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Menstrual Cycle-Related Variation in Autonomic Nervous System Functioning in Women in the Early Menopausal Transition with and without Insomnia Disorder

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Abstract

Insomnia is considered a hyperarousal disorder, in which several psychophysiological domains including the autonomic nervous system (ANS) are over-activated, potentially contributing to increased risk for cardiovascular (CV) disease. Here, we aimed to determine whether insomnia that develops in the context of the transition to menopause (menopausal transition insomnia, MTI) is similarly characterized by autonomic arousal. We also took into account modulation of the ANS by the hormonal changes of the menstrual cycle, a factor that has not previously been considered in studies on insomnia. Twenty one women with insomnia (49.0 ± 3 y) and 25 controls (48.8 ± 2.6 y), also in the menopausal transition, had overnight laboratory-based polysomnographic recordings, including electrocardiograph, during the follicular and/or luteal (progesterone $3 \text{ ng} \cdot \text{ml}^{-1}$) phases of the menstrual cycle, with 21 women having recordings in both phases. Nocturnal time and frequency-domain heart rate variability (HRV) measures were calculated. Heart rate (HR) was significantly elevated (by $\sim 4 \text{ bpm}$) in MTI compared to controls in both follicular and luteal phases, across hours of the night, including during undisturbed periods of NREM and REM sleep ($p < 0.05$). A higher HR tended to be associated with lower frequency- and

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Conflict of interest

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

Contributors

M.d.Z. conducted the data analysis and drafted the manuscript.

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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time-domain vagal HRV indices in MTI compared with controls. In both groups, HR was significantly higher and total and high frequency HRV measures were lower in the luteal phase compared to the follicular phase ($p<0.05$). In addition, REM compared to NREM sleep was characterized by increased HR coupled with decreased vagal modulation and increased sympathovagal balance ($p<0.01$). Insomnia in the menopausal transition is characterized by nocturnal autonomic hyperarousal during both follicular and luteal phases of the menstrual cycle, which could be a factor in the etiology of MTI as well as a potential CV risk factor.

Key terms

Insomnia; menopause; menstrual cycle; autonomic nervous system; heart rate variability; progesterone

Introduction

Insomnia is the most common sleep disorder and a public health concern; it is defined by difficulty initiating or maintaining sleep, with associated distress and impairment in daytime functioning (Levenson et al., 2015). One-third of the general population reports insomnia symptoms with 6–10% meeting the diagnostic criteria for an insomnia diagnosis (Ohayon, 2002). Importantly, female sex is a strong risk factor, with a female/male risk ratio of 1.41/1 (Zhang and Wing, 2006). The prevalence of insomnia increases with age and varies as a function of a woman's reproductive state, being particularly evident during the menopausal transition, with 26% of women in the menopausal transition developing the disorder (Ohayon, 2006). Insomnia that develops in association with the menopausal transition (MTI) is unique because it is time-linked with the menopausal transition and associated hormonal changes (increase in follicle-stimulating hormone (FSH) and decrease in estradiol reflecting aging of the reproductive system). It is consequently linked with menopause-specific symptoms, such as hot flashes, which can act as triggers for insomnia (Ohayon, 2006). Indeed, hot flashes are associated with awakenings from sleep (Baker et al., 2015; Bianchi et al., 2016; Joffe et al., 2013) and account for a significant proportion of total objective awake time during the night (de Zambotti et al., 2014b). Also, greater perceived interference from hot flashes is a predictor of MTI (Sassoon et al., 2014).

Insomnia is considered a hyperarousal disorder in which several psychophysiological domains are over-activated (Levenson et al., 2015). Growing attention is being paid to hyperactivation of the autonomic nervous system (ANS) given that insomnia disorder is an independent risk factor for developing CV disease (Fernandez-Mendoza et al., 2012; Sofi et al., 2014). A number of studies of insomnia disorder in mixed populations of men and/or women of varying ages have reported altered ANS measures in insomnia, including elevated cardiac sympathetic activity (de Zambotti et al., 2014a; De Zambotti et al., 2011; de Zambotti et al., 2013b), elevated HR (Bonnet and Arand, 1998; De Zambotti et al., 2011; Farina et al., 2014), and/or high sympathovagal balance and depressed heart rate variability (HRV) (Bonnet and Arand, 1998; Farina et al., 2014; Spiegelhalder et al., 2011), although alterations are not always evident or even consistent across studies (reviewed in (Riemann et al., 2015)). None of the previous studies focused on MTI, although we recently reported that

women with MTI have elevated beta EEG activity in REM sleep reflecting heightened EEG arousal compared to controls (Baker et al., 2015). We also previously demonstrated (de Zambotti et al., 2016) that women with MTI have blunted vagal recovery during the night following an experimental pre-sleep stressor, suggesting altered ANS function, at least in response to stress.

Previous studies of ANS function in insomnia have not considered the potential impact of the menstrual cycle and associated reproductive hormone changes on the ANS, yet the effect is substantial. Estradiol is cardio-protective, associated with higher vagal activity and lower SNS activity (Saleh and Connell, 2007), while progesterone is associated with higher SNS activity (Genazzani et al., 2000). Most studies based on short-duration ECG recordings found that there is a reduction in cardiac vagal activity and a shift to sympathetic dominance in the luteal phase compared with the follicular phase (see von Holzen et al., 2016, for review). We had similar findings based on recordings made during sleep in young healthy women in the mid-luteal phase relative to the follicular phase (de Zambotti et al., 2013c) and also showed that women with severe premenstrual syndrome had altered sleep-related HRV measures in response to the luteal phase compared with controls (Baker et al., 2008; de Zambotti et al., 2013c). HRV measures also change with age and in association with menopausal hormone changes: there is a reduction in vagal activity and a shift to sympathetic dominance that emerges one year after menopause, when estradiol levels are low (von Holzen et al., 2016). Women in different menstrual cycle phases or different stages of the reproductive lifecycle, therefore, may have altered ANS function due to the hormone environment over and above any effects of insomnia.

Here, we aimed to investigate vagal activity and sympathovagal balance based on HRV measures during sleep in still-cycling women with insomnia disorder, in the early menopausal transition compared with controls. We also evaluated the potential modulatory effect of menstrual cycle-related hormone fluctuations on the ANS in the two groups of women.

Method

Participants

Forty-six women in the menopausal transition (MT) according to Stages of Reproductive Aging Workshop criteria (Soules et al., 2001) (i.e. menstrual cycle lengths that differed by >7 days from normal (early MT) or an amenorrhea interval of >60 days (late MT) but not >12 months), were recruited in the San Francisco Bay Area and constituted the final sample. According to these criteria, 17 women with insomnia and 22 controls were in the early menopausal transition while the remaining women were in the late menopausal transition (4 MTI and 3 controls). They were participants in a larger study of sleep quality in 72 women with and without insomnia in the MT, which involved between one and four recording nights scheduled at different phases of the menstrual cycle for cycling women or across a month for women with infrequent or skipped cycles. Data about sleep macro- and micro-structure have been published elsewhere (Baker et al., 2015), including at different menstrual cycle phases in a subsample of 20 women (de Zambotti et al., 2015). The main polysomnographic variables for the current subset of women, separately by group (insomnia and control

women) and menstrual cycle phase (follicular and luteal), are shown in Supplementary Table 1. The current analysis focuses on measures of heart rate and heart rate variability and includes only women having recordings in the follicular and/or luteal phases of the menstrual cycle.

All women gave written informed consent and received compensation for participation. The study was reviewed and approved by SRI International's Institutional Review Board. Details on sample and screening procedures are fully described in Sassoone et al. (2014).

Briefly, all participants had a structured clinical interview for DSM-IV-TR Axis I Disorders (First et al., 1998) including a customized module evaluating sleep history and DSM-IV criteria for insomnia (Morin and Espie, 2003). Twenty-one women met criteria for an insomnia diagnosis with an onset of insomnia that was coincident with the menopausal transition (menopausal transition insomnia, MTI). 6 MTI participants (28.6%) reported difficulty falling asleep (reporting sleep latency > 30 min), 13 (61.9%) reported experiencing nocturnal awakenings, 8 (38.1%) reported early morning awakening, and 10 (47.6%) reported non-restorative sleep, on at least three nights per week, for at least a month. These symptoms were associated with clinically significant distress or impairment. Twenty-five women did not report clinically-significant sleep difficulties and were categorized as menopausal transition controls.

Exclusion criteria for both groups of women were having a body mass index (BMI) ≥ 33 kg.m⁻², taking hormone therapy or hormonal contraception during the previous 3 months, hysterectomy and/or bilateral oophorectomy, amenorrhea for ≥ 12 months, current severe medical conditions (e.g. hypertension), current Axis I disorders (e.g. major depressive disorder, generalized anxiety disorder) other than insomnia in the MTI group, lifetime history of DSM-IV insomnia, apnea-hypopnea index >5 and/or periodic leg movement index >10 (based on a laboratory clinical PSG assessment), and current use of medication (e.g. hypnotics, antidepressants) and/or supplements (e.g. melatonin, black cohosh, soy products) that could affect sleep quality or menopausal symptoms.

Procedure

Participants had a laboratory adaptation/screening night to adapt to the laboratory and to confirm absence of clinical sleep disorders other than insomnia. Following adaptation, all participants had one or two PSG assessments in the sleep laboratory at SRI International in the follicular (2–10 days after onset of menstruation) and/or luteal phases (plasma progesterone levels ≥ 3 ng.ml⁻¹) of the menstrual cycle. Twenty one women (11MTI and 10 controls) had recordings in both the follicular and luteal phases, 17 other women (8 MTI and 9 controls) had a single recording in the follicular phase, and 8 other women (2 MTI and 6 controls) had a single recording in the luteal phase. Participants slept in sound-attenuated and temperature-controlled bedrooms; they self-selected lights-out and lights-on times. Participants were instructed not to drink beverages containing alcohol or caffeine after 3 pm of each recording day; all women registered 0.0 on a breathalyzer on arrival at the sleep laboratory.

Reproductive hormonal levels

A blood sample was taken in the evening or the morning for each PSG recording, based on availability of the phlebotomist. Blood was immediately centrifuged and serum samples were frozen at -70°C until analysis for follicle-stimulating hormone (FSH), progesterone and estradiol using standard immunoassay kits. The intraassay and interassay coefficients of variations were 2.6% and 5.5%, respectively, for FSH, with a sensitivity of 0.1 IU.l^{-1} (Siemens Healthcare Diagnostics, Los Angeles, CA, USA), 4.0% and 5.7%, respectively, for progesterone, with a sensitivity of 0.2 ng.ml^{-1} (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), and 6.7% and 7.6%, respectively, for estradiol, with a sensitivity of 3 pg.mol^{-1} (Beckman Coulter Inc., Fullerton, CA, USA). Progesterone levels $\geq 3\text{ ng.ml}^{-1}$ was used as a cut-off to classify the luteal phase (Israel et al., 1972). FSH and estradiol values were log transformed before analysis. Blood samples were not obtained from two women in the follicular phase and one woman in the luteal phase (progesterone levels in a saliva sample confirmed an ovulatory cycle).

Self-report measures of menopausal symptoms, sleep quality, and mood

At the clinical interview, severity of psychological, somatic, and vasomotor symptoms was assessed with the Greene Climacteric Scale (GCS) (Greene, 1998). Participants completed the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), to assess sleep habits and quality over the past month. Each evening in the laboratory, current mood was assessed using the Profile of Mood States (POMS) (McNair et al., 1992), which consists of 65 adjectives to which participants respond on a 5-point scale, from 1 (not at all) to 5 (extremely), based on how they are feeling right then. Total mood score was obtained by subtracting the Vigor sub-scale score from the sum of Tension, Depression, Anger, Fatigue and Confusion sub-scale scores (a constant of 50 was added to avoid negative scores).

Polysomnographic sleep and hot flash assessment

Electroencephalographic (F3, F4, C3, C4, O1, O2 referenced to the contralateral mastoids), electromyographic and bilateral electrooculographic signals were acquired through a Grael™ system with Profusion PSG3™ software (Compumedics, Abbotsford, Victoria, Australia) according to American Academy of Sleep Medicine (AASM) criteria (Iber, 2007). EEG was sampled at 256 Hz and 0.3–35 Hz filtered. Sleep stages (wake, N1, N2, N3 and rapid-eye-movement sleep) and arousals ($\geq 3\text{ s}$, $<15\text{ s}$) were manually scored according to AASM rules (Iber, 2007) and by scorers blind to study group.

Nocturnal hot flashes were assessed by measures of sternal skin conductance according to standard criteria (Carpenter et al., 1999) with a BioDerm Skin Conductance Meter (model 2701; UFI, Morro Bay, CA). Objective hot flashes were visually identified on the recordings by fluctuations in skin conductance of 2 micro-Siemens within 30 s (Freedman, 1989). Due to technical problems, skin conductance was missing from 4 recordings (3 luteal phase and 1 follicular phase nights).

Heart rate and autonomic nervous system functioning

An ECG was recorded using 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) through the GraefTM system and Profusion PSG3TM software. The ECG signal was sampled at 512 Hz. R-waves were automatically detected and manually checked and normal-to-normal interbeat-intervals (IBIs) were calculated.

Frequency domain analysis of heart rate variability (HRV) was performed with dedicated software (SRS 5.1, Sleep Research System, School of Behavioural Science, University of Melbourne, Australia) on 2 min bins of artifact-free NREM (N2 and N3) and REM sleep, automatically selected throughout each of the PSG recordings according to rules described by Trinder et al. (2001). Briefly, the 2-min bins had to be free from artifacts and contain four 30-s epochs of the same sleep stage; also, they had to be preceded by four artifact-free 30-s epochs of the same sleep stage. Once a 2-min bin was identified for analysis, another epoch (of the same sleep stage) was not identified for a further 2 min. In addition, consecutive 2-min bins were selected from lights-out to the first epoch of sleep (sleep onset) to obtain a measure of ANS function during the wake period just before sleep onset. For each bin, IBIs were re-sampled (4 Hz) and filtered (3rd order polynomial filter) to remove the slow non-stationary trend; the total power (TP, 0–0.5 Hz, ms²), a measure of total HRV, was divided into 0.02 Hz bands and an algorithm searched for the highest value in the 0.03–0.15 Hz to identify the LF component, and in the 0.15–0.40 Hz to identify the HF component. The absolute integrated power (arbitrary units) was quantified for both the LF (ms²) and HF (ms²) narrow bands as the area between the first frequency bands on either side of the peak to fall to 50% of the peak value (see Trinder et al., (2001) for details). TP, HF power and the ratio of LF/HF power (as a measure reflecting sympathovagal balance) were used in the analysis.

Time domain analysis of HRV was performed using SRS software on consecutive five-min artifact-free periods from lights-out across the first seven hours of the night, irrespective of arousals and sleep stage transitions. Five-min periods were averaged for each hour (periods with major artifacts including body movements were discharged; each hour had at least 10 min of reliable data). Heart rate (HR, bpm), standard deviation of IBIs (SDNN, ms; a measure of total variability), root mean square of differences between adjacent IBIs (RMSSD, ms; a measure of high-frequency activity reflecting vagal modulation) were calculated.

TP, HF power, LF/HF power, SDNN and RMSSD were log transformed before analysis.

Statistical Analysis

Independent t-tests were used to test differences in demographic measures, GCS and PSQI scores, and habitual alcohol and caffeine consumption. Chi-Square tests were used to assess group differences in ethnicity and frequency of women having physiological hot flashes.

Repeated measures hierarchical linear models (HLMs) were used to investigate the pre-sleep wake and nocturnal ANS profile, reproductive hormone levels, and self-report measures of mood at different phases of the menstrual cycle, accounting for multiple observations (11 MTI women and 10 controls had two recordings nights and there were multiple observations for some variables across the night). The statistical models included *group* (MTI and

controls), *menstrual phase* (follicular and luteal), *time* (hours across the night) or *sleep stage* (NREM [N2+N3] and REM sleep), and the interaction terms *group* $\times$ *menstrual phase*.

HLMs included random effects for participants and residual error. If the main effect *time* was statistically significant, we conducted post-hoc tests using a simplified mixed model (analogous to post-hoc t-tests for an ANOVA) to compare response means at different time points. F tests and associated p values are provided for the overall models; Wald Chi-Square test and associated p values are provided for significant main effects and/or interactions.

All analyses were performed using Stata/SE 14.1 for Windows. Results are reported as mean $\pm$ SD unless otherwise indicated. Significance was considered at $p < 0.05$.

Results

Demographic and self-report measures

MTI and control groups did not differ according to age, ethnicity, BMI, education, habitual caffeine and alcohol consumption, or smoking habits (all $p > 0.05$). MTI participants reported more severe psychological ($t = 3.45$, $p < 0.01$) and vasomotor symptoms ($t = 2.40$, $p < 0.05$) on the Greene climacteric scale. As expected, MTI participants had higher PSQI scores, reflecting poorer sleep quality, than controls ($t = 2.02$, $p < 0.001$) (see Table 1).

Reproductive hormones, hot flashes, and mood

HLMs were significant for FSH ($F_{3,17} = 12.04$, $p < 0.001$) and progesterone ($F_{3,17} = 52.75$, $p < 0.001$). A *menstrual phase* main effect indicated that FSH was lower ($t = -5.99$, $p < 0.001$) and progesterone was higher ($t = 12.26$, $p < 0.001$) in the luteal compared to the follicular phase of the menstrual cycle (Table 2). There was no menstrual phase effect for estradiol. There were no significant group or interaction effects for any of the hormones. There was no significant difference between the number of women with and without insomnia having at least one objective hot flash at night (Chi-square, $p > 0.05$) (Table 2). Both groups had a median of 1 hot flash per night. The HLM was significant for mood ($F_{3,19} = 3.33$, $p < 0.05$) with MTI compared to control participants having a worse evening mood (*group* main effect: $t = 3.06$, $p < 0.01$) regardless of menstrual cycle phase. There was no significant *menstrual phase* or *group-phase* interaction effect (see Table 2).

ANS activity during the pre-sleep wake period

None of the HLMs was significant for ANS activity during the pre-sleep wake period, however, there was an overall tendency for higher HR (*group* main effect: $t = 1.85$, $p = 0.08$), lower TP (*group* main effect: $t = -1.99$, $p = 0.06$) and lower HF power (*group* main effect: $t = -2.14$, $p < 0.05$) in MTI women compared to controls.

ANS activity in NREM and REM sleep

Frequency domain HRV measures during NREM and REM sleep in the follicular and luteal phases of the menstrual cycle for MTI and control groups are shown in Figure 1.

The HLM was significant for HR ($F_{4,85}=19.47$, $p<0.001$). There was a main effect of *group*, with MTI participants having a higher HR than controls ($t=2.20$, $p<0.05$). There was also a main effect of *menstrual phase*, with both groups having higher HR in the luteal compared to the follicular phase ($t=6.51$, $p<0.001$), and a main effect of sleep stage, with HR being higher in REM compared to NREM sleep ($t=5.56$, $p<0.001$).

HLMs were significant for TP ($F_{4,85}=4.41$, $p<0.01$), HF power ($F_{4,85}=39.13$, $p<0.001$) and the LF/HF ratio ($F_{4,85}=29.27$, $p<0.001$). TP (*menstrual phase* effect: $F=-2.12$, $p<0.05$) and HF power (*menstrual phase* effect: $F=-2.23$, $p<0.05$) were lower in the luteal compared to the follicular phase of the menstrual cycle. *Sleep stage* main effects indicated that TP ($t=-3.04$, $p<0.01$) and HF power ($t=-12.19$, $p<0.001$) were lower, while LF/HF ratio ($t=10.71$, $p<0.001$) was higher in REM sleep compared to NREM sleep.

MTI showed a tendency to have lower HF power (*group* effect: $t=-1.77$, $p=0.08$) and TP (*group* effect: $t=-1.67$, $p=0.10$) compared to controls. None of the interactions was significant for the frequency-domain HRV measures.

ANS activity across the night

Time domain HRV measures across the first seven hours of the night in the follicular and luteal phases of the menstrual cycle in women with MTI and controls are shown in Figure 2.

The HLM was significant for HR ($F_{9,408}=17.77$, $p<0.001$) such that the pattern of data was consistent with the frequency-domain analysis of HR. HR was higher in MTI participants compared to controls (*group* main effect, $t=2.22$, $p<0.05$) across the night irrespective of arousals, awakenings and sleep stage composition. There was also a main effect of *menstrual phase*; HR being higher in the luteal compared to the follicular phase ($t=9.25$, $p<0.001$) in both groups. A main effect of *time* (Wald Chi-Square=70.2, $p<0.001$) revealed that HR increased from h1 to h2 ($p<0.01$) and then decreased across the night: HR was lower in h6 and h7 compared to h1, in h4-h7 compared to h2, in h5-h7 compared to h3, in h6 and h7 compared to h4, and in h7 compared to h5 (all $p<0.05$) in both groups.

HLMs were significant for SDNN ($F_{9,408}=16.64$, $p<0.001$) and RMSSD ($F_{9,408}=8.45$, $p<0.001$). A main effect of *menstrual phase* indicated that SDNN ($t=-2.78$, $p<0.01$) and RMSSD ($t=-5.25$, $p<0.001$) were lower in the luteal phase compared with the follicular phase in both MTI and controls. Overall, SDNN increased across hours of the night (*time* main effect, Wald Chi-Square=374.38, $p<0.001$): SDNN was higher in h3 and h5-h7 compared to h1, in h3-h7 compared to h2, in h6 and h7 compared to h3-h5, in h5 compared to h4, and in h7 compared to h5 (all $p<0.05$). Similarly, there was a significant *time* main effect for RMSSD (Wald Chi-Square=543.40, $p<0.01$). RMSSD first declined from h1 to h2 ($p<0.01$), and then increased across the night being higher in h3-h7 compared to h2, in h6 compared to h3, and in h7 compared to h1-h6 (all $p<0.05$).

RMSSD showed a tendency to be lower in MTI compared to controls ($t=-1.71$, $p=0.09$). There were no significant interaction effects for any of the time-domain HRV variables.

Discussion

The current study provides insight into cardiac ANS modulation during sleep in insomnia disorder that developed in the context of the approach to menopause. Women with MTI had a higher HR across the night in both follicular and luteal phases of the menstrual cycle compared to controls, with the magnitude of the effect (~4bpm) being equivalent to that for menstrual cycle phase (luteal greater than follicular). HR also tended to be elevated during the pre-sleep wake period albeit not statistically significant. Importantly, the raised HR in the MTI group was evident even during undisturbed periods of NREM and REM sleep. A raised HR in MTI appeared to be associated, in part, with lower nocturnal vagal activity, although the group difference in HF power was not quite statistically significant.

Our finding of a higher HR during sleep suggests that women with MTI are in a state of autonomic hyperarousal. These findings extend our previous work showing altered regulatory control of blood pressure during sleep (de Zambotti et al., IN PRESS) and an altered autonomic profile in response to pre-sleep stress, in women with MTI (de Zambotti et al., 2016). Autonomic hyperarousal is thought to play a role in the etiology of insomnia disorder, with several studies finding higher HR and abnormal ANS modulation in insomnia disorder in mixed groups of men and/or women of varying ages (Bonnet and Arand, 2010; Riemann et al., 2015). Hyperarousal in insomnia disorder is also evident from other measures, such as the EEG, with insomnia patients having greater high frequency EEG activity, indexing cortical arousal during sleep (Levenson et al., 2015; Riemann et al., 2015). Similarly, women with MTI have higher beta EEG activity during REM sleep compared with controls, although the effect was not evident in NREM sleep (Baker et al., 2015). Thus, insomnia in the context of the menopausal transition has unique features, such as hot flashes (Baker et al., 2015; de Zambotti et al., 2014b; Ohayon, 2006), which may trigger and maintain insomnia, and also shares features of hyperarousal common to insomnia disorder in other populations (Sassoon et al., 2014). It is likely that these features interact leading to the development and/or maintenance of insomnia in the menopausal transition.

Importantly, we cannot say from our data whether heightened autonomic arousal during sleep is evident before women develop insomnia (a predisposing factor) or whether it develops in association with the emergence of, or as a consequence of, insomnia and/or other symptoms such as hot flashes. There are phasic ANS modifications in association with hot flashes, with increases in HR accompanied by vagal withdrawal (de Zambotti et al., 2013a). However, the present results are unlikely to be confounded by the presence of nocturnal hot flashes as similar numbers of women with MTI and controls had objectively recorded hot flashes on their recording nights, even though women with MTI had higher scores on the vasomotor subscale of the Greene Climacteric Scale than controls at the clinical interview. It is possible that as women progress through the menopausal transition and may experience more frequent and severe hot flashes, further ANS dysfunction linked with hot flash events at night, may become evident.

Poor PSG-defined sleep quality, short sleep duration, and more wakefulness, which we have previously reported in MTI (Baker et al., 2015) could have played a role in our current finding of an elevated HR in MTI. HR increases in association with arousal from sleep and

is higher during wakefulness than sleep (Trinder et al., 2012). However, the frequency-domain HRV analysis confirmed that a raised HR in MTI was evident even in undisturbed periods of NREM and REM sleep. In addition, women with MTI still showed a decline in HR and increase in HRV across hours of the night, which is attributed in part to circadian and sleep-related factors (Trinder et al., 2012). Finally, all women exhibited similar sleep stage ANS modulation with expected increases in HR coupled with decreased vagal modulation and increased sympathovagal balance in REM sleep compared to NREM sleep (Trinder et al., 2012).

HRV measures are also modified in the presence of depressive disorder (Bassett, 2016) or severe premenstrual syndrome (Baker et al., 2008; de Zambotti et al., 2013c; Matsumoto et al., 2006). Women with MTI had higher scores on the POMS, reflecting poorer current mood, at both menstrual cycle phases, and we have previously reported that they are more likely to have a history of depressive disorders, including PMDD (Sassoon et al., 2014), compared with controls. This clinical history could contribute to the group differences we found here.

To our knowledge this is the first study to investigate ANS functioning, taking into account menstrual cycle phase, in insomnia disorder. As reported in the literature (see von Holzen et al., 2016, for review), we found a profound effects of menstrual cycle phase on HR and HRV, with HR acceleration, reduced HRV, and vagal withdrawal in the luteal phase, compared to the follicular phase of the menstrual cycle in both groups of women. These menstrual cycle related differences in ANS modulation during sleep are similar to those we previously reported in a group of young women studied (de Zambotti et al., 2013c) and are likely related to the influence of reproductive hormones. Estradiol modulates vagal and sympathetic nervous system regulation of HR, with HR being lower and HRV higher in the presence of estradiol (Saleh and Connell, 2007; von Holzen et al., 2016). Progesterone, on the other hand, appears to oppose the beneficial effect of estradiol; postmenopausal women taking combined progesterone and estrogen hormone therapy have lower vagal activity and higher HR than women not taking hormone therapy (Christ et al., 2002; von Holzen et al., 2016). In the luteal phase, therefore, progesterone may oppose the effect of estradiol, leading to vagal withdrawal and an increase in HR. As women progress further through the menopausal transition and post-menopause, additional hormonal changes, including an overall decline in estradiol, also modulate ANS activity, although changes in HRV indices only emerge one year post-menopause (von Holzen et al., 2016).

Remarkably, while HR was already high in women with MTI compared with controls in the follicular phase, they showed a further increase in HR, along with a decrease in vagal activity, in the luteal phase. Our finding of higher HR in MTI during both the follicular and luteal phases of the menstrual cycle, coupled with no group differences in blood hormone concentrations, suggests that both groups responded similarly to the different hormone environment. It is possible, however, that our study may not have been sufficiently powered to find a group-menstrual phase interaction effect. Thus, we cannot exclude the possibility that more direct experimental manipulations across different menstrual cycle phases may capture specific hormone-related ANS alterations in MTI. Whether or not the upshift in the level around which HR is regulated during the night, evident in both menstrual cycle phases,

impacts CV health in women with MTI remains to be determined. The ANS plays a major role in the regulatory control of heart and vasculature and an imbalance in ANS functioning leads to disease and mortality (Thayer et al., 2010). Elevated HR is an independent risk factor for mortality and morbidity in healthy individuals and in those with existing CV pathologies (Cooney et al., 2010; Tardif, 2009) and needs to be treated in the same way as other major risk factors (e.g. high blood pressure, smoking, diabetes). Of clinical relevance is the fact that high HR (Bohm et al., 2015; Tardif, 2009) and reduced HRV (Thayer et al., 2010) are modifiable CV risk factors.

Our study has limitations that need to be considered. This design was a combination of within- and between-participant variables and further studies are needed to investigate within-menstrual cycle changes in a larger group of women, especially when considering potential interactions with insomnia. We only measured HRV and therefore are unable to establish the contribution of altered SNS activity to either the raised HR in women with MTI or the luteal phase increase in HR. Also, the observational nature of the study does not allow controlling for the effect of hormones on ANS. Future studies could manipulate hormone concentration by, for example, direct administration of progesterone and estradiol, to better clarify the relationship between reproductive hormones and ANS measures in women in the MT.

Our study also has several strengths. We assessed HRV measures during sleep, a stable condition in which to record basal ANS functioning (Orr et al., 2000), and also accounted for sleep stage composition, awakenings and arousals as well as time across the night. Finally, we considered menstrual cycle phase effects, and showed that menstrual phase has a critical impact on HR and ANS modulation, an effect that is as substantial as that of insomnia. These results attest directly to the need for considering the reproductive hormone environment when looking at group differences in HRV metrics.

In conclusion, our findings show that women with MTI have increased heart rate during sleep, suggesting a state of autonomic arousal compared with age-matched controls. It remains to be determined whether this state of hyperarousal contributes to the etiology of MTI and whether MTI women may benefit from treatment options targeted at reducing HR and improving ANS functioning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Heart rate is high throughout sleep in menopausal insomnia.
2. Raised heart rate in menopausal insomnia is evident even during undisturbed sleep.
3. Further vagal withdrawal and heart rate increase occurs during the luteal phase.
4. Autonomic arousal in menopausal insomnia may impact cardiovascular health.

Figure 1.

Heart rate (HR), total power (TP), high frequency (HF) and low/high frequency power (LF/HF ratio) heart rate variability measures recorded during artifact- and arousal-free rapid-eye-movement (REM) and non-REM (NREM) sleep across the whole night after lights-out during the follicular and luteal phases of the menstrual cycle for women in the menopausal transition with and without insomnia. To aid interpretation, TP, HF and LF/HF ratio have been plotted in their original units. Vertical bars represent standard errors.

Figure 2.

Heart rate (HR), standard deviation of inter-beat-intervals (SDNN) and root mean square of differences between adjacent inter-beat-intervals (RMSSD) across the first 7 hours of the night after lights-out during the follicular and luteal phases of the menstrual cycle for women in the menopausal transition with and without insomnia. Vertical bars represent standard errors.

Table 1

Demographics and self-report measures of menopausal symptoms, sleep quality, caffeine and alcohol consumption for women in the menopausal transition with and without insomnia.

	Insomnia Mean (SD)	Controls Mean (SD)
Sample, No.	21	25
Caucasian, No.	16	19
Age, y	49.0 (3.0)	48.8 (2.6)
BMI, kg.m⁻²	24.1 (4.0)	24.3 (3.5)
Education, y	16.6 (2.0)	16.0 (1.9)
GCS-psychological	6.6 (3.8)	3.4 (2.6) **
GCS-somatic	2.5 (2.4)	1.6 (1.7)
GCS-vasomotor	1.9 (1.1)	1.1 (1.0) *
PSQI total score	8.5 (3.5)	3.6 (1.8) ***
Current smokers, No.	1/21	0/25
Caffeine, mg/day	175 (127)	126 (127)
Alcohol, drinks/week	2.6 (2.7)	2.2 (3.0)

*
p<0.05;

**
p<0.01;

p<0.001.

Body Mass Index, **BMI**; Pittsburgh Sleep Quality Index, **PSQI**; Greene Climacteric Scale, **GCS**.

Blood hormone levels, frequency of women having nocturnal physiological hot flashes, and self-report measures of state mood in the follicular and luteal phases of the menstrual cycle for women in the menopausal transition with and without insomnia.

Table 2

	Follicular phase Mean (SD)	Luteal Phase Mean (SD)	Follicular phase Mean (SD)	Luteal Phase Mean (SD)	Significant effects (HLM models)
<i>Hormones</i>					
FSH^{a,b} , IU	23.1 (17.1)	5.3 (3.4)	21.3 (16.6)	7.3 (5.2)	▶ Menstrual phase ^{***}
Progesterone^{a,b} , ng ml ⁻¹	0.7 (0.4)	8.2 (4.7)	0.7 (0.7)	10.2 (5.3)	▶ Menstrual phase ^{***}
Estradiol^{a,b} , pg ml ⁻¹	55.1 (34.5)	78.8 (39.3)	72.0 (58.8)	59.9 (23.4)	
<i>Physiological hot flashes</i>					
Women having 1 hot flash at night^c , No.	5/19	2/13	2/19	3/16	
<i>Mood profile</i>					
POMS total score	61.1 (21.8)	63.1 (24.8)	49.2 (12.8)	47.5 (13.6)	▶ Group ^{**}

* p<0.05;
** p<0.01;
*** p<0.001;

^a data have been log transformed before analysis;

^b blood hormones were not collected in two follicular (from one MTI and one control) and in one luteal night (from one MTI; saliva was analyzed to confirm presence of progesterone);

^c due to technical reasons skin conductance data was unavailable for a follicular night (one control) and for three luteal nights (one MTI and two controls).

Follicle-Stimulating Hormone, **FSH**; Profile of Mood States, **POMS**.