to-beat heart rate intervals (i.e., R-R intervals) were calculated, interpolated, and resampled at 2 Hz by computer software (i.e., signal processing Toolbox for MATLAB, The Math Works Inc, Natick, MA), yielding the HRV signal for the identified 15-minute periods of:(1) waking, (2) stage 2 sleep, (3) stage 4 sleep, and (4) REM sleep. (For more details regarding the rationale for the interpolation and resampling of the R-R intervals, we would like to refer the interested reader to a number of published review papers.[1,5]) The HRV signal for each time period was subjected to overall spectral analysis (periodgram method), yielding the LF band power and the HF band power. The LF band power was defined as the percent power in the LF frequency band (0.05-0.15 Hz), and the HF band power was defined as the percent power in the HF band frequency (0.15-0.5 Hz). The LF/HF ratio was calculated by dividing the LF band power by the HF band power.

Table 1.—Polysomnographic Parameters

Variable	Females	Males	p
Time in bed (min.)	434.4 ± 5.1	423.1 ± 8.0	NS
Total Sleep Time (min.)	384.5 ± 10.0	364.2 ± 16.9	NS
Sleep Onset Latency (min.)	19.2 ± 4.9	16.8 ± 4.2	NS
NREM %	77.7 ± 1.5	77.1 ± 1.6	NS
Slow Wave Sleep %	29.5 ± 2.2	27.8 ± 3.2	NS
REM %	19.9 ± 1.4	19.9 ± 1.3	NS
REM Latency (min.)	84.6 ± 9.9	101.2 ± 23.1	NS
Sleep Efficiency (TST/TIB)	88.7 ± 2.3	86.2 ± 4.1	NS

All data are presented as mean ± SEM.
(min.) = minutes
NS = non significant

Design and Statistical Analyses

The study utilized a 2 X 4 mixed factor repeated measures design, with each subject serving as his or her own control for the dependent measures in order to minimize inter-subject variability. The independent variables were gender (between-factor) and sleep stage (within-factor); dependent variables included the LF band power, HF band power, and LF/HF ratio. For statistical analysis, analysis of variance (ANOVA) was computed utilizing a set at 0.05. Pairwise multiple comparisons were carried out with alpha-adjustment using Tukey's HSD method. In order to compare males and females regarding polysomnographic parameters, independent samples t-tests were carried out. All results are presented as mean±SEM (standard error of the mean).

RESULTS

Sleep Data

The sleep data are shown in Table 1. As can be seen in the table, males and females did not differ on any polysomnographic measure. Both groups slept reasonably well, with a total sleep time of 384.5±5.1 minutes (females) and 364.2±16.8 minutes (males), and sleep efficiencies of 88.7±2.3 % and 86.1±4.1 %, respectively.

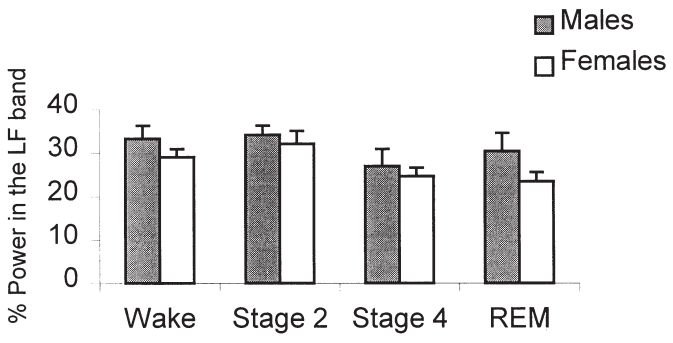

Figure 1—Percent power in the low frequency (LF) band across states of consciousness in males and females.

LF Band Power

The ANOVA on the LF band power revealed a significant main effect for state ($F(3,66)=3.9;p<.05$). However, posthoc comparisons showed no significant changes across sleep stages or significant gender differences. These data are illustrated in Figure 1.

HF Band Power

The ANOVA on the HF band power revealed a significant effect for state ($F(3.66)=26.7;p<.00001$) and a significant interaction of sex and state ($F(3,66)=4.4;p<.05$). As shown in Figure 2, a significant gender difference was found during waking, with males showing significantly decreased HF band power ($p<.05$). In males, there was a significant increase in HF band power from waking to stage 4 sleep ($p<.001$), as well as a trend toward a significant increase from waking to stage 2 sleep ($p=.07$). On the other hand, HF band power did not change significantly from waking to NREM sleep in females.

REM sleep was characterized by decreased HF band power. In females, HF band power during REM sleep was significantly decreased compared to waking ($p<.05$), stage 2 sleep ($p<.05$), and stage 4 sleep ($p<.001$). In males, HF band power during REM sleep was significantly decreased compared to stage 2 sleep ($p<.05$) and stage 4 sleep ($p<.001$), but not compared to waking. No significant gender differences in HF band power were found during any sleep stage.

LF/HF Ratio

LF/HR ratio findings are illustrated in Figure 3. The overall ANOVA on the LF/HF ratio revealed significant main effects for sex ($F(1,22)=6.3;p<.05$) and sleep stage ($F(3,66)=24.0;p<.00001$), as well as a significant interaction ($F(3,66)=6.3;p<.001$). As illustrated in Figure 3, the results showed characteristic changes in the LF/HF ratio across sleep stages. There appeared to be an inverse relation between depth (or synchronization) of sleep and the LF/HF ratio; however, posthoc comparisons showed no statistically significant differences in LF/HF ratio between waking and NREM sleep (stages 2 and 4) in either gender, with the exception of a trend in males toward a significant decrease from waking to stage 4 sleep ($p=.08$).

REM sleep was characterized by an increased LF/HF ratio. In females, the LF/HF ratio during REM sleep was significantly increased compared to stage 4 sleep ($p<.05$). In males the LF/HF ratio during REM sleep was significantly increased compared to NREM sleep (stages 2 and 4)

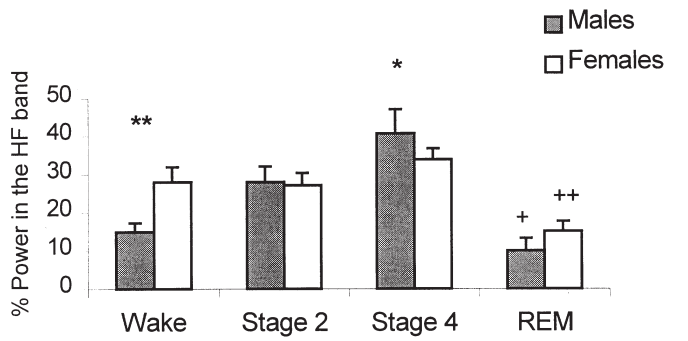

Figure 2—Percent power in the high frequency (HF) band across states of consciousness in males and females.
* Significantly different from waking (p<.001).
+ Significantly different from stage 2 sleep (p<.05) and stage 4 sleep (p<.001).
++ Significantly different from waking (p<.05), stage 2 sleep (p<.05), and stage 4 sleep (p<.001).
** Males significantly different from females (p<.05).

and waking (all p<.001). As for gender differences in LF/HF ratio, there was a statistically significant difference between males and females during REM sleep (p<.001), with males demonstrating a significantly greater LF/HF ratio. No other gender differences were found.

DISCUSSION

Spectral analysis of HRV data during waking, NREM, and REM sleep demonstrated characteristic sleep stage-related changes in ANS activity, and supported the presence of sex differences in the autonomic regulation of cardiac function during waking and sleep. The data suggested an inverse relation between sympathovagal balance, as represented by the LF/HF ratio, and synchronization of sleep. These findings support the concept that the stages of NREM sleep represent a continuum of increasing parasympathetic dominance from waking to stage 4 sleep. In contrast to NREM sleep, REM sleep was characterized by sympathetic dominance, indicated by a significantly increased LF/HF ratio, which confirms previous reports.[7,9-13] However, this study is unique in that it demonstrated a significantly greater sympathetic dominance during REM sleep in males compared to females.

Separate analyses of the LF and HF band components revealed that the power in the LF band showed minor, statistically non-significant changes across sleep stages, with no significant gender differences. In interpreting this finding, it is important to emphasize the above mentioned dual control of the LF band power, i.e., a respiration (vagal) related component can contribute to the power of the component that in relation to its center frequency is classified as LF. This can occur even more easily during NREM sleep, when the respiratory rate is relatively low. This makes an interpretation of the LF band component very difficult, and may be one reason for the finding that the LF band power remains relatively stable across states of consciousness.

In contrast to the LF component, there were marked changes across sleep stages in the HF component. In both males and females, REM sleep was characterized by significantly decreased HF band power, which suggests that the sympathetic dominance during REM sleep primarily results from vagal withdrawal rather than from increased sympathetic activity.

Although no statistically significant gender differences in HF (or LF) band power were found during REM sleep, the data supported an influence of gender on vagal regulation across states of consciousness. Compared to females, males demonstrated significantly lower vagal tone during waking. Unlike females, males significantly increased vagal tone from waking to stage 4 sleep, and the overall analysis indicated a significant interaction of gender and state. These results suggest that males demonstrate a more pronounced regulation of vagal tone (i.e., greater changes) across states of consciousness.

Taken together, the results from this study confirm previous findings about sleep-stage related changes in the autonomic regulation of heart rate. The data clearly substantiate the concept of REM sleep as a period of enhanced sympathetic dominance, which differs markedly from the more parasympathetically dominated NREM sleep stages. While the LF/HF ratio as a global measure of sympathovagal balance characterizes REM sleep as being similar to the waking state in terms of sympathovagal balance, our data suggest that REM sleep is distinguished from waking by marked vagal withdrawal.

The gender differences observed during waking and REM sleep may have clinical implications for the study of cardiovascular and stress-related disorders. Porges sug-

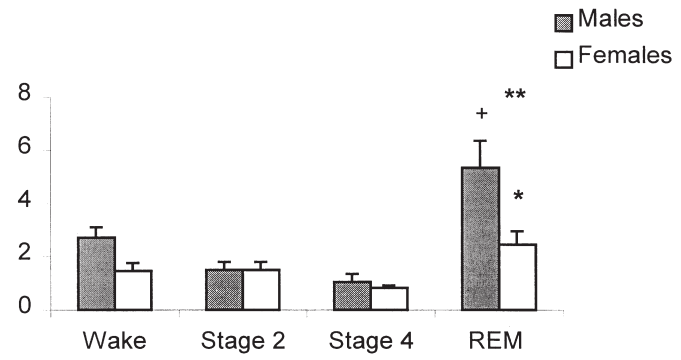

Figure 3—Low frequency to high frequency (LF/HF) ratio across states of consciousness in males and females.
* significantly different from stage 4 sleep (p<.05).
+ significantly different from waking, stage 2 sleep, and stage 4 sleep (all p<.001).
** Males significantly different from females (p<.001).

gested that the parasympathetic nervous system represents a "modulator of stress vulnerability and stress reactivity"' and proposed that a depression of vagal activity is associated with a disruption of homeostasis.[18] Based on this, the marked vagal withdrawal associated with REM sleep may create increased physiological vulnerability or "stress", especially in males. This notion is supported by previous studies that have linked sleep, particularly REM sleep, to the provocation of myocardial ischemia.[6,22,23] Further support comes from accumulating evidence for the role of gender in the expression of cardiovascular disease, including the finding that the risk of early sudden death is greater in males than females with an acute myocardial infarction.[19-21] Based on these results, one goal of future studies could be to investigate whether there is a relationship between cardiac autonomic activity during REM sleep and cardiovascular risk in males compared to females.

The study by Huikuri et al. offers information about the mechanisms underlying gender differences in autonomic regulation. The authors found that when compared to age-matched males, only those middle-aged women (mean age =54 years) demonstrated differences in HRV measures who were undergoing estrogen replacement therapy.[14] These results suggest that hormonal factors (i.e., estrogen) may play a role in the sex differences observed in the current study. It should be noted that all but two females in the current study were pre-menopausal.

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