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Original article

# Sex differences in cardiac sympathovagal balance and vagal tone during nocturnal sleep ☆

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**Abstract**

*Objective:* To analyze sex differences in nocturnal vagal tone and cardiac sympathovagal balance during sleep in healthy men and women.

*Methods:* In two groups of healthy, non-obese adults (15 men and 14 women), sleep was polygraphically recorded and heart rate variability was assessed during an awake period prior to sleep and during sleep. Vagal tone was estimated by the high-frequency (HF) power component of heart rate variability, and sympathovagal balance was indexed by the ratio of low-frequency (LF) power to HF power.

*Results:* As compared to women, men showed decreases in vagal tone and increases in sympathovagal balance. During rapid eye movement (REM) sleep, a withdrawal of vagal tone occurred with an increase in sympathetic dominance. Men showed a greater increase of sympathovagal balance during REM sleep than women. Secondary analyses covarying for differences in reproductive hormone levels, physical activity, and sleep measures did not alter the results.

*Conclusions:* The marked increase in cardiac sympathetic drive during REM sleep in men has implications for understanding sex differences in the risk of cardiovascular events. Additionally, these data offer a pathway to explain the peak in cardiac arrhythmias and sudden cardiac death seen more often in the morning hours.



*Keywords:* Sex differences; Heart rate variability; Cardiovascular risk; Vagal tone; Sympathetic activity

## 1. Introduction

Cardiovascular diseases are the leading cause of mortality in both men and women [1], with men showing a substantially higher risk of cardiac events than women during the fertile age [2]. This greater cardiovascular risk profile in men is thought to be due to sex differences in autonomic nervous system activity [3–6]. Sleep-wake activity, a major modulator of autonomic activity [7,8], is linked to the occurrence of cardiovascular events with rates of sudden cardiac death, myocardial infarction, and ischemic stroke, peaking at the end of sleep or in the morning after awakening [9–13]. Few studies have examined differences in autonomic activity during sleep in men and women [14], despite the need for research on sleep in women, which was recognized over a decade ago by the National Commission on Sleep Disorders Research [15].

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Sleep onset is consistently associated with a decrease in sympathetic activity as measured by a decline in sympathetic nerve fiber activity [7] and decreases in circulating levels of catecholamines [8]. Recent evidence also shows that sleep modulates parasympathetic activity or vagal tone [16–18]. Spectral analysis of heart rate variability (HRV) is a non-invasive method for assessing cardiac autonomic tone. Using this method, spectral power in the high-frequency (HF) band estimates vagal tone by indexing how strongly respiration affects heart rate. The ratio of power in the low-frequency (LF) band to power in the HF band (i.e., LF/HF ratio) is thought to indicate sympathetic output. As compared with wakefulness, rapid eye movement (REM) sleep is associated with decreases in HF power that are coupled with increases in the LF/HF ratio, indicating sympathetic dominance [17,18]. Increases of sympathetic output and/or decreases in parasympathetic tone predispose to the occurrence of ventricular arrhythmias such as ventricular fibrillation [19,20]. This mechanism is invoked as one pathway that may explain the predominant occurrence of cardiac arrhythmias in the early morning when REM sleep predominates.

Men are more likely than women to show increases in sympathovagal balance as measured by increases in the LF/HF ratio, although these data are primarily confined to daytime measures [3–6]. Only one study, to our knowledge, has examined sex differences of HRV during sleep between men and women [16]. Conclusions from that study are, however, constrained by the absence of relevant comparative information for a number of clinical variables that are known to influence HRV, including ethnicity, body mass index (BMI), alcohol consumption, tobacco use, and physical activity. In addition, prior work did not evaluate sex hormone levels, although estrogen replacement therapy is associated with higher levels of HF power and lower LF/HF ratio among post-menopausal women [21]. It is not known whether sex differences in autonomic activity are related to physiologic variations in sex hormone levels. The present study was, therefore, designed to analyze sex differences in sleep-related HRV in healthy men and women, taking into account the possible influence of pertinent clinical variables as well as levels of sex hormones.

## 2. Methods

### 2.1. Subjects

Volunteers were recruited through advertisements that stated the aim of the study was to evaluate ''sleep and health''. Interested participants underwent telephone screening and gave written informed consent. The Institutional Review Board of the University of California, Los Angeles, approved the study protocol.

A total of 35 research participants (19 men and 16 women) were enrolled in the study and underwent eligibility interviews that included administration of the Structured Clinical Interview for a Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) diagnosis, assessment of medical and medication use histories, physical examination, and laboratory screening blood tests (e.g., metabolic panel, complete blood count, thyroid tests, and urine toxicology screen). All participants fulfilled (DSM-IV) criteria for Never Mentally Ill [22], were free of major medical illnesses, had a BMI of $<30\ kg/m^2$, and maintained a regular wake/sleep activity schedule with sleep time between 23:00 and 07:00 h as confirmed by two weeks of sleep diary data. No participant was currently taking any medication known to influence sleep or blood pressure. All participants scored below the cutpoint <6 for clinical sleep impairment as defined by Pittsburgh Sleep Quality Index (PSQI) [23]. Amount of weekly aerobic activity was quantified using the methodology of Sallis [24].

Following completion of eligibility evaluation, participants underwent a night in the sleep laboratory to determine the presence of nocturnal myclonus or sleep apnea, as previously described [18]; two men were excluded due to periodic leg movements in sleep (PLMS > 12/h), two men and one woman were excluded due to sleep apnea (hypopnea index of >10/h), and one woman declined further polysomnographic study. The remaining sample was comprised of 29 research participants (15 men, 14 women).

### 2.2. Sleep recording and analysis

Polysomnographic sleep studies were performed in the UCLA General Clinical Research Center as previously described [18]. Briefly, after the night of adaptation as noted above, participants had two nights of uninterrupted sleep with supine rest from 22:00 to 23:00 h and lights out at 23:00 h. The second night involved intravenous blood sampling, and these polysomnographic data are reported separately, given the effects of blood sampling on sleep [25].

The polysomnographic montage included four electroencephalographic (EEG) channels ($C_3$, $C_4$, $O_1$, $O_2$ referenced to $A_1$–$A_2$, bilateral electrooculography (EOG), bipolar submental electromyography (EMG), pulse oximetry, abdominal respiratory effort, and electrocardiogram (ECG). Sleep records were visually scored as previously described [26,27] by scorers blind to sex status. Briefly, sleep records were visually scored according to the criteria of Rechtschaffen and Kales [28]. Data from each 30-s epoch were entered into a computer program that tallies the summary statistics for each subject. Sleep onset was defined as the first minute of stage 2 or REM sleep followed by at least eight minutes of sleep in the next nine minutes. A REM per-

iod was defined by not less than three consecutive minutes of REM sleep. Sleep efficiency was the ratio of total sleep time to the time in bed, multiplied by 100. Sleep architecture was defined as the duration of time spent asleep in non-REM (NREM) sleep, stages 1 through 4. REM density was an estimate of the number of eye movements per minute of REM sleep, scored on a scale of 0 to 4 per 30-s epoch but expressed on a scale of 0 to 8 per minute. Sleep research technicians were regularly tested on scoring reliability and high standards were maintained: sleep latency ($r = .96$), REM latency ($r = .99$), REM density ($r = .91$), amounts of stages 3 and 4 ($r = .85$), and total sleep time ($r = .99$). Data from each 30-s epoch were entered into a computer program that tallies the summary statistics for each subject.

### 2.3. Heart rate variability (HRV)

ECG signals were acquired and processed utilizing the EMBLA Sleep System and Somnological Studio software package (Somnologica, Flaga hf, Medical Devices, Iceland). The subject's ECG signal was sampled at a frequency rate of 200 Hz, and an interpolation algorithm was used to optimize temporal accuracy of R wave peak detection [29]. The signal was then converted into an RR interval signal and spectral-analyzed using a 12-point autoregressive algorithm (Somnologica, Flaga hf, Medical Devices, Iceland) in accordance with recommended guidelines to generate estimates of high and low frequency [30].

Stringent criteria for ECG segment selection during wakefulness and nocturnal sleep were employed. ECG signals were analyzed using 5 min segments; each utilized segment was free of ECG artifacts and EEG arousals at least 30 s prior and during the recording period. Hence, following visual inspection of each epoch by a rater blind to group status, epochs were included if the following criteria were fulfilled: same stage of sleep across the 5 min segment, no EEG arousals during or 30 s prior to the epoch, no evidence of ECG artifacts, and no ECG arrhythmias. Prior to "lights out", patients were asked to rest quietly in the supine position starting at 22:00 h. HRV assessment during the awake period, prior to sleep onset, was obtained with subjects' eyes closed for 10 min of recording beginning at 22:30 h. The 5 min ECG segments were averaged during wake, stage 2 sleep, and REM sleep. HRV during slow wave sleep was not analyzed due to the lack of HRV epochs on half of the subjects due to decreased slow wave sleep and/or absence of continuous 5 min segments.

Three HRV measures were generated using the Somnologica Studio software package version 3.3.2. (Somnologica, Flaga hf, Medical Devices, Iceland). Very low-frequency power was defined as total spectral power in the 0.003–0.04 Hz frequency band (VLF), low-frequency power as the total spectral power in the 0.04–0.15 Hz frequency band and high-frequency power as the total spectral power in 0.15–0.4 Hz frequency band (HF). From these measures, three HRV indices were produced and examined: low-frequency power in normalized units (LF n.u. $= \mathrm{LF}/[(\mathrm{TP} - \mathrm{VLF}) \times 100)$, high-frequency power in normalized units (HF n.u $= \mathrm{HF}/[(\mathrm{TP} - \mathrm{VLF}) \times 100)$, and ratio of low-frequency power to high-frequency power (LF/HF). Normalized units were employed because of the significant difference in total power between men and women.

Because HF power is related in part to respiratory frequency, breathing rate was assessed across the night using piezo sensing respiratory belts (Pro-Tech Services, Inc., Mukilteo, WA). A breathing index (per minute) was produced for epochs during wake, stage 2 and REM sleep by dividing the number of counted breaths by 5 min.

### 2.4. Reproductive hormone assays

Normal variation of reproductive hormones was compared in men and women without restricting assessment to one point in the menstrual cycle in women. Assays of circulating levels of estradiol, progesterone, and dehydroepiandrosterone (DHEA) were performed on a single plasma sample taken at 20:00 h. Coated-Tube Radioimmunoassay Kits were utilized: DSL-4300 ACTIVE® Estradiol Coated-Tube Radioimmunoassay Kit for assay of estradiol, DSL-5000 ACTIVE® 17 α-OH Progesterone Coated-Tube Radioimmunoassay Kit for assay of 17 α-OH progesterone, and DSL-8900 dehydroepiandrosterone (DHEA) Radioimmunoassay Kit provides materials for assay of DHEA.

### 2.5. Statistical analyses

Data were analyzed using SPSS version 11.5 for Windows and missing values were substituted by single-point multiple imputation using NORM version 2.03 for any participant who had more than 95% of their data. Sex differences on continuously distributed clinical data and on EEG sleep variables were tested using *t*-tests; differences in categorical data were tested using $\chi^2$ tests. Normalized units of HF and LF power were normally distributed. To determine main effects of sex (men vs. women), sleep stage (wake, stage 2, REM sleep), and their interaction on HRV assessments, repeated measures analyses of variance (ANOVA) were used. Secondary analyses of HRV values included a covariate if there was a trend or a significant difference in a background variable between men and women (e.g., reproductive hormones), and the background variable was related to the HRV measures (e.g., physical activity).

## 3. Results

### 3.1. Clinical characteristics

The two groups (15 men and 14 women) were similar on clinical demographic variables including age, income, education, BMI, alcohol consumption and perceived sleep quality (Table 1). Men and women did not differ for ethnic composition ($\chi^2 = 3.2$, $p = 0.2$) or frequency of smokers ($\chi^2 = 0.9$, $p = 0.4$). Reported physical activity was significantly greater in men as compared to women (Table 1). Circulating levels of estradiol were higher and DHEA levels were lower in women as compared to men, with a trend for increases of progesterone in women (Table 1).

### 3.2. Sleep data

Polysomnographic data showed no differences in measures of sleep continuity and sleep architecture between men and women. However, women showed increases in the percentage of REM sleep compared to men, whereas men showed greater REM duration in the first period and greater REM density compared to women (Table 2).

### 3.3. Heart rate variability (HRV)

For heart rate, men showed a lower rate compared to women (sex effect: $F[1,27] = 16.7$, $p < 0.001$), but there was no sex-by-sleep stage interaction. Spectral analyses of HRV during the nocturnal period showed significant sex differences for normalized LF band power, normalized HF band power, and the LF/HF ratio. For normalized LF, men showed higher levels across the night as compared to women (sex effect: $F[1,27] = 12.3$, $p < 0.002$). In addition, there was a significant main effect for sleep stage (i.e., wake, Stage 2, REM sleep), with higher levels of LF during REM sleep (sleep stage effect: $F[2,54] = 12.4$, $p < 0.001$). There was no sex-by-sleep stage interaction. For normalized HF band power, men showed lower levels across the night compared to women (sex effect: $F[1,27] = 16.7$, $p < 0.001$; Fig. 1). There was a sleep stage effect with lower levels of HF during REM sleep (sleep stage effect: $F[2,54] = 46.9$, $p < 0.001$) but no sex-by-sleep stage interaction. For the ratio of LF–HF (LF/HF ratio), men had a significantly higher ratio across the nocturnal period compared to women (sex effect: $F[1,27] = 11.9$, $p < 0.002$), and there was a significant sleep stage effect, evidenced by a marked increase in LF/HF during REM sleep (sleep stage effect $F[2,54] = 22.5$, $p < 0.001$). In addition, there was a sex-by-sleep stage interaction ($F[1,27] = 6.8$, $p < 0.01$), with men showing a greater increase of the LF/HF ratio during REM sleep than women (Fig. 2).

### 3.4. Secondary analyses of heart rate variability (HRV)

Given group differences in physical activity and circulating levels of reproductive hormones, additional analyses were performed, covarying for levels of self-reported physical activity and reproductive hormones (i.e., estradiol, progesterone, and DHEA). Sex differences in the measures of HRV were not altered when these covariates were included; the overall main effects for sex were found for LF ($F[1,25], = 4.4$, $p < 0.05$), HF ($F[1,25], = 7.3$, $p < 0.05$) and ratio of LF/HF ($F[1,25], = 5.2$, $p < 0.05$).

Additional analyses examined whether sex differences in the sleep parameters might account for the HRV findings. Analyses covarying for sleep measures that were marginally different or statistically different between men and women (i.e., total sleep time, sleep efficiency, percentage of stage 2, stage 3, and REM sleep, REM duration and REM density) generated similar sex differences for LF ($F[1,18], = 5.9$, $p < 0.05$), HF ($F[1,18], = 5.6$, $p < 0.05$) and LF/HF ratio ($F[1,18], = 4.6$, $p < 0.05$).

Finally, because oscillations of cardiac HF are related to respiratory rhythm, analyses evaluated sex difference in breathing rate across the night; there were no sex differences nor sex-by-sleep stage interactions for breathing

Table 1
Demographic and clinical variables in men and women

| Variables | Men ($n = 15$) | | Women ($n = 14$) | | Sex effect | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | $t$ | $P$ |
| Age (years) | 36.7 | 10.0 | 36.6 | 9.2 | 0.01 | 1.0 |
| Body mass index (kg/m$^2$) | 24.2 | 3.4 | 23.7 | 4.5 | 0.4 | 0.7 |
| Annual income (× $10,000 per year) | 3.6 | 2.2 | 3.3 | 2.0 | 0.4 | 0.7 |
| Education (years) | 15.5 | 1.7 | 16.2 | 1.3 | −1.2 | 0.2 |
| Alcohol consumption (drinks/day) | 0.8 | 1.1 | 0.6 | 0.9 | 0.5 | 0.6 |
| Sleep Quality (PSQI Global Score) | 4.1 | 3.0 | 2.9 | 2.7 | 1.1 | 0.3 |
| Physical activity (× 1000 kcal/day) | 3.1 | 0.7 | 2.3 | 0.5 | 3.8 | 0.001 |
| Estradiol (pg/ml) | 33.5 | 7.2 | 85.4 | 63.2 | 3.2 | 0.009 |
| Progestersone (ng/ml) | 1.1 | 0.5 | 5.6 | 9.7 | 1.8 | 0.08 |
| DHEA (μg/dl) | 239.5 | 78.1 | 147.1 | 69.1 | 3.4 | 0.002 |

SD, standard deviation; PSQI, Pittsburgh sleep quality index; DHEA, dehydroepiandrosterone.

Table 2
Polysomnographic sleep variables in men and women

| EEG sleep variables | Men ($n = 15$) | | Women ($n = 14$) | | Sex effect | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | $t$ | $p$ |
| *Sleep continuity* | | | | | | |
| Total sleep time (min) | 388.8 | 74.5 | 434.5 | 60.9 | −1.8 | 0.1 |
| Sleep onset (min) | 20.7 | 14.4 | 27.9 | 30.1 | −0.8 | 0.4 |
| Sleep efficiency (%) | 78.0 | 15.0 | 85.5 | 6.9 | −1.7 | 0.1 |
| *Sleep architecture* | | | | | | |
| Stage 1 (%) | 5.9 | 2.2 | 5.3 | 3.2 | 0.6 | 0.6 |
| Stage 2 (%) | 65.6 | 8.6 | 61.0 | 10.3 | 1.3 | 0.2 |
| Stage 3 (%) | 6.6 | 4.1 | 4.2 | 4.0 | 1.5 | 0.1 |
| Stage 4 (%) | 1.8 | 3.8 | 3.7 | 3.8 | −1.3 | 0.2 |
| Delta (%) | 8.4 | 6.3 | 8.4 | 7.8 | −0.01 | 1.0 |
| REM (%) | 20.2 | 6.8 | 25.2 | 3.4 | −2.5 | 0.02 |
| *REM measures* | | | | | | |
| REM latency | 59.9 | 27.6 | 63.6 | 11.9 | −0.5 | 0.6 |
| REM duration (1st period) | 12.7 | 10.3 | 18.8 | 6.4 | −1.9 | 0.07 |
| REM density | 1.9 | 0.7 | 1.2 | 0.6 | 2.7 | 0.01 |

EEG, electroencephalogram; SD, standard deviation; REM, rapid eye movement sleep.

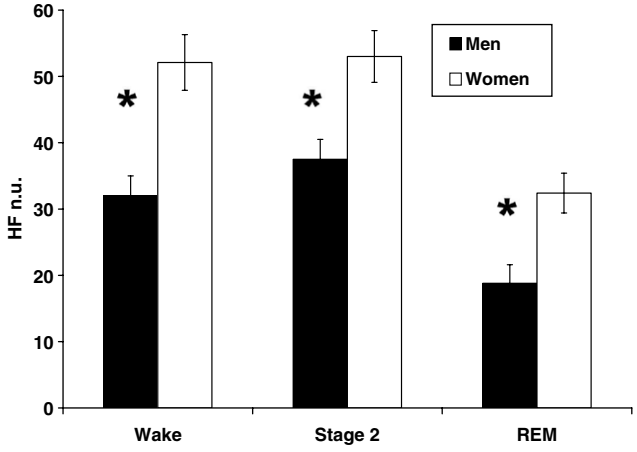


Fig. 1. Comparison of normalized units of the high-frequency component of heart rate variability (HF n.u.; HF/[(TP − VLF) × 100]) during wake prior to sleep, stage 2 sleep and REM sleep in men and women. Data are presented as mean ± SEM. *indicates significant $p < 0.001$ differences between men and women HF n.u, high-frequency power in normalized units; REM, rapid eye movement sleep; SEM, standard error of the mean.

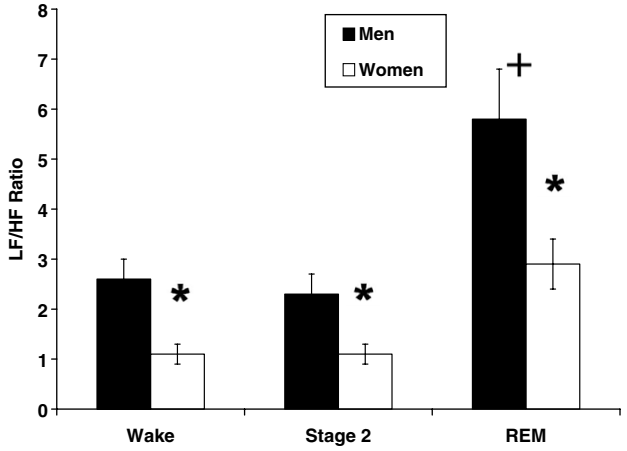


Fig. 2. Comparison of sympathovagal balance as measured by the ratio of low-frequency/high-frequency ratio (LF/HF ratio) during wake prior to sleep, stage 2 sleep and REM sleep in men and women. Data are presented as means ± SEM. *indicates significant $p < 0.001$ differences between men and women; +indicates significantly $p < 0.01$ greater increase for men than women during REM sleep. REM, rapid eye movement sleep; SEM, standard error of the mean.

rate, although respirations tended to increase during REM sleep compared to wake and stage 2 sleep ($F[1, 27] = 2.7$, $p = 0.09$).

## 4. Discussion

The present study demonstrates sex differences in nocturnal measures of HRV during sleep in a small sample of healthy, non-obese men and women. As compared to women, men show decreases in vagal tone as estimated by HF power, and increases in sympathetic output as assessed by the ratio of LF/HF power. Furthermore, men evidence vagal withdrawal during REM sleep, with marked sympathetic dominance compared to women. Together, these findings extend the results of Elsenbruch et al. [16] to demonstrate that sex differences in cardiac autonomic activity occur during sleep and that these differences are not related to confounding clinical variables (e.g., BMI, physical activity) nor to varying levels of reproductive hormones.

Sleep dynamics have been demonstrated to alter vagal tone and sympathovagal balance, resulting in a striking withdrawal of parasympathetic activity and increases in sympathetic output during REM sleep [17]. Our findings

further substantiate the notion of sympathetic dominance during REM sleep and show that this increase of cardiac sympathovagal balance differentially occurs more significantly in men compared to women. Withdrawal of vagal activity with a reciprocal increase in cardiac sympathetic drive is thought to contribute to the occurrence of cardiac arrhythmias such as ventricular fibrillation and is associated with risk of sudden cardiac death [19,20]. REM sleep predominates in the second half of the night, and the frequency of ventricular arrhythmias and sudden cardiac death, as well as other cardiac events such as stroke, increases twofold in the early morning or following awakening from sleep [9–13]. In a prospective study of cardiovascular risk factors and HRV obtained during the day and the first half of the night, Eller reported that two stress hormones, cortisol and noradrenaline, were negatively correlated with HF power in men but not in women [31]. Taken together, these data suggest new targets for future analyses that seek to study sex differences in sleep-related autonomic activity and in cardiovascular disease risk in general.

This is one of the few studies that have simultaneously evaluated measures of HRV along with levels of reproductive hormones in men and women. The findings indicate that sex differences in autonomic activity were independent of physiologic variations in estradiol, progesterone, and DHEA, as these reproductive hormones were not significant covariates in the comparisons between the two groups. Such analyses suggest that categorization of sex is more highly related to differences in HRV than reproductive hormones levels. However, the study was not designed to test whether varying levels of estradiol or progesterone mediate alterations in measures of sympathovagal balance. Pharmacologic studies, for example, show that estrogen supplementation attenuates vagal withdrawal following stress-induced activation of autonomic pathways in animals [32], and that estrogen replacement therapy is associated with higher levels of HF power and lower LF/HF ratio in post-menopausal women [21].

Measures of polysomnographic sleep continuity were similar in the men and women, although both groups showed low levels of sleep efficiency compared to population normative data. Prior to study entry, none of the subjects reported clinical symptoms of sleep difficulty or daytime impairment, and the reasons for the low levels of sleep efficiency are not known; studies were conducted in a clinical research setting with strict monitoring of ambient noise, light exposure, and temperature. However, given that these levels of sleep efficiency are comparable to levels found in three other samples of control subjects we recently studied [18,33,34], it is speculated that recruitment advertisements that target "adults for study of sleep and health" may have solicited persons with modest sleep difficulties as identified by polysomnography.

Sex differences in autonomic activity reported here are consistent with a number of studies that have found that men show lower HF power and higher LF/HF ratio compared to women [3–6]. What is unique about this study is its examination of these differences during sleep and evidence for a withdrawal of vagal activity and a striking increase in sympathetic dominance among men during REM sleep, consistent with the findings of Elsenbruch et al. [16]. These data should motivate further investigations to define the effects of sleep on autonomic mechanisms with implications for cardiovascular disorders in humans. Testing of interventions that target sleep and/or sympathovagal functioning might identify new strategies to ameliorate cardiovascular risk in men and women.

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