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Acute stress alters autonomic modulation during sleep in women approaching menopause:

Stress, sleep and autonomic functioning in women approaching menopause

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Abstract

Hot flashes, hormones, and psychosocial factors contribute to insomnia risk in the context of the menopausal transition. Stress is a well-recognized factor implicated in the pathophysiology of insomnia; however the impact of stress on sleep and sleep-related processes in perimenopausal women remains largely unknown. We investigated the effect of an acute experimental stress (impending Trier Social Stress Task in the morning) on presleep measures of cortisol and autonomic arousal in perimenopausal women with and without insomnia that developed in the context of the menopausal transition. In addition, we assessed the macro- and micro-structure of sleep and autonomic functioning during sleep. Following adaptation to the laboratory, twenty two women with (age: 50.4 ± 3.2 y) and eighteen women without (age: 48.5 ± 2.3 y) insomnia had two randomized in-lab overnight recordings: baseline and stress nights. Anticipation of the task resulted in higher pre-sleep salivary cortisol levels and perceived tension, faster heart rate and lower vagal activity, based on heart rate variability measures, in both groups of women. The effect of the stress manipulation on the autonomic nervous system extended into the first four hours of the night in both groups. However, vagal tone recovered four-six hours into the stress night in controls but not in the insomnia group. Sleep macrostructure was largely unaltered by the stress,

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Conflict of interest

None of the authors has any conflict of interest to report.

Contributors

M.dZ. conducted the data analysis and drafted the manuscript.

D.S. collected the data and assisted with data analysis.

J.T. and I.M.C. assisted with study design and writing of the manuscript.

F.C.B. conceptualized the study, and assisted in the study design and drafting of the manuscript.

All authors read, revised and approved the final manuscript.

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apart from a delayed latency to REM sleep in both groups. Quantitative analysis of non-rapid eye movement sleep microstructure revealed greater electroencephalographic (EEG) power in the beta1 range (15- 23Hz), reflecting greater EEG arousal during sleep, on the stress night compared to baseline, in the insomnia group. Hot flash frequency remained similar on both nights for both groups. These results show that presleep stress impacts autonomic nervous system functioning before and during sleep in perimenopausal women with and without insomnia. Findings also indicate that women with insomnia had increased EEG arousal and lacked recovery in vagal activity across the stress night suggesting a greater sensitivity to stress in this group.

Keywords

insomnia; menopause; Trier social stress task; cortisol; hyperarousal; heart rate variability

1.1. INTRODUCTION

Sleep difficulties are more common in midlife women transitioning to menopause compared with pre-menopause, with prevalence rates of insomnia symptoms ranging between 40-56% in the menopausal transition (Joffe et al., 2010; Kravitz et al., 2003; Kravitz et al., 2008; Nowakowski et al., 2009; Ohayon, 2006; Polo-Kantola, 2011) and with 26% qualifying for a DSM-IV diagnosis of insomnia (Ohayon, 2006). We have shown that women who developed severe first-onset insomnia in the menopausal transition have a significant sleep deficit, with almost 50% of them having polysomnographic (PSG)-defined short sleep duration (<6 hours). They also had more wakefulness after sleep onset (WASO) and poorer sleep efficiency compared with controls (Baker et al., 2015). Sleep difficulties and insomnia disorder in the menopausal transition have been linked to several factors, including changing reproductive hormone levels (decrease in estradiol and increase in follicle stimulating hormone) (de Zambotti et al., 2015a; Kravitz et al., 2008), hot flashes (Baker et al., 2015; Kravitz et al., 2008; Ohayon, 2006), and psychosocial factors (Sassoon et al., 2014; Woods and Mitchell, 2010). Another important factor that could contribute to the development and/or exacerbation of insomnia in midlife women is susceptibility to stress.

It is hypothesized that there exists a trait-like vulnerability to insomnia driven by an augmented response to stress (Harvey et al., 2014). Stress exposure at baseline is a significant predictor of insomnia one year later, particularly in individuals with high sleep reactivity (more likely to have difficulty sleeping in stressful situations, such as before an important meeting the following day), an effect mediated by the extent of cognitive intrusion elicited by the stress exposure (Drake et al., 2014). Insomnia that develops in response to stress could be related to underlying levels of chronic hyperarousal (Bonnet and Arand, 2003), a core feature of the hyperarousal model of insomnia, indexed by increased heart rate, presleep cognitive arousal, sympathetic activity, and brain activation (reviewed in (Levenson et al., 2015; Riemann et al., 2015). While individuals with insomnia in the general population may not necessarily experience more frequent stressful life events, they perceive the impact of these events more negatively and consider their lives to be more stressful compared with good sleepers, with the pathway between daily stress and poor sleep being mediated by high levels of pre-sleep arousal (Morin et al., 2003).

There are limited studies that have investigated associations between stress and insomnia in the context of the menopausal transition. The Study of Women Across the Nation (SWAN) reported that midlife women (51.2 ± 2.1 years) who showed a chronic stress profile characterized by more annual events that were “very and still upsetting” over a period of up to 9 years before a PSG study, had a poorer subjective sleep quality and more PSG-defined wakefulness, and were more likely to report insomnia than women with low or moderate stress profiles (Hall et al., 2015). These findings suggest that high levels of chronic stress may precipitate sleep continuity disturbances in midlife women (Hall et al., 2015). While Shaver and colleagues (2002) did not find a difference in perceptions of stress exposure in midlife women with and without insomnia, they found that the insomnia group had more psychological distress and a greater morning-evening difference in urine cortisol levels compared with controls.

Hot flashes (transient periods of flushing, sweating, and a sensation of heat (Kronenberg, 1990) are a major contributor to sleep disturbance in midlife women. They emerge as estrogen levels decline but their mechanism is more complex than just estrogen withdrawal, with several lines of evidence implicating involvement of the autonomic nervous system (de Zambotti et al., 2013; Freedman, 2014; Thurston et al., 2012). Stress or emotional situations are cited as the most frequent trigger for hot flashes (Kronenberg, 1990) raising the possibility that stress in symptomatic perimenopausal women could induce more frequent hot flashes during the night, which could further disturb sleep.

Given the evidence of a relationship between stress exposure/reactivity and insomnia, studies have used acute experimental stress protocols to investigate physiological pathways linking stress and sleep disturbance. Findings are mixed regarding the impact of an acute stress on sleep architecture and sleep continuity, with some finding a longer sleep onset latency and/or more frequent awakenings in response to stress and others finding no effect (see Kim and Dimsdale, 2007, for review). Quantitative electroencephalographic and electrocardiogram measures of physiological arousal during sleep may be sensitive to the effects of stress, as shown by Hall and colleagues (2007; 2004). A group of young healthy adults exposed to an acute experimental anticipatory stress task before bedtime had similar sleep architecture but lower vagal activity during sleep, as indexed by high frequency power derived from spectral density analysis of heart rate variability, with the effect persisting across the night, compared to a control group (Hall et al., 2007). Broesschot and colleagues (2007) showed that daily stress, mediated by worry duration, is associated with a prolonged physiological response (higher heart rate and lower heart rate variability) that persists during sleep even when the stressor is no longer present. In patients with primary insomnia, high levels of perceived stress and more frequent avoidance behaviors are associated with electroencephalographic (lower EEG delta power and higher EEG beta power) and/or electrocardiographic (lower high frequency power) indicators of physiological arousal during NREM sleep (Hall et al., 2007). No relationships between psychological stress and sleep architecture were found.

Here, we aimed to investigate the effect of stress on sleep, autonomic nervous system (ANS) functioning during sleep, as well as the frequency of hot flashes, in perimenopausal women without a history of insomnia but with a current diagnosis of insomnia that developed in the

approach to menopause. We used an acute experimental psychosocial stress anticipation protocol (impending Trier Social Stress Test, Kirschbaum et al., 1993) implemented before bed. We hypothesized that women with menopausal-onset insomnia would be more sensitive to the stress paradigm (greater sleep disturbance, higher heart rate and lower vagal functioning during the stress night compared to a baseline night) compared to a control group of women who were transitioning to menopause without the onset of insomnia.

1.2. MATERIAL and METHODS

1.2.1. Participants and Screening

The study was reviewed and approved by SRI International's Institutional Review Board. Participants were recruited from the San Francisco Bay Area community and gave written informed consent. Sample characteristics and screening procedures are fully described in Sassoon et al. (2014).

Briefly, all women had to be in the menopausal transition (menstrual cycle lengths that differed by more than 7 days from normal or skipping cycles, but not with amenorrhea longer than 11 months), according to Stages of Reproductive Aging Workshop (STRAW) criteria (Soules et al., 2001), have an intact uterus and at least one ovary, and a body mass index (BMI) of 32 kg.m^{-2} or lower. Exclusion criteria were use of hormone therapy, severe medical conditions (e.g., hypertension or diabetes), or current sleep medication and/or antidepressant use.

All women underwent a structured clinical interview (SCID, First et al., 1998) including a customized module querying DSM-IV criteria for insomnia (Morin and Espie, 2003). Sleep disturbance needed to be coincident with the onset of the menopausal transition; none of the participants had a lifetime history of DSM-IV insomnia. None of the participants met criteria for current other Axis-I disorders (except nicotine dependence, 2 in the control group and 5 in the insomnia group). All women had a clinical/adaptation in-lab PSG assessment, to confirm absence of sleep-disordered breathing (apnea-hypopnea index >5) and/or periodic limb movement disorder (periodic limb movement index >10).

Comparisons of sleep composition between women with insomnia and controls (Baker et al., 2015) and between different menstrual cycle phases in both groups of women (de Zambotti et al., 2015b) as well as hormone-sleep relationships (de Zambotti et al., 2015a) have been previously published. The current data were drawn from an additional experimental night, for which participants were exposed to a pre-sleep anticipatory stress condition, compared to an undisturbed (baseline) night.

Twenty two women with (Age, 50.4 ± 3.2 y; BMI, $24.6 \pm 4.0 \text{ Kg.m}^{-2}$; 16 Caucasian) and 18 women without (Age, 48.5 ± 2.3 y; BMI, $24.8 \pm 4.4 \text{ Kg.m}^{-2}$; 13 Caucasian) a diagnosis of insomnia constituted the final sample.

1.2.2. General Laboratory Procedure

Participants arrived at the Human Sleep Research Laboratory at SRI international at approximately 8pm and had sensors attached. Baseline and anticipatory stress nights were

randomly scheduled after the clinical/adaptation night based on availability of participants, lab schedule and availability of technicians. Baseline and stress nights were never consecutive. In nine controls and six women with insomnia the stress night preceded the baseline night. Blood samples were collected either on arrival at the laboratory or the following morning of each visit to establish menstrual cycle phase. Nine women in the insomnia group and eight controls had both their baseline and stress nights recorded in the luteal phase of the menstrual cycle as confirmed by progesterone levels $\geq 3 \text{ ng.ml}^{-1}$ (see de Zambotti et al., 2015b). Progesterone was $<3 \text{ ng.ml}^{-1}$ on the remaining nights.

On each visit, subjective indices including pre-sleep measures of perceived stress, and cortisol levels (obtained by analysis of saliva samples collected immediately before bedtime) were obtained. Women self-selected their bedtimes and wake-up times and slept in temperature-controlled, sound-attenuated bedrooms. All participants registered 0.0 on a breathalyzer on arrival at the sleep laboratory.

On the stress night women underwent the anticipatory stress procedure (see below) while on the baseline night they did not receive any additional instructions.

1.2.3. Anticipatory Stress Procedure

Approximately 30 min before lights-out, after participants had been prepared for PSG recordings, completed any evening questionnaires, and were ready for bed, they underwent the anticipatory stress protocol. The protocol was adapted from the Trier Social Stress Test, a well-established psychosocial stress protocol (Allen et al., 2014; Kirschbaum et al., 1993). Participants were introduced to a role-playing scenario by one of the research team (DS). They were instructed that they would need to prepare a 5-min speech the following morning, which they would need to give in front of a panel of assessors, who would judge their performance and ask questions. They were also told that the assessors would assign them a 5-min mental arithmetic task to complete following the speech. Participants were shown the speech assessment room, which was already set up with chairs in place for the assessors, a video camera and microphone, and it was emphasized that their speech would be recorded for subsequent evaluation. Participants were not given the speech topic; they were told they would receive the topic the following morning.

Finally, they were reminded to sleep well in order to be at their best for their speech the following morning (Hall et al., 2004).

1.2.4. Subjective Evaluations

Each evening, women completed a questionnaire that included questions about caffeine use (none of the women reported consuming caffeine after 3 pm that day) and how stressed they had been feeling during the day on a 100 mm visual analogue scale (anchor points: 'not at all stressed', 'very stressed') (this rating was missing from 3 control participants).

In addition, immediately before bedtime, participants rated how tense they felt "*right now*" on a 100 mm visual analogue scale, ranging from "not at all tense" to "very tense" (this rating was only implemented later in the study so is missing for 4 women with and 4 women without insomnia).

1.2.5. Polysomnographic Recording and Electroencephalographic Spectral Analysis

Electroencephalographic (EEG), electrooculographic and electromyographic signals were acquired using Compumedics Graef amplifiers (Compumedics, Abbotsford, Victoria, Australia) according to American Academy of Sleep Medicine (AASM) criteria (Iber C et al., 2007). EEG signals were sampled at 256Hz and band-pass filtered (0.03-35Hz). Sleep stages (Wake, N1, N2, N3, rapid-eyes-movement [REM]) and brief arousals (>3 s, < 15 s) were scored according to AASM rules (Iber C et al., 2007) by experienced scorers. Inter-rater reliability was set at 90% and if concordance was below 90%, discrepancies were resolved by a third scorer. Time in bed (TIB, min) was calculated as the time from lights-out to lights-on, total sleep time (TST, min) as the TIB minus time spent to fall asleep and time awake during the night, sleep efficiency (SE; %) as $TST/TIB \times 100$, sleep onset latency (SOL, min) as the time from lights-out to the first epoch of any stage of sleep, REM onset latency (REML, min) as the time between sleep onset and the first epoch of REM sleep. Time spent in each sleep stage was calculated as a percentage of TIB.

EEG analysis was performed on NREM sleep epochs across the first 6 hours after lights-out, divided into three 2-hour periods. EEG absolute power density ($\mu V^2 \cdot Hz^{-1}$) in delta (0.3- 4 Hz), theta (4- 8 Hz), alpha (8- 12 Hz), sigma (12- 15 Hz), beta1 (15- 23Hz) and beta2 (23- 30Hz), was calculated on each artifact-free 30-s epoch of NREM (N2 and N3) sleep from C4 using a sliding 4096ms Hanning window, using EEGLAB Matlab toolbox. (Delorme and Makeig, 2004) One woman each in the insomnia and control groups was excluded from the EEG analysis due to excessive artifact.

1.2.6. Cardiac Autonomic Nervous System Assessment

Electrocardiographic (ECG) signals from Ag/AgCl Meditrace surface spot electrodes in a modified Lead II Einthoven configuration (leads were placed under the right clavicle and on the lower left abdomen within the rib cage frame) were acquired using Compumedics amplifiers. Data were continuously collected at 512Hz. Autonomic regulation was assessed with time- and frequency-domain measures of heart rate variability (HRV). Power spectral HRV analysis was performed using dedicated software developed at the University of Melbourne and according to procedures previously described (Trinder et al., 2001). Briefly, R waves were automatically detected by the software, visually checked and manually adjusted by trained technicians when necessary. Inter-beat-intervals were re-sampled at 4Hz and filtered with a third-order polynomial filter to remove very low frequency components. Power spectrum analysis was applied to two-min periods of artifact-free wakefulness between lights-out and sleep onset, and to two-min periods of artifact- and arousal-free sleep across the night. Results are reported as the averages of all 2-min bins selected between lights-out and sleep onset (pre-sleep period) and for all 2-min bins selected in NREM sleep (N2+N3) and REM sleep using the procedure described in Trinder et al. (2001). To identify the high frequency (HF) component, the peak frequency band between 0.15 and 0.40 Hz was identified. The width of the HF component was then defined by the first frequency bands either side of the peak to fall to 50% of the peak value. The area so defined was then integrated. The same procedure was used to identify the low frequency (LF) component between 0.03 and 0.15 Hz.

We derived the following measures: heart rate (HR; bpm); absolute HF integrated power in arbitrary units (HF_a , ms^2), an indicator of vagal activity; total absolute integrated power between 0.03 and 0.4 Hz (TP, ms^2); high frequency power as a proportion of total power (HF_{np}), an indicator of sympathovagal balance (higher values indicate a shifting of the balance toward vagal modulation); and peak frequency in the high frequency range (HF_{pf} , Hz), a measure of respiratory rate (Brown et al., 1993).

Time domain analysis of HRV was performed on the first 6 hours after lights-out (including all time spent sleeping and any intermittent wakefulness), divided into three 2-hour periods. The normal-to-normal (NN) R intervals were converted to HR (bpm) and the following statistical measures were derived: standard deviation of all NN intervals (SDNN, ms), which is a measure of total variability, and the root mean square of differences between adjacent NN intervals (RMSSD, ms), a measure of high-frequency activity reflecting vagal modulation (Cacioppo et al., 2007).

1.2.7. Hot Flash Measurement

Two silver/silver chloride electrodes filled with 0.05 M potassium chloride Velvachol/glycol gel (Dormire and Carpenter, 2002) were placed on either side of the sternum. Skin conductance was continuously recorded across the night using a BioDerm Skin Conductance Meter (model 2701; UFI, Morro Bay, CA) using a 0.5 V constant voltage circuit (Lykken and Venables, 1971). Hot flashes were evaluated by two independent scorers (see de Zambotti et al., 2014, for details) for fluctuations meeting accepted criteria for physiological hot flashes, i.e. an increase of 2 micromhos within 30 seconds (Freedman, 1989). Number of hot flashes was calculated. Skin conductance measures were missing in one control on both nights and from one woman in the insomnia group on the baseline night and from another on the stress night.

1.2.8. Saliva Cortisol

Saliva samples were collected immediately before bedtime (ranging between 21:00 and 01:00 depending on the participants' self-selected bedtime) using the Salivette system (Salimetrics, Carlsbad CA, USA). Samples were frozen at $-70^{\circ}C$ for subsequent analysis of cortisol by a commercial laboratory (ZRT laboratory, Beaverton, OR, USA) using a standard immunoassay kit (inter- and intra-assay coefficients of variation, 7.1% and 5.4%, respectively; sensitivity 0.1 ng/mL). Insufficient saliva was obtained for three controls on baseline nights and for two women with insomnia on stress nights.

1.2.9. Statistical Analyses

PSG variables, pre-sleep cortisol and self-report tension levels, daily stress, and pre-sleep frequency-domain HRV variables were analyzed using repeated measure ANOVAs with *Group* (insomnia and control) as a between factor and *Night* (baseline and stress nights) as a within factor. Frequency domain HRV variables during NREM and REM sleep were analyzed using repeated measure ANOVAs with *Group* (insomnia and control) as a between factor, *Night* (baseline and stress nights) and *Sleep Stage* (NREM and REM) as within factors. Time domain HRV variables were analyzed across the first 6 hours of the night using repeated measures ANOVAs with *Group* as a between factor and *Night* (baseline and

stress nights) and *Time* (first, second and third 2-hour periods of the night) as within-factors. A similar analysis was used for absolute power in EEG bands across NREM sleep within the first, second, and third 2-hour periods of the night. Newman–Keuls (N–K) post-hoc comparisons were performed on the significant effects, and the Geisser–Greenhouse (G–G) correction was applied when variables with more than 2 levels were involved; only results remaining significant are reported. A secondary analysis was performed on HF_a averaged within stable artifact-free epochs of NREM sleep within the first, second, and third 2-hour periods of the night, with *Group*, *Night*, and *Time* as factors. HRV Total power, HF power, SDNN, RMSSD and absolute power in EEG bands were log-transformed before analyses. Self-report measures of daily stress and pre-sleep level of tension were transformed using the asine sqrt transformation. Participants with missing data or unreliable data (as described above) for a particular variable were not included in the statistical models that included those variables. $P < 0.05$ was considered significant for all analyses.

1.3. RESULTS

1.3.1. Daily stress, pre-sleep tension, cortisol, and pre-sleep cardiac autonomic functioning

There was a significant *Group* effect for daily ratings of perceived stress, with women in the insomnia group reporting higher levels of daily stress on both baseline and stress nights compared with controls (*Group*: $F_{1,35}=4.69$, $p=0.037$). There was no main effect of *Night* or any interaction effects. There was a main effect of *Night* for ratings of pre-sleep perceived tension on the stress night compared to the baseline night (*Night*: $F_{1,30}=75.88$, $p<0.001$), indicating that the anticipatory stress procedure was effective in both groups; there was no effect for *Group* or interaction effects.

Pre-sleep levels of cortisol (*Night*: $F_{1,33}=4.16$, $p=0.049$) and HR (*Night*: $F_{1,38}=6.53$, $p=0.015$) were elevated, and HF_a was reduced (*Night*: $F_{1,38}=4.97$, $p=0.032$), indicating lower vagal modulation, during the presleep period on the stress night compared to the baseline night in both groups. There were no significant *Group* or *Group* $\times$ *Night* interactions for pre-sleep cortisol, HR, and HF_a , and no significant effects for TP, HF_{np} or HF_{pf} . Pre-sleep measures are provided in Table 1.

1.3.2. Macrostructure and Microstructure of Sleep

There were no main effects of *Night* for any of the variables other than REML. REML was delayed on the stress night compared to the baseline night in both groups of women (*Night*: $F_{1,38}=9.32$, $p=0.004$). Overall, the insomnia group had lower N2 sleep percentage compared to controls (*Group*: $F_{1,38}=5.00$, $p=0.031$). There were no significant *Night* $\times$ *Group* interactions for any variables. PSG variables are summarized in Table 2.

Analysis of NREM sleep microstructure revealed significant *Night* $\times$ *Group* interactions for EEG power in alpha ($F_{1,38}=4.28$, $p=0.046$) and beta1 ($F_{1,36}=9.67$, $p=0.004$; Figure 1) frequency bands. Post-hoc tests revealed that power in the beta1 band was higher in all three time periods on the stress night compared to the baseline night in the insomnia group ($p=0.004$) whereas beta1 power did not differ between baseline and stress nights for

controls. Post-hoc tests failed to reveal any significant differences in alpha power between stress and baseline nights, although alpha power tended to be higher on the stress night only in the insomnia group. Delta (*Time*: $F_{2,72}=80.82$, $p<0.001$), theta (*Time*: $F_{2,72}=115.34$, $p<0.001$) and alpha (*Time*: $F_{2,72}=17.04$, $p<0.001$) power progressively decreased across the first, second and third 2-h periods of both nights (all p 's <0.05) while sigma (*Time*: $F_{2,72}=10.58$, $p<0.001$), beta1 (*Time*: $F_{2,72}=6.77$, $p=0.002$) and beta2 (*Time*: $F_{2,72}=6.11$, $p=0.003$) powers were lower in the second compared to both first and third 2-h periods of both nights (all p 's <0.05). EEG spectral measures are shown in Table 3.

1.3.3. Physiological hot flashes

More women in the insomnia group had physiological hot flashes on the stress night than did controls (9/21 in the insomnia group and 2/17 in the control group; $\chi^2=4.42$, $p=0.036$). The number of participants with hot flashes in each group did not differ on baseline nights (11/21 in the insomnia group and 6/17 in the control group; $\chi^2=1.11$, $p=0.292$). Irrespective of group, in women having at least one hot flash during baseline and/or stress nights, the overall number of hot flashes did not significantly increase on the stress night (mean $\pm$ SD: 2.9 ± 2.4) compared to the baseline night (mean $\pm$ SD: 2.1 ± 2.3) ($p>0.1$).

1.3.4. Cardiac autonomic control during NREM and REM sleep

Frequency domain HRV measures are provided in Table 4. ANOVAs revealed significant *Night* ($F_{1,38}=7.89$, $p=0.008$) and *Sleep Stage* ($F_{1,38}=29.29$, $p<0.001$) main effects for HR. Both groups had a faster HR during NREM and REM sleep on the stress night compared to the baseline night. HR was faster during REM sleep compared to NREM sleep on both nights. Similarly, both groups of women had lower TP ($F_{1,38}=4.62$, $p=0.038$), indicating a lower total variability, during the stress night compared to the baseline night and lower TP ($F_{1,38}=12.47$, $p=0.001$) during REM sleep compared with NREM sleep. There were significant *Sleep Stage* main effects for HF_a ($F_{1,38}=127.92$, $p<0.001$) and HF_{np} ($F_{1,38}=161.52$, $p<0.001$), which were lower (indicating reduced vagal modulation) during REM sleep compared with NREM sleep. There were no significant effects for HF peak frequency. There were no significant main effects for *Group* or any interaction effects for any of these HRV measures.

1.3.5. Autonomic control across the night

Nocturnal patterns for time domain HRV measures (HR, RMSSD and SDNN) are displayed separately for women with and without insomnia in Figure 2.

ANOVAs revealed significant *Night* ($F_{1,38}=7.22$, $p=0.011$), *Time* ($F_{2,76}=20.18$, $p<0.001$) and *Night* $\times$ *Time* ($F_{2,76}=4.33$, $p=0.017$) interaction effects for HR. Post-hoc analysis revealed that both groups of women had elevated HR during the first and second two-hour periods of the stress night compared to the baseline night ($p<0.001$) whereas in the third two-hour period, HR no longer differed between nights. On the baseline and stress nights, HR was higher in the first two-hour period than the second ($p<0.05$) and third ($p<0.001$) two-hour periods. On the stress night only, HR was also slower in the third two-hour period compared to the second two-hour period ($p<0.001$).

There was a main effect of *Time* ($F_{2,76}=35.62$, $p<0.001$) and *Night* $\times$ *Time* ($F_{2,76}=3.58$, $p=0.033$) interaction for SDNN. In both groups of women, SDNN was lower in the second ($p=0.001$) and third ($p=0.044$) two-hour periods of the stress night compared with the baseline night. SDNN progressively increased across first, second and third periods of the night ($p<0.001$) on the baseline night, but on the stress night, SDNN was similar in the first and second two-hour periods, and only increased in the third two-hour period, compared with the first and second two-hour periods ($p<0.001$).

There was a significant main effect of *Time* ($F_{2,76}=6.88$, $p=0.002$) and a *Night* $\times$ *Time* $\times$ *Group* ($F_{2,76}=4.95$, $p=0.009$) interaction for RMSSD. While both groups showed increases in RMSSD, i.e. increase in vagal modulation, across the baseline night [control: first vs. second period ($p=0.021$); insomnia: first vs. third period ($p=0.012$)], only controls showed within-night increases in RMSSD on the stress night [first vs. third period ($p=0.012$) and second vs. third period ($p=0.010$)]. In the insomnia group, RMSSD did not change significantly across any of the three periods on the stress night. Also, RMSSD was lower in the second two-hour period of the stress night compared to the same period on the baseline night in controls ($p<0.001$). Similarly, a secondary analysis performed on HF_a (also reflecting vagal modulation), recorded within stable artifact-free NREM sleep, confirmed an increase in vagal activity (increase in HF_a) across the stress night in the control group but not in the insomnia group (*Night* $\times$ *Time* $\times$ *Group* interaction; $F_{2,76}=4.20$, $p=0.019$).

1.4. DISCUSSION

These findings show that anticipation of a psychosocial stress the following morning in women in the menopausal transition with and without an insomnia disorder is associated with: 1) increased pre-sleep psychophysiological activation (higher cortisol levels, faster heart rate and lower vagal activity, and greater perceived levels of tension); 2) greater autonomic activation during sleep, when heart rate continued to be faster and total heart rate variability was lower in the first 4 hours, relative to a baseline night; and 3) a delay in REM sleep latency but no change in sleep onset latency or sleep efficiency relative to a baseline night. In addition, our findings show different responses between women with and without insomnia: 1) women with insomnia showed no within-night recovery in vagal activity across the stress night whereas controls showed recovery in vagal activity four-six hours into the stress night; 2) women with insomnia showed increased beta1 EEG power in NREM sleep on the stress relative to the baseline night whereas beta1 EEG power did not differ between nights in the control group.

Overall, these results from an experimental anticipatory stress protocol show that perimenopausal women are sensitive during sleep to the impact of pre-sleep stress anticipation, with the effect being greatest in women with insomnia.

Our data are the first to experimentally show that the ANS during sleep is sensitive to the impact of stress anticipation in midlife women approaching menopause. Our findings complement those of Hall and colleagues (2004), who reported that exposure to a similar pre-sleep stress anticipation paradigm was associated with altered heart rate variability measures (reduced vagal activity) during NREM and REM sleep in a group of young men

and women (age 19.6 ± 1.8 years, 97% without a history of insomnia) compared with a control group. Others have shown that the impact of stress on the ANS during sleep is evident in real-life situations: higher levels of daily worry and stressors were associated with faster HR and lower HRV during waking and the subsequent sleep period in healthy men and women, with worry duration mediating the effect (Brosschot et al., 2007). On the other hand, higher resting vagal activity before bed is associated with better subjective and objective sleep quality in young women (Werner et al., 2015).

Sleep is a recovery period for the cardiovascular system. Heart rate and blood pressure decline and vagal activity increases across the sleep period (Trinder, 2007). There also is a prominent circadian influence on HR and vagal activity, which contributes to the decline in HR across the night (Burgess et al., 1997). This nocturnal reduction in HR and increase in vagal activity is evident in our study from both frequency domain analysis of HRV during stable NREM sleep epochs and time domain analysis of HRV across the baseline night in both groups. The normal within-night increase in vagal activity overlays sleep-stage specific fluctuations in autonomic regulation, as evident from our data showing a higher HR, lower vagal activity and a shift towards sympathetic dominance during REM sleep compared with NREM sleep, as also documented by others (Trinder et al., 2001).

The pattern of nocturnal recovery in vagal activity differed on the stress night compared with the baseline night, and diverged between insomnia and control groups. In controls on the stress night, vagal activity was lower and the within-night recovery was delayed, with an increase in RMSSD and HF power becoming apparent only 4-6 hours after lights-out. In the insomnia group on the stress night, vagal activity failed to ever increase across the 6 hour period analyzed. This divergence in recovery between groups was evident from time domain (which incorporates epochs of intervening wakefulness) and frequency domain (which includes only epochs of artifact-free and arousal-free NREM sleep) analyses of HRV, indicating that the lack of recovery in vagal activity on the stress night in the insomnia group was not mediated by movement artifacts, wake time, or sleep-stage composition. The effect of anticipatory stress on the vagal system during sleep, therefore, is prolonged in women with menopausal insomnia.

Although vagal tone remained unchanged across the stress night in the insomnia group, HR still declined and total HRV (SDNN) increased in the third two-hour period of the stress night. HR is influenced by both vagal and sympathetic branches of the ANS and changes in the sympathetic branch across the night may have contributed to the decline in HR. While vagal activity and sympathovagal balance can be inferred from HRV analysis, other methods (e.g. the measurement of pre-ejection period using the Impedance Cardiography technique) are needed to measure cardiac sympathetic nervous system activity, which would be a worthwhile focus of future studies about the impact of stress on sleep and the ANS.

While ANS function during sleep was altered by the anticipatory stress exposure, sleep quality and sleep macrostructure were not, apart from a delay in latency to REM sleep. Together, our findings suggest that the effects of anticipatory stress on nocturnal ANS function are not mediated by a disruption in overall sleep quality and reflect the sensitivity of the ANS to mild stress exposure before sleep. These results are similar to those of Hall et

al. (2004), who found no differences in sleep architecture between young participants exposed to experimental stress and a control group. Findings from the literature of how stress exposure impacts sleep have been mixed, and depend on the type, duration, and severity of the stressor (Akerstedt, 2006; Kim and Dimsdale, 2007). Some studies of acute experimental stressors found delayed sleep onset latency and/or more frequent awakenings or changes in sleep architecture, however, others found no effects. Exposure to moderate “real-life” work stress negatively impacted sleep efficiency (Petersen et al., 2013), and apprehension of the following work day was associated with a lower amount of slow wave sleep (Kecklund and Akerstedt, 2004). The first night in the sleep laboratory is considered to be stressful, with several studies showing reduced total sleep time, increased sleep onset latency, and a poorer sleep efficiency on the first night compared to subsequent nights (see Kim and Dimsdale, 2007, *for review*). Work-related stress and the first night in the laboratory may have a greater impact on sleep architecture because they are likely more stressful conditions than anticipating an experimental stressful situation the following morning. Some studies reported a delayed latency to REM sleep in response to stress, particularly on the first laboratory night (Agnew et al., 1966; Coble et al., 1974; Hirschner et al., 2015; Toussaint et al., 1995; Webb and Campbell, 1979), but this delay can be interpreted in the context of the other sleep disturbances reported on that night. Our finding of a small delay in latency to REM sleep in the absence of any other changes in sleep macrostructure might reflect a sensitivity of REM sleep mechanisms to either the stress anticipatory procedure, or the heightened autonomic arousal that was apparent in the first part of the night. Further studies are needed to replicate this effect and to explore possible mechanisms.

Analysis of sleep microstructure revealed an impact of the anticipatory stress paradigm only in the group of women with insomnia, who had increased high frequency EEG beta1 power, indicative of increased arousal during NREM sleep, compared with the baseline night. Our findings in the insomnia group, of both raised high frequency EEG power during NREM sleep and lack of recovery in vagal activity across the stress night, support our hypothesis, in part, that perimenopausal women with insomnia are more sensitive to the impact of stress.

We did not find group differences in the NREM sleep EEG spectrum on baseline nights, similar to what we previously reported in the larger group of women with and without insomnia developed in the context of the menopausal transition that included women in this dataset (Baker et al., 2015). We also did not find any evidence of autonomic hyperarousal in this group on baseline nights: HR and HRV measures, and evening cortisol levels, did not significantly differ between insomnia and control groups. These findings are in contrast to reports of autonomic and NREM EEG arousal in some studies of individuals with insomnia in the general population (Riemann et al., 2015). The women with insomnia in our study did, however, report higher levels of perceived daytime stress on both recording days compared with controls, which is similar to findings for individuals with insomnia in the general population (Morin et al., 2003). The women in the insomnia group in our study had all developed insomnia in the context of the menopausal transition, and had no prior history of insomnia. It still needs to be clarified what is the extent of overlap between insomnia that develops in this context and insomnia that develops in the context of other life events. As previously published by our group, women with perimenopausal insomnia are more neurotic

and have higher levels of subclinical depression, and have increased REM sleep beta EEG power compared with controls (Baker et al., 2015; Sassoone et al., 2014), similar to findings for insomnia in other populations (Levenson et al., 2015; Riemann et al., 2015; van de Laar et al., 2010). Data presented here suggest that a stress challenge reveals underlying differences in responsivity in both the autonomic and central nervous systems in perimenopausal women with insomnia that are not apparent under baseline conditions.

Hot flashes are a critical component of menopausal insomnia, uniquely contributing to sleep disruption in perimenopausal women (de Zambotti et al., 2014; Joffe et al., 2010). Indeed, we have previously reported (Baker et al., 2015), and show here, that women with insomnia are more likely to have hot flashes than perimenopausal women without insomnia (reaching significance on the stress night). However, women susceptible to hot flashes did not have more frequent hot flashes during the stress night, despite evidence of increased autonomic activation on that night. We can, therefore, exclude the potential confounding factor “hot flashes” in the interpretation of our results. There is limited work about stress as a trigger for hot flashes; postmenopausal women who reported frequent hot flashes were more likely to report and have physiological hot flashes during a daytime experimental stress session, which consisted of a series of stress exposures including the paced arithmetic task and giving a speech, compared with a non-stress session (Swartzman et al., 1990). Differences in experimental design may explain the different findings; our study focused on a stressor centered around the anticipation of a public speech and arithmetic task the following morning and data were collected during sleep. Increased frequency of hot flashes may only be evident in response to more immediate, short-term and highly stressful situations. Also, the relatively low sympathetic dominance that characterizes sleep may counteract stress-related activation. Finally, women were not recruited based on hot flash frequency or severity, and it remains to be determined whether women with severe/frequent nocturnal hot flashes may have an increased number of events in response to stress.

Our findings should be interpreted in the context of the study limitations. We focused on a group of midlife women, with and without DSM-IV insomnia developed in the context of the menopausal transition, recruited from the community. Our results cannot be extrapolated to clinical populations or to groups of individuals who developed insomnia in other contexts. Further work is required to establish both the similarities and differences between insomnia in the context of menopause and other times, particularly with regard to its pathophysiology. The anticipatory stress procedure was relatively mild, and it is possible that a more noxious stress could be associated with greater sleep disruption. It is also possible that individual differences in reactivity to stress may impact the extent of sleep or autonomic disturbances in response to an acute stress regardless of a current diagnosis of insomnia. This study is a cross-sectional investigation of women approaching menopause and we cannot rule out the possibility that some women in the control group may develop insomnia later in the transition. Further work is needed to determine whether traits such as worry, neuroticism, and stress reactivity, as well as history of chronic stress exposure, may influence responses to an acute pre-sleep stress during the menopausal transition.

CONCLUSIONS

Our study shows that stress anticipation impacts the autonomic nervous system before and during sleep in perimenopausal women with and without insomnia, with effects persisting for longer in women with insomnia. In addition, perimenopausal women with insomnia but not controls showed increased EEG arousal in response to stress. Together, these results suggest that women with insomnia developed in the menopausal transition have an altered reactivity to stress, which may play a role in the development and persistence of insomnia in midlife.

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ACRONYMS

AASM	Academy of Sleep Medicine
ANS	autonomic nervous system
BMI	body mass index
EEG	electroencephalography
HF_a	absolute high frequency integrated power in arbitrary units
HF_{np}	high frequency power as a proportion of total power
HF_{pf}	peak frequency in the high frequency range
HR	heart rate
HRV	heart rate variability
NREM	non-rapid-eye-movement sleep
PSG	polysomnography
REM	rapid-eye-movement sleep
REML	rapid-eye-movement sleep latency
RMSSD	root mean square of differences between adjacent normal-to-normal R intervals

SDNN	standard deviation of normal-to-normal R intervals
SE	sleep efficiency
SOL	sleep onset latency
STRAW	stages of reproductive aging workshop
TIB	time in bed
TP	total power
TST	total sleep time
WASO	wake after sleep onset

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Highlights

1. Stress could be a contributing factor to insomnia in perimenopausal women.
2. An acute anticipatory stress leads to increased pre-sleep autonomic arousal.
3. Autonomic arousal persists during the night, more so in women with insomnia.
4. EEG arousal in response to stress is evident in women with insomnia.
5. Perimenopausal women with insomnia showed high sensitivity to stress.

Figure 1.

Electroencephalographic spectral power ($\mu\text{V}\cdot\text{Hz}^{-1}$) in the Beta1 band (15- 23Hz) averaged for all artifact-free epochs of NREM (N2+N3) sleep across the first 6 hours on baseline and anticipatory stress nights, in perimenopausal women with (N=21) and without (N=17) insomnia. Vertical bars represent standard errors. **, $p<0.01$.

Figure 2.

HR (heart rate, bpm), RMSSD (root mean square of differences between adjacent NN intervals, ms) and SDNN (standard deviation of NN intervals, ms) averaged across first, second and third two-hour periods (after lights-out) on a baseline and anticipatory stress night in perimenopausal women with (N=22) and without (N=18) insomnia. Vertical bars represent standard errors.

Table 1

Pre-sleep perceived tension and cortisol levels, and cardiac autonomic measures during a 10-min pre-sleep period, on baseline and anticipatory stress nights in perimenopausal women with (N=22) and without (N=18) insomnia.

	Group	Pre-sleep Baseline night Mean (SD)	Pre-sleep Stress night Mean (SD)	Significant ANOVA effects
“How tense do you feel right now?” (0-100 mm) ^{a, ‡}	Control	11.4 (5.2)	75.6 (17.1)	◀ <i>Night</i> [*]
	Insomnia	20.2 (25.7)	62.0 (27.0)	
Cortisol (nmol/L) ^b	Control	2.0 (1.2)	2.3 (1.2)	◀ <i>Night</i> [*]
	Insomnia	1.8 (1.0)	2.5 (1.6)	
HR (bpm)	Control	63.9 (8.6)	67.6 (10.4)	◀ <i>Night</i> [*]
	Insomnia	64.9 (8.3)	66.9 (7.5)	
TP (ms ²) [‡]	Control	519.8 (463.3)	371.6 (356.5)	
	Insomnia	304.9 (252.6)	314.7 (334.1)	
HF_a (ms ²) [‡]	Control	62.9 (66.6)	48.6 (49.9)	◀ <i>Night</i> [*]
	Insomnia	41.6 (45.2)	27.7 (25.6)	
HF_{np} [‡]	Control	0.16 (0.12)	0.15 (0.11)	
	Insomnia	0.16 (0.10)	0.11 (0.07)	
HF_{pf} (Hz)	Control	0.22 (0.04)	0.25 (0.05)	
	Insomnia	0.24 (0.05)	0.24 (0.06)	

^{*}, p<0.05. **HR**, heart rate; **TP**, total power; **HF**, high frequency (**a**= absolute integrated power; **pf**=peak frequency; **np**=narrow proportion calculated as the proportion of absolute high frequency integrated power on the total power);

^a, n = 14 controls and 18 women with insomnia;

^b, n = 15 controls and 20 women with insomnia;

[‡], analyses based on log-transformed data;

[‡], analyses based on arcsine transformed data.

Table 2

Polysomnographic variables on a baseline and anticipatory stress night in perimenopausal women with (N=22) and without (N=18) insomnia.

	Group	Baseline night Mean (SD)	Stress night Mean (SD)	Significant ANOVA effects
TIB (min)	Control	430.0 (46.7)	450.0 (34.9)	
	Insomnia	433.8 (57.4)	435.7 (53.2)	
TST (min)	Control	378.5 (50.5)	388.9 (54.3)	
	Insomnia	368.8 (50.4)	375.6 (56.9)	
SE (%)	Control	87.9 (6.2)	86.3 (8.8)	
	Insomnia	85.3 (7.7)	86.2 (7.1)	
SOL (min)	Control	11.2 (8.4)	14.0 (11.3)	
	Insomnia	11.2 (10.0)	13.9 (13.1)	
REML (min)	Control	67.7 (22.0)	89.4 (36.3)	◀ <i>Night</i> **
	Insomnia	68.6 (27.0)	90.4 (46.1)	
WASO (min)	Control	40.3 (22.0)	47.2 (34.3)	
	Insomnia	53.8 (36.7)	46.2 (25.0)	
Arousal Index	Control	8.2 (2.7)	7.9 (3.1)	
	Insomnia	8.1 (2.9)	8.7 (3.5)	
Awakening Index	Control	3.5 (1.4)	3.1 (1.2)	
	Insomnia	3.7 (1.0)	3.8 (1.5)	
REM (%) ^a	Control	19.4 (4.0)	18.5 (3.1)	
	Insomnia	19.3 (4.6)	19.2 (5.9)	
N1 (%) ^a	Control	6.6 (2.5)	6.1 (2.3)	
	Insomnia	7.4 (3.3)	8.6 (4.8)	
N2 (%) ^a	Control	51.0 (6.4)	50.7 (8.6)	◀ <i>Group</i> *
	Insomnia	47.1 (6.9)	47.2 (6.2)	
N3 (%) ^a	Control	10.8 (4.1)	10.9 (4.2)	
	Insomnia	11.1 (6.5)	11.2 (6.2)	

* , p<0.05;

** , p<0.01. **TIB**, time in bed; **TST**, total sleep time; **SE**, sleep efficiency; **SOL**, sleep onset latency; **REM**, rapid-eye-movement sleep (**L**=latency); **WASO**, wake after sleep onset;

^a, calculated as percentage of time in bed.

Table 3

Electroencephalographic spectral power ($\mu\text{V}^2\cdot\text{Hz}^{-1}$) for delta (0.3- 4 Hz), theta (4- 8 Hz), alpha (8- 12 Hz), sigma (12- 15 Hz), beta1 (15- 23Hz) and beta2 (23- 30Hz) bands during artifact-free epochs of NREM (N2+N3) sleep across first, second and third 2-h periods on baseline and anticipatory stress nights in perimenopausal women with (N=21) and without (N=17) insomnia.

Group		Baseline night Mean (SD)			Stress night Mean (SD)			Significant ANOVA effects
		0-2h	2-4h	4-6h	0-2h	2-4h	4-6h	
Delta ($\mu\text{V}^2\cdot\text{Hz}^{-1}$)	Control	120.5 (58.4)	99.9 (46.8)	61.4 (25.9)	107.2 (48.6)	111.1 (69.1)	65.8 (29.8)	◀ <i>Time</i> ***
	Insomnia	117.0 (71.5)	100.2 (64.9)	63.3 (28.8)	135.8 (90.1)	114.4 (60.0)	69.2 (39.7)	
Theta ($\mu\text{V}^2\cdot\text{Hz}^{-1}$)	Control	9.0 (4.0)	8.2 (4.1)	7.0 (4.0)	9.1 (4.3)	8.3 (4.3)	7.0 (4.0)	◀ <i>Time</i> ***
	Insomnia	9.1 (4.1)	7.8 (3.5)	6.5 (2.8)	9.8 (4.8)	8.6 (3.9)	7.2 (3.4)	
Alpha ($\mu\text{V}^2\cdot\text{Hz}^{-1}$)	Control	4.8 (4.1)	4.6 (4.0)	4.4 (3.5)	4.8 (4.0)	4.5 (4.1)	4.2 (3.2)	◀ <i>Time</i> *** and <i>Night</i> $\times$ <i>Group</i> *
	Insomnia	4.8 (2.7)	4.2 (2.3)	4.0 (2.4)	5.1 (2.8)	4.6 (2.7)	4.4 (2.4)	
Sigma ($\mu\text{V}^2\cdot\text{Hz}^{-1}$)	Control	2.6 (1.1)	2.4 (1.2)	2.6 (1.2)	2.6 (1.1)	2.4 (1.0)	2.6 (1.3)	◀ <i>Time</i> ***
	Insomnia	2.5 (1.2)	2.3 (1.2)	2.6 (1.5)	2.9 (1.8)	2.7 (1.9)	3.0 (1.9)	
Beta1 ($\mu\text{V}^2\cdot\text{Hz}^{-1}$)	Control	0.39 (0.17)	0.38 (0.19)	0.43 (0.19)	0.39 (0.15)	0.37 (0.15)	0.38 (0.16)	◀ <i>Time</i> ** and <i>Night</i> $\times$ <i>Group</i> **
	Insomnia	0.39 (0.16)	0.38 (0.18)	0.43 (0.21)	0.46 (0.21)	0.45 (0.24)	0.51 (0.26)	
Beta2 ($\mu\text{V}^2\cdot\text{Hz}^{-1}$)	Control	0.12 (0.05)	0.11 (0.04)	0.12 (0.05)	0.12 (0.04)	0.12 (0.04)	0.11 (0.04)	◀ <i>Time</i> **
	Insomnia	0.12 (0.06)	0.11 (0.04)	0.13 (0.06)	0.13 (0.05)	0.12 (0.06)	0.13 (0.06)	

* , $p < 0.05$;

** , $p < 0.01$;

*** , $p < 0.001$. All analyses based on log-transformed data.

Table 4

Cardiac autonomic measures derived from frequency domain analysis of heart rate variability recorded during undisturbed REM and NREM sleep on a baseline and anticipatory stress night in perimenopausal women with (N=22) and without (N=18) a diagnosis of insomnia.

	Group	Baseline night Mean (SD)		Stress night Mean (SD)		Significant ANOVA effects
		NREM	REM	NREM	REM	
HR (bpm)	Control	61.2 (5.3)	63.7 (6.6)	63.0 (63.4)	65.3 (6.0)	◀ <i>Night** and Sleep stage***</i>
	Insomnia	63.1 (6.4)	65.9 (5.9)	64.7 (6.7)	67.5 (6.8)	
TP (ms ²) [†]	Control	383.4 (218.2)	368.6 (350.7)	381.0 (289.0)	370.7 (371.2)	◀ <i>Night* and Sleep stage**</i>
	Insomnia	337.0 (355.3)	271.6 (335.2)	287.2 (254.6)	200.2 (185.4)	
HF_a (ms ²) [†]	Control	80.8 (63.7)	38.2 (45.8)	77.1 (62.2)	37.9 (32.1)	◀ <i>Sleep stage***</i>
	Insomnia	72.1 (82.2)	27.0 (36.4)	64.6 (56.4)	18.4 (13.7)	
HF_{np} [†]	Control	0.22 (0.09)	0.11 (0.06)	0.22 (0.09)	0.11 (0.06)	◀ <i>Sleep stage***</i>
	Insomnia	0.23 (0.07)	0.11 (0.04)	0.24 (0.08)	0.10 (0.05)	
HF_{pf} (Hz)	Control	0.24 (0.04)	0.25 (0.04)	0.25 (0.04)	0.25 (0.04)	
	Insomnia	0.25 (0.03)	0.25 (0.04)	0.25 (0.03)	0.25 (0.04)	

* , p<0.05;

** , p<0.01;

*** , p<0.001. **HR**, heart rate; **TP**, total power; **HF**, high frequency (**a**= absolute integrated power; **pf**=peak frequency; **np**=narrow proportion calculated as the proportion of the absolute high frequency integrated power on the total power);

[†] , analyses based on log-transformed data.