Circadian Rhythms and Sleep of Postmenopausal Women

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Abstract

Women have a high risk of developing sleep-wake disturbances after the menopausal transition. Such disturbances have been reported in 40-60% of postmenopausal women. Several factors may contribute to this increased risk, such as the high incidence of chronic medical conditions and intrinsic sleep disorders such as sleep-disordered breathing. However, the contribution of the circadian timing system to sleep-wake disturbances remains understudied and requires further investigation. This thesis aims to investigate differences in the circadian and diurnal variation of core body temperature, salivary melatonin, self-reported alertness, sleep, electroencephalographic (EEG) activity, heart rate, and heart rate variability of postmenopausal women compared to younger women in their mid-follicular phase. An ultradian sleep-wake cycle procedure (USW) was employed, consisting of alternating 1-hour wake periods and 1-hour sleep opportunities over 48 to 72 hours. The USW is a specialized procedure used in human chronobiology to record several parameters across circadian phases. Overall, our study revealed shallower and a more disrupted sleep architecture and EEG activity in postmenopausal women, along with reduced heart rate variability and parasympathetic activity of the heart during sleep of the baseline period and throughout the USW procedure. Moreover, postmenopausal women showed a dampened or absent circadian rhythm in several measures across the USW procedure: melatonin, self-reported alertness, total sleep time, sleep onset latency, stage N3 sleep, delta power, theta power, sigma power, heart rate, heart rate variability, and parasympathetic activity. Altogether, these results indicate a dampened output signal from the circadian pacemaker influencing physiological and behavioural processes in postmenopausal women. In the present thesis, we discuss the probable role of aging and declining ovarian hormones after menopause on these variables. We further comment on the paucity of circadian rhythm studies at menopause, and we highlight the potential contribution of our study to understanding the increased risk of sleep disturbances and cardiovascular events in this population. The work presented in this thesis is expected to direct future research to improve treatment options for postmenopausal women experiencing sleep-related disturbances and related conditions.

Résumé

Les femmes ont un risque élevé de développer des perturbations du sommeil après la transition vers la ménopause, avec une prévalence rapportée de 40 à 60% chez les femmes ménopausées. Plusieurs facteurs peuvent contribuer à ce risque accru, tels que l'incidence élevée de maladies chroniques et de troubles intrinsèques du sommeil tels que les perturbations de la respiration nocturne. Cependant, la contribution du système de synchronisation circadien aux troubles du sommeil et de l'éveil reste peu étudiée et nécessite des recherches plus approfondies. Cette thèse vise à étudier les différences entre les variations circadiennes et diurnes de la température corporelle centrale, de la mélatonine salivaire, de la vigilance auto-déclarée, du sommeil, de l'activité électroencéphalographique (EEG), de la fréquence cardiaque et de la variabilité de la fréquence cardiaque chez les femmes ménopausées par rapport aux femmes plus jeunes en phase mi-folliculaire. Une procédure de cycle veille-sommeil ultradien (USW) a été utilisée, consistant à alterner des périodes d'éveil d'une heure et des périodes de sommeil d'une heure sur une période de 48 à 72 heures. L'USW est une procédure spécialisée utilisée en chronobiologie humaine pour enregistrer plusieurs paramètres au cours des phases circadiennes. Dans l'ensemble, notre étude a révélé une architecture du sommeil et une activité EEG moins profondes et plus perturbées chez les femmes ménopausées, ainsi qu'une réduction de la variabilité de la fréquence cardiaque et de l'activité parasympathique du cœur pendant le sommeil de la période de référence et tout au long de la procédure USW. En outre, les femmes ménopausées ont montré un rythme circadien atténué ou absent dans plusieurs mesures à travers la procédure USW: mélatonine, vigilance auto-déclarée, temps de sommeil total, latence d'endormissement, stade N3 du sommeil, puissance delta, puissance thêta, puissance sigma, fréquence cardiaque, variabilité de la fréquence cardiaque, et activité parasympathique. Dans l'ensemble, ces résultats indiquent un signal de sortie atténué du pacemaker circadien influençant les processus physiologiques et comportementaux chez les femmes ménopausées. Dans la présente thèse, nous discutons du rôle probable du vieillissement et du déclin des hormones ovariennes après la ménopause sur ces variables. Nous commentons également la rareté des études sur le rythme circadien à la ménopause, et

nous soulignons la contribution potentielle de notre étude à la compréhension du risque accru de troubles du sommeil et d'événements cardiovasculaires parmi cette population. Le travail présenté dans cette thèse devrait orienter les recherches futures afin d'améliorer les options de traitement pour les femmes ménopausées souffrant de troubles du sommeil et d'affections connexes.

List of Abbreviations

AHI	Apnea-Hypopnea Index
aMT6	6-sulfatoxymelatonin
ANS	Autonomic nervous system
BMI	Body Mass Index
CBT	Core body temperature
CVD	Cardiovascular disease
DMH	Dorsomedial hypothalamus
DRN	Dorsal raphe nucleus
dSPZ	Dorsal subparaventricular zone
EEG	Electroencephalogram
FMP	Final menstrual period
FSH	Follicle stimulating hormone
GnRH	Gonadotropin-releasing hormone
HF	High-frequency band
HR	Heart rate
HRT	Hormone replacement therapy
HRV	Heart rate variability
HSFA	High spindle frequency activity
LC	Locus coeruleus
LF	Low-frequency band
LH	Luteinizing hormone
LHA	Lateral hypothalamic area
LSFA	Low spindle frequency activity
MnPO	Median preoptic nucleus
MPO	Medial preoptic nucleus
Namb	Nucleus ambiguous
NREM	Non-rapid eye movement

NTS	Nucleus of the solitary tract
PLMS	Periodic leg movements during sleep
PMW	Postmenopausal women
POA	Preoptic area
PSG	Polysomnography
PVN	Paraventricular nucleus
REM	Rapid eye movement
ROL	REM sleep onset latency
RMSSD	Root mean square of successive differences in RR intervals
RPA	Raphe pallidus
RVLM	Rostral ventrolateral medulla
SCN	Suprachiasmatic nucleus
SE	Sleep efficiency
SDNN	Standard deviation of RR intervals
SFA	Spindle frequency activity
SOL	Sleep onset latency
SPZ	Subparaventricular zone
STRAW	Stages of Reproductive Aging Workshop
SWAN	Study of Women Across the Nation
SWA	Slow wave activity
SWS	Slow wave sleep
TMN	Tuberomammillary nucleus
TST	Total sleep time
USW	Ultradian sleep-wake cycle
VLPO	Ventral lateral preoptic area
VMS	Vasomotor symptoms
WASO	Wake after sleep onset
vPAG	Ventral periaqueductal gray
vSPZ	Ventral subparaventricular zone
YW	Young women

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Contribution of Authors

Chapter 2:

Rafael Perez-Medina-Carballo, Anastasi Kosmadopoulos, Philippe Boudreau, Manon Robert, Claire-Dominique Walker, and Diane B. Boivin. **The circadian variation of sleep and alertness of postmenopausal women.** Sleep (2023); 46(2), zsac272.

Experiments in time isolation were conducted at the Centre for Study and Treatment of Circadian Rhythms at the Douglas Mental Health University Institute under the supervision of Diane B. Boivin, MD, PhD. Diane B. Boivin, MD, PhD participated in the study design, supervised data collection and analysis, and manuscript preparation. Anastasi Kosmadopoulos, PhD, contributed to the study design, experimental data collection, data analysis, and manuscript preparation. Philippe Boudreau, PhD, participated in data analysis, visualization, and manuscript preparation. Manon Robert, MSc, supervised and conducted sleep scoring, and participated in data analysis and visualization. Assays of salivary melatonin concentration were performed in the laboratory of Claire-Dominique Walker, PhD, who also contributed to the manuscript preparation. I acted as project leader for the study, contributed to experimental design and data collection, conducted all the data analyses, and prepared the manuscript.

Chapter 3:

Rafael Perez-Medina-Carballo, Anastasi Kosmadopoulos, Philippe Boudreau, Christophe Moderie, Manon Robert, and Diane B. Boivin. **Dampened circadian amplitude of EEG power in women after menopause.** Journal of Sleep Research (2024): e14219.

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participated in data analysis, visualization, and manuscript preparation. Christophe Moderie, MD, MSc, participated in data preprocessing, analysis, and manuscript preparation. Manon Robert, MSc, supervised and conducted sleep scoring, and participated in data analysis and visualization. I acted as project leader for the study, contributed to experimental design and data collection, conducted all the data analyses, and prepared the manuscript.

Chapter 4:

Rafael Perez-Medina-Carballo, Anastasi Kosmadopoulos, Philippe Boudreau, Linda Ma, Manon Robert, and Diane B. Boivin. **Circadian modulation of heart rate variability in postmenopausal women: implications for cardiovascular health.** Submitted for publication.

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Contribution to Original Knowledge

Chapter 2:

Rafael Perez-Medina-Carballo, Anastasi Kosmadopoulos, Philippe Boudreau, Manon Robert, Claire-Dominique Walker, and Diane B. Boivin. **The circadian variation of sleep and alertness of postmenopausal women.** Sleep (2023); 46(2), zsac272.

This study investigated the circadian rhythms of core body temperature (CBT) and melatonin, as well as circadian variations of sleep and alertness in postmenopausal women. A highly controlled laboratory procedure called the USW procedure was used for the study. The results showed that postmenopausal women had dampened circadian variations in several parameters, including melatonin, alertness, and sleep compared to young women.

Although some groups have previously described changes in circadian rhythms after menopause, the circadian variation of sleep and alertness in postmenopausal women had not yet been studied. The present study is innovative as it is the first to demonstrate a significant decrease in the circadian variation of sleep and self-reported alertness in this population. This finding contributes significantly to our knowledge of the effect of aging on women's sleep and their increased susceptibility to insomnia after menopause. Moreover, the findings of this study support the hypothesis that aging weakens the circadian signal, which affects sleep and wakefulness. This hypothesis is further described in Chapter 5.4.

Chapter 3:

Rafael Perez-Medina-Carballo, Anastasi Kosmadopoulos, Philippe Boudreau, Christophe Moderie, Manon Robert, and Diane B. Boivin. **Dampened circadian amplitude of EEG power in women after menopause.** Journal of Sleep Research (2024): e14219.

This study aimed to explore the changes in EEG activity during sleep after menopause and its modulation by the circadian timing system. Our results demonstrated a clear disruption of the circadian pattern of EEG activity during sleep in postmenopausal

women, specifically in delta, theta, and sigma power. Additionally, we observed that postmenopausal women have disturbed nocturnal sleep, which is characterized by increased power within the alpha band, and decreased power within the delta and sigma bands. Despite being good sleepers, these changes in EEG activity are similar to those seen in individuals with insomnia, indicating an increased risk for sleep disturbances. Our results complement the findings from Chapter 2 regarding the sleep architecture and provide further insight into the impact of the circadian timing system on the regulation of sleep in postmenopausal women.

Chapter 4:

Rafael Perez-Medina-Carballo, Anastasi Kosmadopoulos, Philippe Boudreau, Linda Ma, Manon Robert, and Diane B. Boivin. **Circadian modulation of heart rate variability in postmenopausal women: implications for cardiovascular health**. Submitted for publication.

The purpose of this study was to investigate the circadian variations in heart rate (HR) and heart rate variability (HRV) in healthy postmenopausal women. This is of particular significance since the circadian timing system also regulates the cardiovascular system. During the naps of the USW procedures, we observed a circadian pattern in HR, HRV, and parasympathetic activity. Postmenopausal women exhibited a decrease in HRV and parasympathetic activity, as well as dampened circadian variations.

Previous research has explored HRV in menopause during wakefulness, while our study specifically focused on measuring HRV during sleep under highly controlled laboratory conditions. This allowed us to obtain reliable circadian patterns of HR, HRV, and parasympathetic activity. Our findings align with those presented in Chapters 2 and 3 and are particularly noteworthy as they indicate an elevated risk of cardiovascular disease, an increased susceptibility to insomnia, and a disruption of the circadian timing system in postmenopausal women.

Preface

In the current thesis, chapters 2, 3, and 4 consist of complete manuscripts that have been published (Chapters 2 and 3) or submitted to peer-reviewed journals for publication (Chapter 4). The bibliography for each manuscript is included at the end of the respective chapter. The individual prefaces for each chapter serve as a connecting text, providing a logical progression through the manuscripts and the experimental aims and findings. The complete reference list for the Introduction (Chapter 1), chapter prefaces (Chapters 2.1, 3.1, and 4.1), and the Discussion (Chapter 5) can be found in the Non-manuscript References section at the end of the thesis.

Chapter 1. Introduction

1.1 General introduction

Menopause marks the end of the reproductive period in women as a result of the cessation of ovarian function. This leads to hormonal fluctuations that may affect various physiological functions in the body. Sleep disturbances affect a significant percentage of women after menopause, ranging from 40-60% (Salari et al., 2023; Shaver & Woods, 2015; Woods & Mitchell, 2010). These sleep disturbances may be caused by different factors, such as mental health disorders, sleep-disordered breathing, and hot flashes. Furthermore, changes in the circadian timing system may also impact sleep in postmenopausal women, which may further exacerbate sleep disturbances. Therefore, the studies conducted in this thesis aim to highlight the changes in the circadian timing system in women after menopause that could potentially impact their sleep.

1.2 The sleep-wake cycle

Sleep is a fundamental aspect of human physiology and is defined as a reversible and quiescent behavioural stage characterized by disengagement from the environment. Sleep can be divided into rapid eye movement (REM) sleep stage and non-rapid eye movement sleep stage (NREM). During NREM sleep, sleep progresses from stage N1 to N3, which is considered the deep sleep stage. REM sleep, on the other hand, is characterized by rapid eye movements and increased brain activity compared to NREM sleep, similar to that during wakefulness. The complex sleep architecture is controlled by

a network of various brain regions, including the suprachiasmatic nucleus, which acts as the master pacemaker coordinating the sleep-wake cycle. Age and sex differences have been described in human sleep, including changes in sleep duration, efficiency, and overall sleep architecture. This Chapter aims to cover these various aspects of the sleep-wake cycle.

1.2.1 Sleep architecture and EEG activity during sleep

Polysomnography (PSG) is a method for recording and classifying sleep stages. This method involves various components, such as an EEG to measure brain activity, a chin electromyogram (EMG) to measure muscle tone, and an electrooculogram (EOG) to measure eye movements. Additionally, an electrocardiogram (EKG), respiratory parameters, and a leg electromyogram are recorded to diagnose sleep disorders (Rowley & Badr, 2022).

The American Academy of Sleep Medicine has classified sleep into two main categories: NREM and REM sleep. NREM sleep is divided into three stages: N1, N2, and N3. The N1 stage represents shallow sleep and transitioning from awake to falling asleep. K-complexes and sleep spindles characterize the N2 stage and represent most of the time spent asleep. The N3 stage, also called slow wave sleep (SWS), is characterized by slow wave oscillations and is considered deep sleep. On the other hand, the REM sleep stage is characterized by rapid eye movements, mixed-frequency low-amplitude EEG waves, and loss of muscle tone. The average sleep cycle consists of alternating NREM and REM sleep periods, with the average cycle lasting about 90 minutes. At the beginning of the sleep period, there is a higher occurrence of deep sleep, while more REM sleep appears towards the end. This polysomnographically-scored sleep is considered the macrostructure of sleep (Berry et al., 2020; Rowley & Badr, 2022).

On the other hand, the sleep microstructure reflects oscillatory EEG activity in the brain. This is called the EEG power spectra and reflects the power distribution across different frequency bands, namely delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (12-16 Hz) beta (16-30 Hz), and gamma (>30 Hz) (Zhang, 2019). Each frequency band is associated with different states of consciousness and cognitive processes. Delta activity in the brain is characteristic of deep sleep and is more commonly recorded in the

frontal lobe. Theta activity has been associated with memory processes and in response to inhibition tasks. Sigma activity is linked to sleep spindle activity, which protects sleep against external stimuli. Alpha and beta activity are associated with active wakefulness, attention, and cognitive processing. Gamma activity is present during wakefulness only and is related to information processing and memory (Fernandez & Luthi, 2020; Herrmann et al., 2016).

1.2.2 Anatomical Regions Regulating the Sleep-Wake Cycle

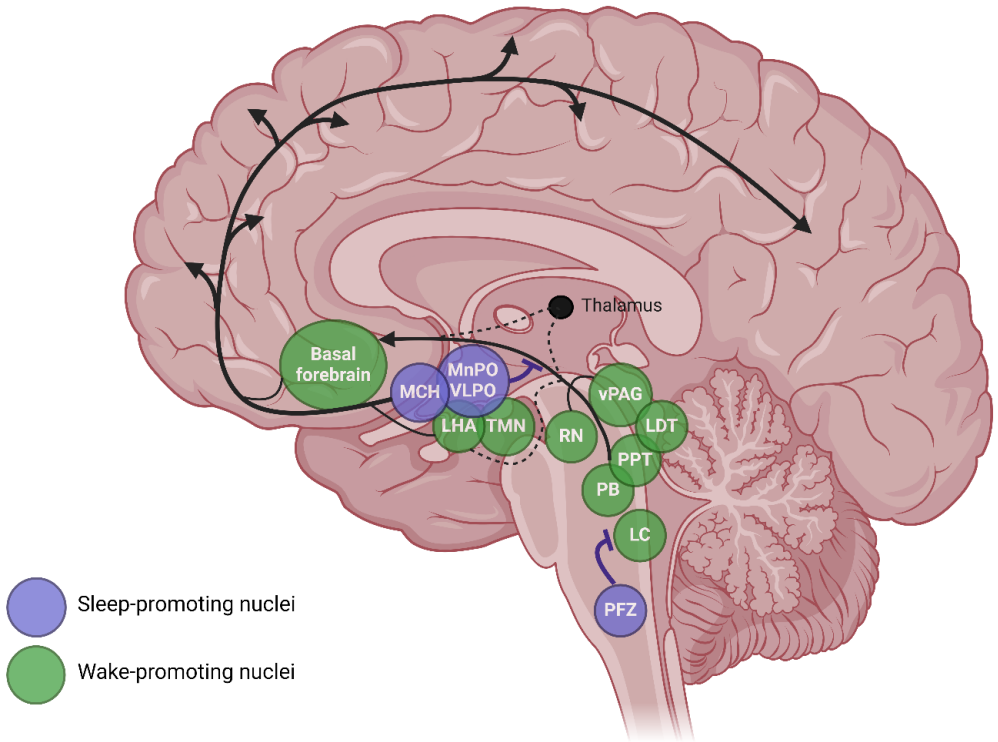

Figure 1. 1. Regulation of the sleep-wake cycle.

LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone neurons; MnPO, median preoptic area; MPO, medial preoptic area; PB, parabrachial nucleus; PFZ, parafacial zone; PPT, pedunculopontine tegmental nucleus; RN, raphe nucleus; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic area; vPAG, ventral periaqueductal gray. Created with BioRender.com.

The sleep-wake cycle regulation is controlled by sleep-promoting and wake-promoting nuclei in the brain, as illustrated in Figure 1.1. The nuclei that promote sleep are located in the preoptic area (POA) of the hypothalamus, specifically the median preoptic area (MnPO) and the ventrolateral preoptic area (VLPO). Activation of these nuclei induces deep sleep. In addition, the parafacial zone (PFZ) is another essential nucleus that provides inhibitory input to a wake-promoting nucleus, the parabrachial nucleus (PB). The population of melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus is also considered to promote sleep. The MCH neuronal population is more specifically a REM-promoting area since it fires maximally during REM sleep but not during wakefulness (Anaclet & Fuller, 2017; Deboer, 2020; Saper & Fuller, 2017).

On the other hand, wakefulness is achieved due to the activity of a different set of brain nuclei, mainly the PB, the pedunculopontine tegmental nucleus (PPT), and the basal forebrain. These three regions contain glutamatergic and cholinergic neurons capable of activating neurons in the cortex, resulting in a vigilance state. PB and PPT neurons reach the cortex through different pathways. First, PB and PPT reach the BF through glutamatergic neurons, ultimately causing neuronal activation of the cortex. Second, through cholinergic projection to the thalamus that is followed by glutamatergic diffused excitation in the cortex. Alternatively, after reaching the thalamus, a secondary pathway through cholinergic neurons to the orexin neurons of the lateral hypothalamic area (LHA) leads to glutamatergic activation of the cortex. Damage to these three regions leads to behavioural unresponsiveness with increased SWS (Deboer, 2020; Jones, 2017; Saper & Fuller, 2017). The ventral periaqueductal gray (vPAG), the ascending reticular arousal system, and the orexin neurons of the LHA are secondary nuclei that contribute to the regulation of wakefulness. The ascending reticular arousal system includes four brain nuclei: the histaminergic neurons of the tuberomammillary nucleus (TMN), noradrenergic neurons of the locus coeruleus (LC), serotoninergic neurons of the raphe nucleus (RN), and cholinergic neurons of the laterodorsal tegmental nucleus (LDT) (Saper & Fuller, 2017).

The flip-flop switch is a remarkable characteristic of the sleep- and wake-promoting nuclei. These nuclei are in an antagonistic relationship, whereby the activation of one

results in the suppression of the other. For instance, during the wake-to-sleep transition, the basal forebrain, the LC, and TMN firing rates are elevated. Once the sleep-promoting nuclei of the POA increase their neuronal fire rate, the firing rate in the LC, TMN, and basal forebrain declines until it is null a few seconds after activation of POA neurons. The NREM-REM sleep transition functions in a similar fashion. The apparent origin of REM sleep in humans is the subcoeruleus region. Activating the subcoeruleus region (REM-on neurons) mainly inhibits the ventrolateral periaqueductal gray and the lateral pontine tegmentum (REM-off neurons). The subcoeruleus region simultaneously activates other REM-on and sleep-on neurons and inhibits wake-on neurons, while the orexin-expressing neurons mediate this network. As a result, the flip-flop switch circuitry of the wake-sleep regulatory system produces the typical sleep pattern in healthy adults, with consolidated waking during the day and alternation between NREM and REM sleep at night (Deboer, 2018; Deboer, 2020; Saper et al., 2010).

1.2.3 Age and sex differences in sleep

Human sleep exhibits evident variations across age and between sexes. In the context of aging adults, sleep organization can be influenced by various factors such as chronic diseases, psychiatric conditions, a higher incidence of sleep disorders like sleep-disordered breathing, and the use of medications. Commonly reported sleep changes in aged adults include decreased total sleep time (TST) and increased awakenings (Klerman et al., 2004). Indeed, age-related changes in sleep have been commonly reported in the literature. A meta-analysis exploring age-related changes in sleep parameters revealed a decline in SWS, REM sleep, TST, and sleep efficiency (SE), with these changes becoming more pronounced after the age of 60 (Ohayon et al., 2004). Additionally, older individuals exhibit differences in the EEG power spectral, particularly reduced slow wave activity (SWA) and sleep spindles during NREM sleep (Carrier et al., 2001; Dijk, Beersma, & van den Hoofdakker, 1989).

Moreover, human sleep present variations with sex (Dib et al., 2021). Overall, women self-report poorer sleep quality, more frequent arousals, and difficulties in maintaining sleep compared to men (Mong et al., 2011). PSG recordings, paradoxically, indicate that women exhibit more total sleep time, higher SE, increased SWS, and sleep

spindles measured by sigma power, along with less wakefulness and N1 sleep than men (Carrier et al., 2001; Carrier et al., 2017; Dijk, Beersma, & Bloem, 1989). Furthermore, for a similar sleep schedule, women present an earlier circadian rhythm of melatonin and CBT than men, thereby setting the timing of their sleep period at a later circadian phase (Boivin et al., 2016; Cain et al., 2010; Duffy et al., 2011). Over women's lifespan, they consistently present a higher prevalence of insomnia disorder compared to men, with a major increase at menopause (Mong & Cusmano, 2016). Significant evidence supports the effect of ovarian hormones on the sleep of women, which will be discussed in detail in Chapter 5, Discussion.

1.2.4 Sleep and the cardiovascular system

The autonomic nervous system (ANS), which is regulated by the central nervous system, controls various physiological functions such as HR, blood pressure, breathing, digestion, metabolism in the liver, and saliva secretion, among others. The ANS is further categorized into two branches: the sympathetic and parasympathetic nervous systems. Generally, the parasympathetic system is responsible for the "rest" functions, while the sympathetic system responds to stressful situations (Chokroverty & Bhat, 2021).

Various brain regions play a central role in regulating the ANS of the cardiovascular system. Among these regions, the caudal portion of the nucleus of the solitary tract (NTS) receives afferent fibres from carotid baroreceptors and chemoreceptors, as well as vagal afferents from the heart and lungs. Information from cardiovascular afferent fibres is then integrated into two other regions: the nucleus ambiguous (NAmb) and the rostral ventrolateral medulla (RVLM). The NAmb and RVLM work together to control autonomic reflexes that help regulate blood pressure and HR. This network also controls autonomic reflexes to maintain adequate blood oxygen perfusion (Shouman & Benarroch, 2021).

Sleep and the ANS are closely connected and primarily regulated by the circadian system. The PB and the NTS nuclei, situated in the brainstem, have been hypothesized to establish a bidirectional connection between the ANS and the sleep-wake cycle (Silvani et al., 2015). This is due to their role in regulating cardiovascular function and their projections to various wake-promoting brain areas. When the baroreflex is triggered in animal studies, the NTS nucleus stimulation has been found to promote EEG

synchronization. Conversely, cardiovascular changes occur as a response to arousals during sleep (Silvani et al., 2015).

ANS activity fluctuations are evident during sleep, reflecting the dynamic cardiovascular adjustments across different sleep stages. As such, transitioning from wakefulness to NREM sleep involves a shift from sympathetic to parasympathetic activity (de Zambotti et al., 2018). In NREM sleep, there is a pronounced decrease in HR, blood pressure, sympathetic nervous system activity, and increased parasympathetic activity. These changes are particularly marked during deep sleep (Silvani, 2019; Tobaldini et al., 2013). Conversely, REM sleep is characterized by increased HR and blood pressure, similar to patterns observed during wakefulness. These changes are particularly marked during phasic REM sleep when sympathetic activity is elevated (Tobaldini et al., 2013). Notably, ANS activity changes are observable during both nocturnal sleep and daytime napping, suggesting a significant influence of the sleep period on the ANS activity fluctuations (Whitehurst et al., 2018).

HRV analysis, derived from EKG recordings, serves as a valuable tool for studying cardiovascular changes during sleep (Laborde et al., 2017). Various measurements can be obtained through the time-domain and frequency-domain analysis of HRV. The time-domain analysis calculates various measures in the EKG within a specific period. These measures include peak-to-peak RR intervals of the EKG, HR, the standard deviation of RR intervals (SDNN), and the root mean square of successive differences in RR intervals (RMSSD). The frequency-domain analysis filters different frequency bands obtained from the time domain, using a Fast Fourier Transform. The frequency-domain analysis yields the low-frequency (LF) band between 0.04 and 0.15 Hz and the high-frequency (HF) band between 0.15 and 0.4 Hz (Laborde et al., 2017). SDNN reflects overall HR variability and is associated with cardiovascular health. RMSSD and HF represent parasympathetic activity, specifically respiratory sinus arrhythmia. The interpretation of LF, previously considered a measure of sympathetic activity, is controversial. LF reflects a combination of parasympathetic activity, sympathetic activity, and arterial baroreceptor reflexes, making the interpretation of LF unclear and inaccurate (Laborde et al., 2017).

1.2.5 The two-process model

The two-process model of sleep regulation was first described in a publication by Borbely (1982), and it was based on sleep observations in rats. This model proposes that human sleep regulation is a result of the interplay between two processes: a sleep-wake-dependent process (Process S) and a circadian process (Process C). The model simulates the timing and intensity of sleep in various human experimental protocols (Borbely, 2022).

The Process S, which is referred to as the sleep homeostat, is based on the SWA oscillations of the EEG. The rise in sleep pressure during the wake period explains the declining SWA of the subsequent sleep period. During sleep, SWA declines exponentially, and this decline is steeper after sleep deprivation. On the contrary, process C is controlled by the endogenous circadian system and is illustrated by the vigilance rhythm throughout the day and night during a prolonged sleep deprivation protocol (Borbely et al., 2016). The model also established that the interaction of processes S and C determines sleep and wake propensity. For instance, when sleep pressure is high at night, the circadian rhythm of vigilance is dampened (Borbely et al., 2016). Other observations have been described and complemented the model, such as the regional dynamics of SWA. This means that SWA differs between cortical areas, with a more substantial EEG power and declining response after sleep deprivation in the frontal compared to the occipital cortex (Guillaumin et al., 2018).

The basis of the two-process model has been confirmed by various experiments. For instance, Dijk and Czeisler (1995) showed that various sleep parameters (latency to sleep, SE, and REM sleep) display a robust circadian variation. This model is still widely used for understanding the interaction of sleep and the circadian timing system. Current research aims to unveil the synaptic mechanisms by which the homeostat functions (Borbely, 2022).

1.3 Circadian rhythms

The word circadian originates from the Latin roots “*circa*” (around) and “*diem*” (day). Circadian rhythms are endogenous biological oscillations that follow an approximately 24-hour cycle and are present in most living organisms. Circadian rhythms

are genetically encoded throughout the body and organized in a hierarchical structure of central and peripheral oscillators. These oscillators are orchestrated by the suprachiasmatic nucleus (SCN) of the anterior hypothalamus and synchronized by external cues called *zeitgebers*, with environmental light being the primary factor that aligns them with the earth's rotation. Exposure to *zeitgebers* can shift the timing of the circadian clock, either advancing or delaying it (Patke et al., 2020; Yao & Silver, 2022).

1.3.1 The SCN and the molecular clock

The SCN serves as the central pacemaker in the mammalian circadian system, playing a fundamental role in orchestrating the timing of biological processes. The SCN comprises two regions, the core and the shell, each with separate neurotransmitter expressions from their neuronal population. The core contains neurons that express vasoactive intestinal polypeptide, while the shell contains neurons expressing arginine vasopressin that provide high-amplitude oscillations (Takahashi, 2017).

To synchronize the circadian clock to the external environment, light is sensed in the mammalian retina by intrinsically photosensitive retinal ganglion cells, with the signal conveyed to the core of the SCN through the retinohypothalamic tract. The SCN neurons in the core and the shell display a robust, autonomous, and stable circadian rhythm in their membrane potential and spontaneous firing rate. The SCN integrates the input from the core and shell regions and conveys information to peripheral clocks in organs such as the liver, kidneys, muscles, and ovaries through neurotransmitters and neuromodulators. It is believed that the central nervous system, hormones, and body temperature synchronize the central and peripheral clocks (Patke et al., 2020; Yao & Silver, 2022).

Within the SCN, a complex molecular clock mechanism operates, involving a set of genes known as clock genes (Clock, Bmal1, Per, and Cry) and their corresponding proteins. This molecular clockwork regulates the transcription and translation of these genes in a rhythmic manner, creating transcription-translation feedback loops. Although the rhythms of the SCN are synchronized by environmental cues such as light, the functioning of this molecular clock is autonomous and continues in the absence of external entraining cues (Patke et al., 2020; Takahashi, 2017).

The functioning of the molecular clock involves the dimer CLOCK/BMAL1 in the neuron nucleus and translocation of this dimer to the E-box of the promoter regions of clock genes Per1, Per2, Cry1, Cry2, and Cry3. PER and CRY proteins are synthesized in the cytosol, which suppresses the translational activity of dimer CLOCK/BMAL1. A secondary transcriptional loop regulates CLOCK/BMAL1 dimer expression, composed of Rev-Erb α and β, and Ror α and β. Both proteins bind to the retinoic acid-related orphan receptor response element and exert different functions. REV-ERB protein suppresses BMAL1 transcription, while ROR protein activates it. The activity of these transcriptional loops promotes other clock-controlled genes and their protein products, which form the output of the SCN (Patke et al., 2020; Yao & Silver, 2022).

The regulation of the molecular clock is attained through the coordinated expression and degradation of clock gene products, which contribute to the 24-hour oscillations that characterize circadian rhythms. The phosphorylation and stability of PER and CRY proteins determine the length of the circadian period, while REV-ERBα degradation modulates its length and amplitude (Patke et al., 2020; Takahashi, 2017).

1.3.2 Outputs of the SCN

The SCN is responsible for regulating the circadian rhythms through the output to different anatomical regions (Figure 1.2). The subparaventricular zone (SPZ) receives the majority of the SCN output and is divided into two parts: the dorsal and the ventral regions (dSPZ and vSPZ, respectively) (Saper, Scammell, et al., 2005).

The dSPZ connects the SCN to the MnPO and median preoptic area (MPO), which are responsible for regulating CBT. On the other hand, the vSPZ integrates the SCN output into the dorsomedial hypothalamus (DMH). The DMH sends efferent fibres to various hypothalamic regions that control the circadian component of different body functions (Saper & Machado, 2020; Saper, Scammell, et al., 2005). The connection between the DMH and the paraventricular nucleus (PVN) relays information toward the superior cervical ganglion, which then reaches the pineal gland for melatonin secretion (Borjigin et al., 2012). The connection between the DMH and PVN also regulates the circadian rhythm of corticosteroid secretion. Moreover, the connection between the DMH and the VLPO and the LHA regulates the sleep-wake cycle, wakefulness, and feeding

behaviour (Saper & Fuller, 2017). Through the PVN, the connection between the SCN and the NTS contributes to the circadian regulation of heart rate and autonomic activity (Aryan et al., 2020).

The complex pathways between these regions are crucial for maintaining healthy circadian rhythms. Dysregulation of these pathways may contribute to a range of health issues associated with circadian disturbances, which are further described in this thesis.

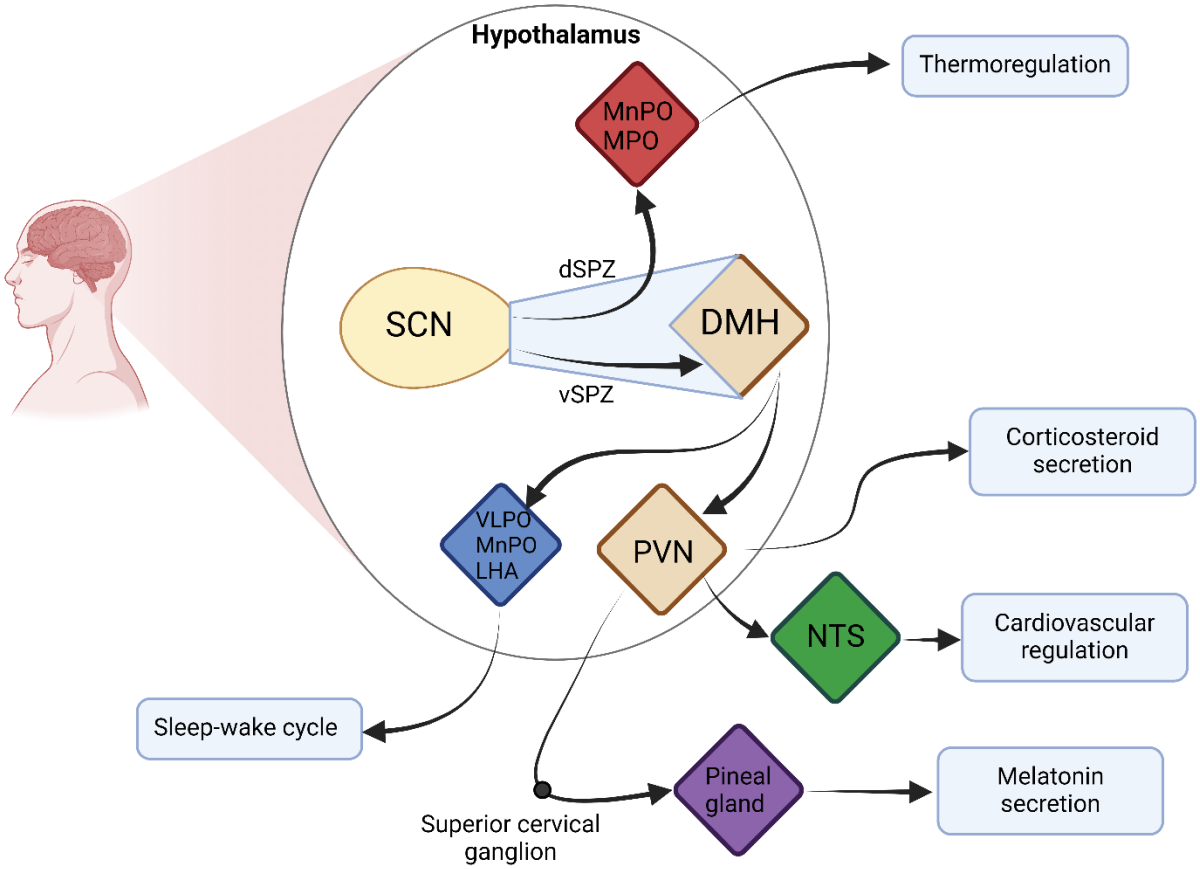

Figure 1. 2. Circadian rhythm regulation by the suprachiasmatic nucleus (SCN).
DMH, dorsomedial hypothalamus; LHA, lateral hypothalamic area; MnPO, median preoptic area; MPO, medial preoptic area; PVN, paraventricular nucleus; SPZ, subparaventricular zone and its dorsal (d) and ventral (v) regions; VLPO, ventrolateral preoptic area. Created with BioRender.com.

1.3.2.1 Core Body Temperature (CBT)

The POA of the hypothalamus integrates thermoregulatory information, particularly the MnPO and the MPO. Approximately 21% of POA neurons are thermosensitive and can be further divided into warm-sensitive and cold-sensitive neurons (Szymusiak et al., 2007). When an external thermal stimulus is applied to the skin, this activates warm-sensitive neurons through a synaptic pathway that connects the thermoreceptors present on the skin to the spinal cord. The signal is then conveyed to the lateral PB, which connects to the MnPO and the MPO. Warm-sensitive neurons can promote heat dissipation by inducing vasodilation, which can be achieved by inhibiting the RVLM and raphe pallidus (RPA). Conversely, cold-sensitive neurons can promote heat retention by inducing vasoconstriction (Nakamura & Morrison, 2008, 2010). Other neuronal populations within the hypothalamus also contribute to body temperature modulation, including the ventromedial hypothalamus (VMH) and the DMH involved in brown adipose tissue thermogenesis, and the arcuate nucleus (ARC) involved in cutaneous vasoconstriction (Zhang et al., 2021).

In humans, CBT exhibits a circadian rhythm under highly controlled protocols used in chronobiology, including the “constant routine”, “forced desynchrony”, and “USW procedure”. These protocols are performed in dim light conditions and minimize the environmental and behavioural factors that can mask the expression of an endogenous rhythm (i.e., obscure its underlying rhythm) (Klerman et al., 2022). In these laboratory-controlled protocols, CBT reaches its nadir during the biological night and its peak during the biological day (Krauchi & Wirz-Justice, 1994). Since the SCN activity in the brain cannot be measured directly in humans, CBT is considered a reliable marker of the circadian timing system when collected in these conditions. This rhythm is generated by the interaction between the SCN and thermoregulatory centers in the hypothalamus, including the MnPO and MPO (Saper, Scammell, et al., 2005). The peak of CBT is given by heat production due to daytime metabolic activity and heat retention through peripheral vasoconstriction, whereas the nocturnal nadir is provided by heat dissipation through peripheral vasodilation (Refinetti, 2020).

Body temperature recordings can be safely measured in different body sites such as the mouth, axilla, and rectum. As such, CBT recording is frequently used in chronobiology research (Klerman et al., 2022). However, under non-laboratory conditions, CBT is not a reliable marker as several factors can mask its expression, including body posture, physical activity, food intake, and the sleep-wake cycle (Klerman et al., 1999).

1.3.2.2 Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone synthesized and secreted by the pineal gland. Melatonin secretion follows a robust circadian rhythm regulated by the master circadian pacemaker. The SCN indirectly connects to the pineal gland through the PVN and the superior cervical ganglion. During the day, the SCN inhibits the PVN by sending GABA projections and ultimately inhibiting activity in the pineal gland, whereas at night, the SCN activates the PVN through glutamate projections. At night, the sympathetic projection of the superior cervical ganglion releases norepinephrine into β and α1 adrenergic receptors of pinealocytes, which stimulates melatonin synthesis from tryptophan via serotonin (Borjigin et al., 2012; Cipolla-Neto et al., 2022; Pevet & Challet, 2011). Melatonin is exclusively secreted at night and plays a crucial role in synchronizing the organism to the day-night cycle through melatonin receptors in the SCN. Melatonin also promotes sleep through its interaction with the reticular thalamic nucleus and through peripheral vasodilation in the evening (Ng et al., 2017).

Due to its robust and stable rhythm, melatonin is considered a reliable marker of the circadian system (Kalsbeek et al., 2006). Particularly, the dim light melatonin onset is often used to mark the beginning of the biological night and is closely linked to other physiological processes, such as sleepiness and the decline of the evening core body temperature (Klerman et al., 2022). Melatonin secretion can be measured in saliva, urine, and blood, though it is highly inhibited by light exposure. Thus, light exposure must be controlled when assessing melatonin secretion and, ideally, it would be measured in controlled laboratory conditions. However, ambulatory measures at home have been validated and are becoming more frequently used. Monitoring melatonin secretion

provides valuable insights into the functioning of the circadian system, providing useful information in the diagnosis and treatment of sleep and circadian rhythm disorders (Burgess et al., 2015).

1.3.2.3 The Sleep-Wake Cycle

The timing and duration of sleep are regulated by the two processes: a homeostatic process (process S) and a circadian process (process C), as described in Chapter 1.2.5 (Borbely, 2022; Jones, 2020). The circadian variation of sleep is regulated by the contribution of the SCN output to specific hypothalamic nuclei. However, the direct output from the SCN to hypothalamic regions regulating sleep (VLPO) and alertness (LHA) is limited. Instead, most projections from the SCN travel through the SPZ and the DMH to reach the VLPO and LHA (Saper & Fuller, 2017; Saper, Scammell, et al., 2005). The DMH regulates the flip-flop switch via neuron projections to the LHA and VLPO. The DMH stimulates the activity of orexin neurons through glutamate and thyrotropin-releasing hormone in the LHA, promoting wakefulness. The DMH also inhibits the activity of the VLPO by releasing the neurotransmitter GABA, thereby promoting wakefulness (Chou et al., 2003; Deboer, 2020).

These interactions between the SCN, DMH, VLPO, and LHA synchronize the sleep-wake cycle with the 24-hour day-night cycle. During the biological day, the SCN promotes wakefulness by inhibiting VLPO and stimulating LHA activity. Conversely, during the biological night, the SCN reduces its output to these brain regions, allowing for increased sleep-promoting activity in the VLPO and decreased arousal in the LHA (Saper, Lu, et al., 2005; Saper, Scammell, et al., 2005). The circadian variation of sleep is further synchronized by melatonin secretion, as it provides the timing of the biological night and permits sleep initiation (Cipolla-Neto et al., 2022).

1.3.2.4 The Cardiovascular System

In terms of the circadian regulation of the cardiovascular system, the SCN sends projections to the PVN for autonomic control (Scheer et al., 2003). The PVN connects to the NTS and might be involved in regulating HR and cardiovascular reactivity (such as blood vessel plasticity) (Spary et al., 2009). The SCN, in turn, receives glutamatergic

projections from the NTS to complete the loop and modulate the cardiovascular response (Buijs et al., 2014).

HR follows a circadian pattern, peaking during the afternoon and reaching a trough at night. The circadian variation of HR and parasympathetic activity have been observed in various studies in laboratory conditions, such as constant routine and forced desynchrony protocols (Boudreau et al., 2013; Shea et al., 2011), but the circadian variation of sympathetic activity is less clear (de Zambotti et al., 2018). On the other hand, blood pressure follow a diurnal variation with a peak in the evening and a trough at night but has not been reproduced in various studies, possibly since BP is highly affected by the sleep period (de Zambotti et al., 2018). Importantly, the precise mechanism by which the SCN may modulate the autonomic components of the cardiovascular system is still unclear and is currently under investigation.

1.4 Menopause

Menopause is defined as the end of the reproductive stage in women due to the cessation of ovarian function with a subsequent decline in estrogen and progesterone production. These hormonal fluctuations trigger various systemic changes. Fluctuations in hormone levels can lead to hot flashes, night sweats, and mood swings. Sleep disturbances are common, often characterized by difficulties falling and staying asleep. Vaginal dryness and atrophy can occur, contributing to discomfort and potential disruptions in sexual health. Metabolic changes can influence body composition and increase the likelihood of increased body fat and decreased muscle mass. Changes in bone density may also increase the risk of osteoporosis (El Khoudary et al., 2019; Harlow et al., 2012; Motlani et al., 2023).

According to the Stages of Reproductive Aging Workshop (STRAW) criteria, menopause is determined retrospectively when women reach 12 months after their final menstrual period (FMP) (Harlow et al., 2012). The average age of natural menopause in Canada is 52 years, and it typically arises between the ages of 45 and 60 years. Early menopause is defined as occurring between the ages of 40 and 45 years and represents less than 9% of Canadian women (Velez et al., 2019). Premature menopause or primary ovarian insufficiency is defined as occurring before the age of 40 years, and it represents

less than 4% of Canadian women (Mehra et al., 2023; Velez et al., 2019). There are also various conditions that can cause ovarian insufficiency and lead to menopause, including bilateral oophorectomy, chemotherapy, radiation therapy, and genetic disorders such as Turner syndrome (Gravholt et al., 2019; Harlow et al., 2012; Poniatowski et al., 2001).

1.4.1 Classification of women's reproductive stages

Menopause occurs in different stages, which can be classified according to the STRAW criteria. These criteria divide the reproductive lifespan of women in the late reproductive stage, early and late menopausal transition, and postmenopause (Figure 1.3) (Harlow et al., 2012). Each of these stages has specific characteristics related to the menstrual cycle and hormonal levels.

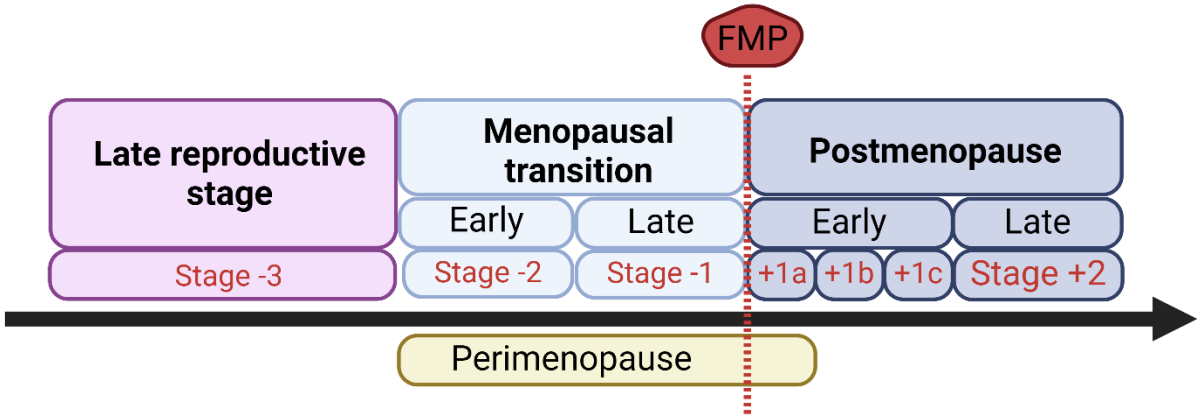

Figure 1. 3. Reproductive stages in women's lifespan.

FMP, final menstrual period. Created with BioRender.com.

1. Late reproductive stage (stage -3): This stage is characterized by the period preceding the onset of menopausal transition. Follicle-stimulating hormone (FSH) levels may increase during the follicular phase of the menstrual cycle. This increase is accompanied by a decline in antimüllerian hormone, inhibin-b levels, and antral follicle counts, which may result in shorter menstrual cycles (Santoro et al., 2021; Van Voorhis et al., 2008).
2. Early menopausal transition (stage -2): This stage is defined by a difference of 7 days or more in the length of consecutive menstrual cycles, which

is persistent for at least 10 cycles. Hormonal changes in this stage are similar to those in the late reproductive stage, with increased and variable levels of FSH, and reduced levels of antimüllerian hormone and antral follicle counts (Harlow et al., 2012).

3. Late menopausal transition (stage -1): This stage is marked by amenorrhea lasting for at least 60 days. This period is characterized by significant hormonal fluctuations, which can last from one to three years on average. As a result, FSH levels might be found at reproductive levels or with increased values greater than 25 IU/L, which is close to postmenopausal levels (~40-50 IU/L). Women are likely to experience hot flashes at this stage (Harlow et al., 2012; Motlani et al., 2023).

4. Early postmenopause (stage +1): This stage is further divided into substages:

    a. Stage +1a is the period that finalizes with the 12 months of amenorrhea necessary to define the FMP. The diagnosis of menopause is made retrospectively at the end of stage +1a, once 12 months have elapsed since the FMP. Perimenopause comprises the menopausal transition and stage +1a. Throughout this stage, FSH levels further rise, and estrogen levels continue to decline.

    b. Stage +1b represents the final hormonal fluctuations in FSH and estradiol levels, estimated to last approximately 1 year (i.e., up to two years after the FMP).

    c. Stage +1c represents the period of stable high FSH levels and low estrogen levels. This stage lasts, on average, three to six years (i.e., up to eight years after the FMP).

5. Late postmenopause (stage +2): This stage is defined as the remaining lifespan of postmenopausal women, with very limited hormonal changes but increased urogenital symptoms such as vaginal dryness and urogenital atrophy. It has also been described that FSH levels may decline late in women's lifespan (Harlow et al., 2012).

1.4.2 The neuroendocrine system at menopause

During the reproductive stage of ovulating women, the menstrual cycle is controlled by the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus to the pituitary gland. Subsequently, FSH and luteinizing hormone (LH) are synthesized and secreted, stimulating the ovaries to secrete estrogen and progesterone. Stimulatory and inhibitory pathways from ovarian hormones can affect the pituitary gland and the GnRH pulses in the hypothalamus to create feedback loops necessary for regulating the menstrual cycle. At the beginning of the menstrual cycle, named the follicular phase, the FSH secreted by the pituitary gland promotes follicle development in the ovary, which secrete estradiol. Then, estradiol, inhibin A and B secreted by ovarian follicles, inhibit FSH secretion and promote LH secretion from the pituitary gland. The LH peak and the pulsatile GnRH secretion from the hypothalamus trigger ovulation. The following stage of the menstrual cycle is named the luteal phase, in which the corpus luteum (the remainder of the developed follicle) secretes progesterone in preparation for implantation of the fertilized oocyte. If the oocyte is not fertilized, menstruation occurs at the end of the luteal phase as estrogen and progesterone levels decline, allowing FSH to increase and initiate a new menstrual cycle (Hall, 2019).

Early and progressive hormonal alterations arise before the clinical onset of the menopausal transition. First, the declining number of follicles in the late phase of the reproductive stage of women leads to decreased inhibin B and antimüllerian hormone produced in the granulosa cells of the oocyte. The decreasing levels of inhibin B and antimüllerian hormone lead to increased FSH levels. As a response to this loss of ovarian feedback, estrogen levels decrease, promoting GnRH pulses and further raising FSH levels. Once this physiological mechanism cannot be compensated, irregular menstrual cycles arise, indicating the onset of the menopausal transition (Hall, 2015; Santoro et al., 2021).

The Study of Women's Health Across the Nation (SWAN) is a multicentric longitudinal cohort study in the USA in 1996, comprising 3,302 middle-aged women, which has advanced research on the menopausal transition (El Khoudary et al., 2019). The SWAN study has provided important insights into hormonal trajectories of FSH and

estrogen throughout menopause (El Khoudary, 2017). On average, FSH begins increasing about six years before the FMP, with increasing values and a greater rate of change at about two years before the FMP. FSH levels start decreasing around the time of the FMP and reach stable levels two years after FMP. On the other hand, estradiol levels begin decreasing about two years before the FMP and continue declining until two years after the FMP, with a stabilization period afterward (Randolph et al., 2011). As described lately in the SWAN study, estrogen and FSH levels presented a wide variability among participants (Tepper et al., 2012). As such, Tepper et al. (2012) described different FSH and estradiol trajectories throughout the menopausal transition in a subsequent analysis. Firstly, their study showed three trajectories for FSH levels throughout the menopausal transition based on their blood concentration levels. FSH low pattern was present in 10.6% of women in their study, medium pattern in 48.7%, and high pattern in 40.7%. In the low and medium patterns, FSH levels began increasing at about two years before FMP, whereas in the high pattern, FSH levels may begin up to 5.5 years before FMP (Tepper et al., 2012). Secondly, their study showed that estradiol levels follow four patterns as follows:

1. Rise/steep decline (31.5% of women): Estradiol rises steeply about five years before FMP, followed by a steep decline one year before FMP.
2. Flat pattern (28.6%): Medium estradiol levels are observed before and after FMP, with a limited drop in values throughout the menopausal transition.
3. Slow decline (26.9%): Consistently low estradiol levels are observed before and after FMP, with a subtle decline throughout the FMP.
4. Rise/slow decline (13.1%): Estradiol rises steeply about 4.5 years before FMP, followed by a slow decline around FMP and up to 2.5 years afterward.

1.4.3 Vasomotor symptoms

Vasomotor symptoms (VMS) are the most common symptoms experienced by menopausal women, which can greatly affect their quality of life. VMS include hot flashes (or hot flushes) and night sweats (Crandall et al., 2023). These symptoms occur due to peripheral vasodilation, causing a sudden and transient increase in blood flow and skin

temperature in different parts of the body, such as the face, chest, abdomen, hands, arms, and legs (Freedman, 2014).

VMS are more frequently present during the menopausal transition affecting 50-80% of women at this stage (Avis et al., 2015). According to the SWAN study, the duration of VMS is typically 7.4 years in women who experience frequent VMS (at least six days in the previous two weeks (Avis et al., 2015)) and four years for women who report any frequency of VMS (Freeman et al., 2014). The duration appears to be dependent on the time of VMS onset. For women who start having VMS during the menopausal transition, the median duration of VMS is 11.8 years, while women who start having VMS after menopause experience them for a median duration of 3.4 years (Avis et al., 2015). Although the exact mechanism of hot flashes is not entirely understood, it has been suggested that decreased estrogen levels and a transient increase in norepinephrine levels in the POA are required in triggering hot flashes (Freedman, 2014).

1.5 Sleep at menopause

As previously mentioned, menopause is commonly accompanied by various physiological and psychological changes. Sleep disturbances, among them, may affect 40-60% of menopausal women (Salari et al., 2023; Shaver & Woods, 2015; Woods & Mitchell, 2010). Several factors may contribute to the sleep disturbances occurring in menopausal women, such as the high incidence of mental health disorders (e.g., depression and anxiety symptoms (El Khoudary et al., 2019)), the high incidence of sleep disorders (e.g., periodic leg movements during sleep [PLMS] and sleep-disordered breathing (Gabbay & Lavie, 2012; Pennestri et al., 2006)), vasomotor symptoms, chronic health conditions (e.g., cancer, chronic pain, hypothyroidism (Jehan et al., 2015)), changes in the circadian timing system (detailed in Chapter 1.6), among others.

1.5.1 Objective vs self-reported measures of sleep

Among the most common sleep complaints reported by menopausal women are difficulties falling and maintaining sleep, fragmented sleep with increased wake time during the night, and early morning awakenings (Baker, 2023b; Carrier et al., 2017). Increased sleep complaints have been commonly described in research using self-reported measures of sleep at menopause and have clinical significance for quality of life

and cognition (Shieu et al., 2023). However, some controversies persist as some articles have not reported changes in polysomnographically-recorded sleep during menopause (Shaver & Woods, 2015).

The Canadian Longitudinal Study on Aging has shed light on the effect of menopause on self-reported sleep disturbances (Zolfaghari et al., 2020). Within their study, a cross-sectional analysis of 6,179 women between 45 and 60 years old found that postmenopausal women are more likely to report sleep disturbances on questionnaires than pre- and perimenopausal women. More specifically, postmenopausal women more frequently reported sleep onset longer than 30 minutes, sleep onset insomnia, and were at higher risk of developing obstructive sleep apnea (Zolfaghari et al., 2020). Similarly, a meta-analysis conducted by Xu and Lang (2014) analyzed data from pre- peri- and postmenopausal women and found that peri- and postmenopausal women self-reported more sleep disturbances compared to premenopausal women, as measured by the Pittsburgh Sleep Quality Index, the Insomnia Severity Index, and the Athens Insomnia Scale. Moreover, their study found a higher risk for sleep disturbances in Asian and White compared to Hispanic peri- and postmenopausal women, but other factors such as socioeconomic status may have affected their results (Xu & Lang, 2014).

Regarding objective measures of sleep, only a few research has described PSG and actigraphic changes in sleep at menopause. Importantly, PSG and actigraphy results are heterogeneous as inconsistent methodologies have yielded mixed results on the effect of menopause on sleep. Research studies have demonstrated that women in the peri- and postmenopausal stages experience longer sleep onset, increased wake after sleep onset (WASO), and more frequent nighttime arousals than premenopausal women (de Zambotti et al., 2015; Kalleinen et al., 2020; Lampio et al., 2017; Xu et al., 2011; Young et al., 2003). However, it is important to note that some studies have produced contradictory or inconsistent results (Freedman & Roehrs, 2004; Hachul et al., 2009; Hachul et al., 2015; Kalleinen et al., 2008). These inconsistencies have been attributed to the severity of hot flashes or the absence of screening procedures for other sleep disturbances, such as sleep-disordered breathing. Moreover, sleep quality has been reported to deteriorate with aging among postmenopausal women (Ahmady et al., 2022).

Various studies have also described changes in the sleep architecture as a function of age but did not account for menopausal status (Mitterling et al., 2015; Ohayon et al., 2004; Schwarz et al., 2017). Studies on aging populations have commonly reported decreased SE, TST, SWS, and REM sleep. These results are particularly evident in groups aged over 60 years, but studies that have factored in menopausal status have produced inconsistent findings. These inconsistencies underline the importance of further research to investigate the sleep disturbances that menopausal women experience.

1.5.2 Vasomotor symptoms and sleep

VMS or hot flashes are frequently linked to sleep disturbances at menopause, particularly in the perimenopausal phase (Kravitz et al., 2017). VMS episodes may occur during the day or night, lasting from a few seconds to several minutes and causing disruptions in sleep. Several studies have shown that self-reported instances and severity of hot flashes at night are often associated with poor sleep quality and insomnia (Kingsberg et al., 2023; Ohayon, 2006). According to a study conducted by Song et al. (2022), women aged between 40 and 65 who experience hot flashes commonly have poorer sleep quality, as measured by the Pittsburgh Sleep Quality Index, compared to women who do not experience hot flashes. Racial and ethnic differences may play a role in the occurrence of hot flashes, as African-American women experience them more often than White women. On the other hand, White women are more likely to report experiencing sleep disturbances when they have hot flashes (Kingsberg et al., 2023).

The extent to which hot flashes impact postmenopausal sleep has been questioned due to the potential influence of memory bias and sleep inertia on the recall of nighttime hot flashes (Baker, 2023a). Research conducted by Freedman and Roehrs (2004) has demonstrated that objectively measured hot flashes, as identified via skin conductance during sleep, coincide with brief arousals and awakenings in postmenopausal women experiencing at least six hot flashes during the day. Nonetheless, the average number of hot flashes recorded per participant during the sleep period was 5.2 (range 1-18), which accounted for only about 5% of the number of arousals occurring during the sleep period (Freedman & Roehrs, 2004). Despite the accepted notion that hot flashes widely affect the sleep of menopausal women, sleep changes in

these women may also be influenced by various factors, as nighttime VMS only account for a small fraction of arousal events (Baker, 2023a).

1.5.3 Hormone replacement therapy for menopausal sleep disturbances

Hormone replacement therapy (HRT) is commonly used for the relief of VMS in menopausal women, particularly when VMS are present frequently and of moderate-to-severe intensity (Pan et al., 2022). Various presentations of HRT are commercially available (e.g., oral, patches, or vaginal tablets) and include various formulations of estrogen, progesterone, or a combination of both.

A number of studies have investigated the effect of HRT on the sleep of postmenopausal women. A recent meta-analysis conducted by Pan et al. (2022) evaluated 15 studies on the effects of HRT on sleep quality between 2003 and 2018. The study found that HRT improved self-reported measures of sleep quality but did not significantly impact objective measurements such as TST, SE, sleep onset latency (SOL), or number of arousals. Specifically, estrogen treatment alone was found to improve self-reported sleep quality, with transdermal patches yielding the best results. Moreover, combined estrogen and progesterone HRT was found to improve self-reported sleep disturbances when present. A study by Geiger et al. (2019) compared the effectiveness of using estradiol alone, estradiol with progesterone, and placebo for a period of 12 months among 172 women who were going through perimenopause. The authors found that combined HRT improved self-reported latency to fall asleep and the number of awakenings, regardless of their VMS and depressive symptoms. A systematic review by Attarian et al. (2015) evaluated the effect of HRT and various hypnotics on postmenopausal women with insomnia. The review included 23 studies, out of which 14 showed improved VMS (vasomotor symptoms) and sleep (i.e., self-reported and PSG recordings), while 9 reported mixed or no significant effects on sleep. The authors concluded that HRT could be used for insomnia when accompanied by VMS or when other sleeping medication is ineffective. Notably, several factors, such as different types of hormones used, doses, and the definition of insomnia, may lead to variability and account for inconsistent results.

According to the available literature, HRT has the potential to improve sleep, although some results have been inconsistent. HRT may be particularly effective in postmenopausal women who experience VMS or meet the criteria for insomnia.

1.5.4 HRV

Research has shown that men are at a higher risk of developing CVD than women during youth. However, the risk of CVD in women increases in middle age, a time coincidental with menopause. Coronary heart disease, which includes conditions such as coronary artery spasm and coronary microvascular dysfunction, is the primary cause of CVD during menopause (Anagnostis & Stevenson, 2024). Additionally, menopause is associated with a higher incidence of metabolic diseases such as dyslipidemia, arterial hypertension, and type 2 diabetes, which further increase the risk of CVD. Studies with longitudinal designs have provided a better understanding of the contribution of menopause to CVD (El Khoudary et al., 2020). At menopause, it has been described that parasympathetic activity (measured by HF and RMSSD) and HRV (measured by SDNN) decrease, possibly contributing to this increased risk of CVD (von Holzen et al., 2016).

This increase in CVD risk at menopause has been associated with hormonal changes that occur during this stage of life (El Khoudary, 2017). However, the contribution of sex hormones to the risk of CVD is not yet fully understood. Several studies have been conducted to investigate the effect of exogenous hormonal administration to HRV measures. Most studies have revealed that estrogen HRT (oral, transdermal, or nasal) in postmenopausal women increases measures of HRV (Liu et al., 2003; Neves et al., 2007; Yang et al., 2013), but some studies have shown no effect (Fernandes et al., 2005; Hautamaki et al., 2013; Virtanen et al., 2023). The effect of oral contraceptives on the HRV of young women also presents conflicting results. Most studies have shown no significant effect of oral contraceptives on HRV measures (Nisenbaum et al., 2014; Teixeira et al., 2015; Wilczak et al., 2013), except for one longitudinal study showing a reduction of parasympathetic activity (Ahokas et al., 2023). Thus, the current literature shows discrepant effects of exogenous hormonal treatment on HRV measures. While it appears that estrogen treatment has a beneficial impact on the HRV of postmenopausal women, more research is required to clarify various aspects. These include establishing

the causal impact of endogenous and exogenous sex hormones on cardiovascular changes, examining the impact of different hormonal preparations, distinguishing the impact of progesterone from estrogen, and validating the results obtained in studies with larger sample sizes.

The studies of HRV are mostly performed in awake individuals. However, the ANS and sleep are closely related as both are outputs of the circadian timing system and are centrally controlled by neural complex networks. Indeed, a bidirectional connection between the ANS and sleep has been suggested, as described in Chapter 1.2.4. Recently, a growing interest in the relationship between CVD and sleep disorders has arisen, particularly insomnia. This relationship may have important implications in the developing of insomnia in postmenopausal women. A recent meta-analysis presented some research showing a decreased parasympathetic and HRV in insomnia patients, but the result did not reach significance ($p = 0.075$) (Zhao & Jiang, 2023). Notably, this meta-analysis included 17 studies, most with small sample sizes, suggesting that further research is necessary for clarifying the relationship between HRV and insomnia (Zhao & Jiang, 2023). The group of Zambotti et al. conducted a study in perimenopausal women to compare the HRV changes during sleep between insomniacs and good sleepers(de Zambotti, Trinder, Colrain, et al., 2017; de Zambotti, Trinder, Javitz, et al., 2017). Their findings indicated increased HR and blood pressure, as well as decreased parasympathetic activity in insomniacs during both NREM and REM sleep (de Zambotti, Trinder, Colrain, et al., 2017; de Zambotti, Trinder, Javitz, et al., 2017).

Only a few studies have focused on HRV changes during the sleep of menopausal women. Magri et al. (2006), using a cross-sectional design, showed that postmenopausal women with no HRT had lower SDNN and RMSSD than younger premenopausal women both during daytime and nighttime. They also showed that postmenopausal RMSSD and SDNN values were lower during nighttime vs daytime, although a direct statistical comparison was not performed. Conversely, Virtanen et al. (2023) used a prospective, randomized, double-blind, placebo-controlled study to analyze the effect of combined HRT on HRV variables of postmenopausal and perimenopausal women during their sleep. In this study, HRV and parasympathetic activity were comparable between peri-

and postmenopausal women at baseline. After six months of treatment, HRT caused an increased RR interval in both peri- and postmenopausal women, with no differences in HRV measures (Virtanen et al., 2023). Despite some inconsistent results, it is possible that the declining HRV and parasympathetic activity at menopause might be part of sleep disruptive changes, akin to individuals with insomnia (de Zambotti, Trinder, Colrain, et al., 2017; de Zambotti, Trinder, Javitz, et al., 2017; Zhao & Jiang, 2023), and deserve further investigation.

1.6 Circadian regulation of sleep and alertness of postmenopausal women

As previously described, postmenopausal women are vulnerable to sleep disturbances. As described by the two-process model, the circadian timing system contributes to the timing and duration of sleep (Borbely et al., 2016), and it is thus essential to understand the changes in this system that may influence postmenopausal sleep.

Limited research has been conducted on the circadian rhythms of postmenopausal women. However, insights can be gained from studying sex and age-related changes in the human circadian timing system. Table 1.1 provides a comprehensive summary of the relevant literature regarding age and sex differences in human circadian rhythms. In summary, research has shown that there are gender differences in the intrinsic circadian period of CBT and melatonin, with women presenting, on average, a shorter period compared to men (Duffy et al., 2011). Accordingly, women exhibit earlier circadian phases of CBT, melatonin, and circadian clock gene expression (PER2, PER3, ARNTL1) than men (Boivin et al., 2016; Lim et al., 2013; Santhi et al., 2016). Furthermore, women typically have earlier bedtimes than men but initiate sleep at a later circadian phase, which may contribute to their increased susceptibility to sleep disruptions at the end of the night (Boivin et al., 2016; Lazar et al., 2013). These findings have important implications for understanding and addressing sleep-related disturbances among women.

In comparison to younger populations, aged individuals have been observed to experience the following changes in their circadian rhythms (Duffy et al., 2015; Kim et al., 2022):

1. Advanced circadian phase of CBT, melatonin, and cortisol (Carrier et al., 2002; Duffy et al., 1999; Kim et al., 2014; Van Cauter et al., 1996; Youngstedt et al., 2019).

2. Reduced circadian amplitude of CBT and melatonin (Buysse et al., 2005; Dijk et al., 1999; Kim et al., 2014; Kripke et al., 2005; Munch et al., 2005; Yoon et al., 2003).

3. Decreased circadian drive for sleep and wakefulness (Dijk et al., 1999; Munch et al., 2005).

These age-related circadian changes have been associated with sleep-wake disturbances commonly present in aged populations, including early morning awakenings and daytime sleepiness (Duffy et al., 2015; Roenneberg et al., 2022).

Moreover, age-related changes in the homeostatic sleep process may also contribute to sleep disturbances at menopause. Studies have shown that older adults present a reduced sleep consolidation across all circadian phases (Dijk et al., 1999) and a decline in SWA as a response to prolonged wakefulness (Munch et al., 2004). It has been hypothesized that the changes in the homeostatic sleep process that occur with aging could be a result of diminished sleep pressure and a subsequent decline in the ability to sleep or, conversely, a reduction in the necessity for sleep (Carrier et al., 2017).

The existing literature suggests that the circadian rhythms and sleep patterns of aged populations are affected; however, a complete understanding of circadian rhythms in postmenopausal women is hindered by certain limitations. Firstly, the majority of participants included in these studies recruited old populations aged over 60, probably disregarding the early postmenopausal phase, as the average age of menopause is 52 years. Secondly, studies that have included aged women have not adequately accounted for their menopausal status. Only a few studies investigated circadian rhythms in women while considering menopausal status. Walters et al. (2005) conducted a study using a 22-h constant routine protocol (i.e., sleep deprivation in laboratory-controlled conditions) to compare melatonin rhythms between postmenopausal and younger premenopausal women. The study found that postmenopausal women exhibit an advanced melatonin acrophase compared to their younger counterparts. The authors of the study concluded that it is difficult to determine whether this advanced melatonin rhythm in postmenopausal

women is due to aging, hormonal changes at menopause, or both (Walters et al., 2005). Another study by Toffol et al. (2014) examined melatonin secretion in women at different stages of menopause before and after the administration of hormone therapy during nocturnal sleep. The study found that postmenopausal women experienced a delayed melatonin acrophase compared to pre- and perimenopausal women after receiving hormone therapy, but statistical comparisons before and after treatment were not performed. Notably, hormone therapy administered to premenopausal and perimenopausal women only consisted of estradiol, whereas the combined HRT was administered to postmenopausal women. The authors of the study attributed the observed delayed melatonin phase in postmenopausal women to the presence of this progesterone component as it has been shown to prolong the circadian period in animal models (Toffol et al., 2014).

Although circadian rhythm changes may play a significant role in sleep disturbances experienced by postmenopausal women, research is scarce in this area. According to current literature, disruptions in circadian rhythms can impact the sleep of aged women, and these rhythms can be influenced by external hormonal administration.

Author (year)	Sample size	Protocol	Circadian rhythms changes
Boivin et al. (2016)	11 healthy women in the mid-follicular phase aged 25.8 ± 3.5 y 15 healthy men aged 23.4 ± 3.7 y	72-h USW: 60/60 min sleep-wake cycle	Women had an advanced circadian phase of the rhythms of SE, SOL, ROL, N1, N2, SWS, REM, NREM, and TST vs men.
Bullock et al. (2017)	168 participants aged 18-30 y (68F, 100 M)	30- or 50-h Constant Routine protocol.	Women had an earlier CBT circadian phase than men. No sex differences in melatonin and morningness-eveningness were observed.
Buysse et al. (2005)	19 young participants aged 23.2 ± 2.6 y (9F, 10M) 17 older participants aged 76.3 ± 5 y (10F, 7M)	60-h USW: 30/60 min sleep-wake cycle.	Older adults tended to have a lower CBT average than young adults, with no differences in amplitude or phase. Older adults presented a decreased circadian amplitude of TST than young adults.
Cain et al. (2010)	28 women and 28 men aged 18-30 y	27–50-hour Constant Routine protocol	Women presented an earlier CBT and MLT rhythm relative to habitual sleep-wake time and had higher MLT amplitude.
Carrier et al. (2002)	11 young adults aged 25-38y (6 F, 5 M) 16 middle-aged adults aged 40-58 y (10 F, 6 M)	25-h Constant Routine protocol	Middle-aged individuals showed earlier habitual wake time and CBT minimum. Group differences in circadian CBT amplitude of phase angles were not observed.
Czeisler et al. (1992)	27 healthy young men aged 18-31 y. 21 healthy older people aged 65-85 y (10 F, 11 M)	~40-hour Constant Routine protocol	Mean CBT and circadian amplitude were higher in older women vs older men. Older individuals showed a lower circadian amplitude and earlier phase of CBT than young individuals.
Dijk et al. (1999)	11 young men aged 21-30 y. 13 older individuals aged 64-74 y (4 F, 9 M)	40-h Constant Routine protocol followed by a 28-h Forced Desynchrony protocol for a month	Older adults had a lower circadian amplitude and mesor of CBT and a dampened circadian drive of sleep and wakefulness.
Duffy et al. (1998)	44 older subjects aged 64–81 y (25 F, 19 M) 101 young men aged 18–30 y	26-53-hour Constant Routine protocol followed by a Forced Desynchrony protocol for a month	The circadian phase of CBT was earlier, with shorter phase angles relative to rise time in oldervs younger adults.
Duffy et al. (2002)	33 young men aged 23.4 ± 3.3 y 15 older men and women aged 67.8 ± 3.1 y	31.6 to 52.8 h Constant Routine protocol	Older adults showed earlier sleep times and dim-light melatonin onset vs younger adults.
Duffy et al. (2011)	52 women and 105 men aged 18-74 y	A month-long Forced Desynchrony protocol	Women had circadian periods of CBT and melatonin shorter by about 6 minutes vs.men. No differences in the circadian period were observed with aging neither in women nor in men.
Gunn et al. (2016)	28 participants aged 19-33 y (14 F, 14 M) All females were on oral contraceptives	~36-hour Constant Routine protocol	Women showed greater levels and higher circadian amplitude of plasma melatonin and cortisol vs.men. Women excreted less urinary cortisol than men, but no sex differences in urinary melatonin were observed. No sex differences in the circadian phase of melatonin or cortisol were observed.
Kim et al. (2014)	29 healthy young participants aged 20-35 y (21 F, 8 M)	Constant Posture protocol for three days	Older adults presented advanced CBT and melatonin phases, as well as a decreased melatonin amplitude, compared to younger adults.

	16 healthy older subjects aged 60-80 y (11 F, 5 M)		
Kripke et al. (2005)	62 older participants aged 58-84 y 25 young participants aged 19-40 y	~72h USW 30min sleep, 60min awake	Older adults had lower 6-sulfatoxymelatonin (aMT6s) and salivary melatonin circadian amplitudes than younger adults. The circadian phase of urinary-free cortisol was advanced in older adults. Age differences in the circadian phase of CBT, melatonin, or phase angles were not observed.
Kripke et al. (2007)	50 young adults aged 18-31 y (31 F, 19 M) 56 older adults aged 59-75 y (28 F, 28 M)	90-min USW procedure for 4.7 to 5.6 days	Older adults presented phase-advanced sleep, cortisol, and aMT6s onset but not aMT6s or temperature rhythms.
Lazar et al. (2013)	35 healthy participants aged 20.5-32.4 y (18 F, 17M)	28-hour Forced Desynchrony protocol for nine days	Women had earlier bedtimes than men. No sex differences were observed in the melatonin period or dim-light melatonin onset.
Lim et al. (2013)	490 older deceased individuals: 298 females aged 88.8 ± 6.5 y 192 males aged 85.7 ± 6.4 y	Postmortem study	Significant diurnal variations of PER2/3 and ARNTL1 (BMAL1) were observed in the human cerebral cortex. PER2/3 peaked in the morning, while ARNTL1 peaked at night. The timing of expression of these clock genes occurred earlier in women than in men.
Munch et al. (2005)	17 young participants aged 20-31 y (9F, 8M) 15 older participants aged 57-74 y (7F, 8M)	40-h USW 75/150 min sleep/wake schedule	Older adults showed attenuated melatonin secretion at night and an average 24-hour secretion compared to younger adults. Age differences were not observed in melatonin duration or circadian phase.
Santhi et al. (2016)	16 men aged 24.54 ± 0.72 y 18 women aged 26.67 ± 0.83 y	28-h Forced desynchrony protocol	Melatonin circadian amplitude was greater in women vs men, with no differences in phase. Women had a higher circadian amplitude in performance measures than men. Women were earlier chronotypes than men.
Van Cauter et al. (1996)	90 healthy men and 87 healthy women aged 18-83 y	Data was obtained from 7 laboratory studies	Cortisol secretion was lower in women vs men. With aging, the cortisol circadian amplitude was dampened, and the timing of the circadian phase elevation was advanced.
Yoon et al. (2003)	67 young adults aged 18 - 32 y (44 F, 25 M) 56 older adults aged 60 - 75 y (37 F, 22 M)	USW protocol for 30 h: 60-min awake, 30-min sleep	Women tended to have an earlier acrophase of aMT6s and cortisol than men. The acrophase of cortisol, sleep time, and sleep propensity was advanced in older vs younger adults.
Youngstedt et al. (2019)	48 young adults aged 18-32 y (26 F, 22 M) 53 older adults aged 59-75 y (29 F, 22 M)	90-min USW procedure (60 min awake, 30 sleep)	Women presented earlier aMT6s onset, acrophase, and offset than men. Older adults had an earlier aMT6 acrophase, onset, offset, and lower mesor than younger adults.
Zeitzer et al. (1999)	98 young men aged 18-30 y 34 older individuals aged 56-81 y (20 F, 14 M)	Constant Routine protocol for at least 30 hours	There was no significant age or sex differences in the circadian amplitude, duration, mean, and integrated area under the curve of the nocturnal peak of plasma melatonin.

Table 1. 1. Summary of age and sex differences in circadian rhythms.

1.7 Thesis rationale and objectives

The high prevalence of sleep disturbances among menopausal women can be attributed to various factors, but the contribution of the circadian timing system remains largely unexplored. Multiple variables potentially relevant to sleep after menopause must be considered for a comprehensive analysis.

As described in Chapter 1.6, research has suggested that circadian changes in older individuals, such as a dampened and advanced rhythm of CBT or melatonin, may contribute to the sleep disturbances observed in this population (Duffy et al., 2015). However, few studies have investigated circadian rhythms considering menopausal status (Toffol et al., 2014; Walters et al., 2005). Based on the literature available, sex differences in sleep and circadian rhythms have been described, suggesting a role for ovarian hormones in these physiological components. This is further supported by the effect of HRT, which can potentially improve sleep and shift the circadian rhythm of melatonin (Toffol et al., 2014).

It has been found that HRV measures change during sleep, and a decline in parasympathetic activity and HRV has been linked to insomnia (de Zambotti, Trinder, Colrain, et al., 2017; de Zambotti, Trinder, Javitz, et al., 2017). Moreover, studies have observed decreased HRV and parasympathetic activity during menopause. It is important to investigate the associations between sleep and HRV to understand better the link between sleep disruption and cardiovascular changes in postmenopausal women.

Direct measurement of circadian rhythms from the SCN in humans is not feasible. However, CBT, melatonin, alertness, sleep, EEG activity, and HRV measures exhibit a circadian variation modulated by the SCN and can be measured in laboratory-controlled conditions. As part of this thesis, we aim to measure these various outputs of the circadian system to explore the potential sleep and circadian rhythm changes that may affect postmenopausal women.

Accordingly, our study compared healthy postmenopausal women to young women studied in their mid-follicular phase. Both groups were comprised of healthy sleepers. We conducted an 8-hour baseline sleep recording at night, followed by an

Ultradian sleep-wake cycle (USW) procedure, a specialized protocol used in human chronobiology. This procedure involved alternating 1-hour wake periods in dim light (< 10 lux) and 1-hour nap opportunities in darkness (~0 lux) for 48 hours in postmenopausal and 72 hours in young women. The PSG was set up at the beginning of the experiment and was kept at all times throughout the USW procedure. Electrode impedance was verified every 2 hours to ensure proper data collection. The USW procedure is conducted in a laboratory setting with controlled ambient temperature, light exposure, body posture and regular isocaloric snacks. This design allows us to obtain endogenous circadian rhythms and record sleep at different circadian phases. Furthermore, the uniform distribution of sleep throughout the USW procedure minimizes the impact of sleep deprivation.

As such, the present thesis aims to test the following specific hypotheses:

I. **Circadian rhythms differ between postmenopausal and young women.** In both groups, rectal CBT and salivary melatonin rhythms will be measured throughout the first 48 hours of the USW procedure. Chapter 2 will present specific postmenopausal changes in these circadian rhythms.

II. **The circadian variation of sleep and alertness differs in postmenopausal women.** Nocturnal sleep and the circadian variation of sleep parameters will be presented. Group differences will be analyzed in various measures, namely TST, SOL, NREM sleep stages N1, N2, N3, REM sleep, REM sleep onset (ROL), number of arousals, self-reported sleep quality, and self-reported alertness. Specific postmenopausal changes will be observed in the circadian variation of sleep and alertness.

III. **There are differences between groups in the circadian variation of EEG activity.** The EEG signal will provide a refined analysis of the visually-scored sleep parameters. Chapter 3 investigates postmenopausal changes in the EEG activity of nocturnal sleep and its circadian variation, including delta, theta, alpha, sigma, and beta power.

IV. **HRV measures during sleep differ in postmenopausal women.** The study in Chapter 4 will investigate how sleep and the circadian system affect

HRV of postmenopausal women. HRV changes during the nocturnal sleep episode will be compared between the two groups. The circadian variation of various HRV measures during naps s of the USW procedure will also be analyzed. More specifically, HR, SDNN, RMSSD, and HF will be measured.

In Chapters 2, 3, and 4, we will present complete manuscripts to address our hypotheses. The following manuscripts have been published (Chapters 2 and 3) or submitted to peer-reviewed journals for publication (Chapter 4).

Chapter 2.

The circadian variation of sleep and alertness of postmenopausal women

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SLEEP (2023), 46(2), zsac272

2.1 Preface

As humans age, changes in their circadian rhythm may lead to sleep disturbances. Although a few studies have focused on circadian rhythms in older adults, only a few have examined the circadian rhythms of women accounting for their menopausal status (Toffol et al., 2014; Walters et al., 2005). Therefore, the objective of the current study is to investigate the circadian rhythms of CBT and melatonin, as well as the circadian variation of sleep and alertness in postmenopausal women. These changes in circadian rhythms may contribute to their high risk of developing sleep disturbances.

2.2 Abstract

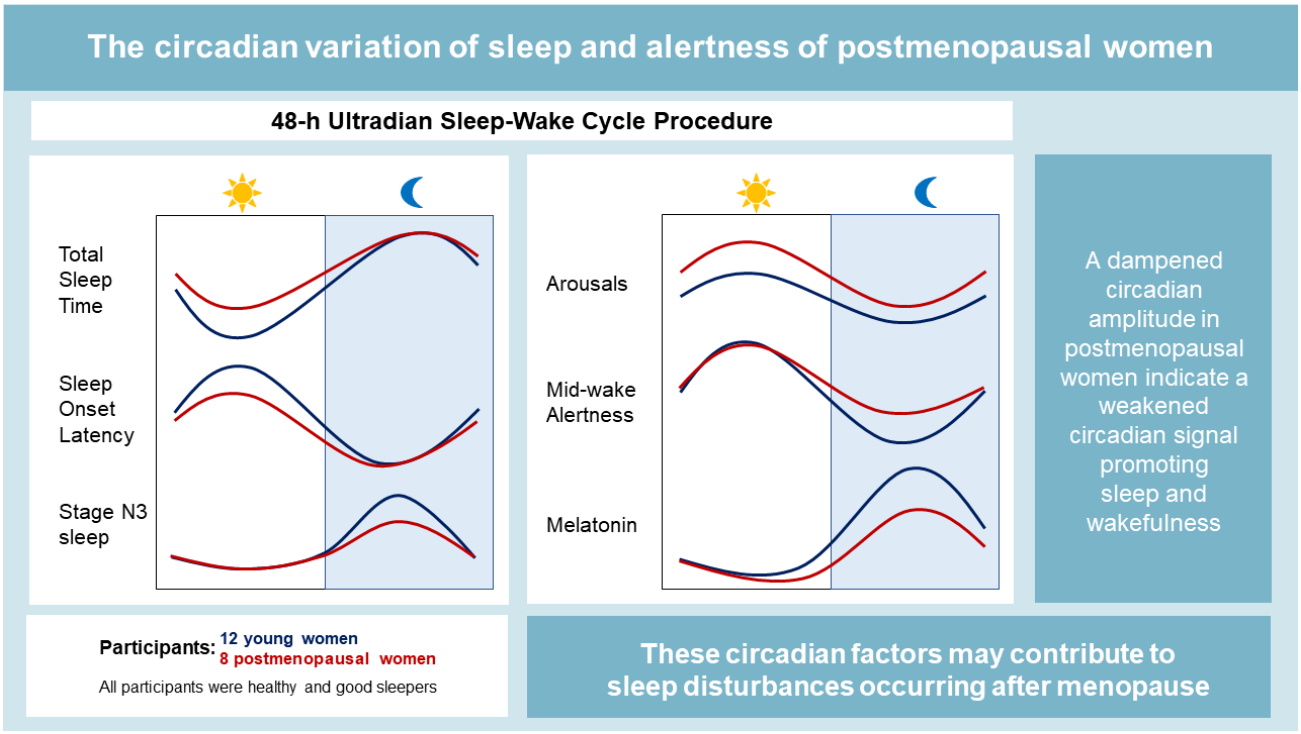
Study Objectives: Several factors may contribute to the high prevalence of sleep disturbances occurring in postmenopausal women. However, the contribution of the circadian timing system to their sleep disturbances remains unclear. In the present study, we aim to understand the impact of circadian factors on changes of sleep and alertness occurring after menopause.

Methods: Eight healthy postmenopausal women and 12 healthy young women in their mid-follicular phase participated in an ultradian sleep–wake cycle procedure (USW). This protocol consisted of alternating 60-min wake periods and nap opportunities for ≥ 48 h in controlled laboratory conditions. Core body temperature (CBT), salivary melatonin, self-reported alertness, and polysomnographically recorded sleep were measured across this procedure.

Results: In both groups, all measures displayed a circadian variation throughout the USW procedure. Compared to young women, postmenopausal women presented lower CBT values, more stage N1 and N2 sleep, and number of arousals. They also showed a reduced amplitude of the circadian variation of melatonin, TST, SOL, stage N3 sleep, and alertness levels. Postmenopausal women fell asleep faster and slept more during the biological day and presented higher alertness levels during the biological night than young women.

Conclusion: These results support the hypothesis of a weakened circadian signal promoting sleep and wakefulness in older women. Aging processes including hormonal changes may be main contributors to the increased sleep–wake disturbances after menopause.

2.3 Graphical Abstract



2.4 Statement of significance

Circadian factors may play an important role in the sleep–wake changes that occur after menopause. We investigated the effect of menopause on the circadian variation of body temperature, melatonin, sleep, and alertness. Our results demonstrate that postmenopausal women have more shallow and fragmented sleep throughout day and night, as well as a reduced circadian variation of sleep, alertness, and melatonin. Postmenopausal women tend to fall asleep faster and sleep more during the biological day and be more alert during the biological night than young women. These results are consistent with a diminished circadian organization of sleep and wakefulness, possibly due to hormonal changes and/or aging. Circadian factors could be main contributors to the high prevalence of sleep–wake disturbances in postmenopausal women.

2.5 Introduction

Women present a higher prevalence of sleep complaints than men throughout their life (see Mong & Cusmano, 2016, for review), and these complaints steeply increase in the peri- and postmenopausal period, affecting 40-60% of women (Kravitz et al., 2008; Shaver & Woods, 2015; Woods & Mitchell, 2010; Zolfaghari et al., 2020). According to

epidemiological studies, sleep disturbances at menopause mainly present as sleep fragmentation and difficulties falling and staying asleep (Kravitz et al., 2008; Woods & Mitchell, 2010; Zolfaghari et al., 2020). Several factors have been reported to influence the sleep of postmenopausal women, including vasomotor symptoms, chronic health conditions, and higher incidence of sleep apnea/hypopnea syndrome and periodic limb movements disorder (Arnardottir et al., 2016; Pennestri et al., 2006; Xu & Lang, 2014). Furthermore, age- and hormone-related changes in the circadian timing system can have an important role in the regulation of sleep and may additionally contribute to the sleep disturbances observed in postmenopausal women.

Menopause is characterized by decreased levels of sex hormones and increased levels of gonadotropins due to loss of ovarian function (Harlow et al., 2012) which may contribute to their sleep disturbances. Indeed, research has identified estradiol and progesterone receptors in brain regions that regulate sleep, wakefulness and circadian rhythms, in female rodents (Dorsey et al., 2020), although the precise mechanisms by which sex hormones regulate these systems in women remain to be elucidated.

The circadian timing system, controlled by the suprachiasmatic nucleus, present age-related changes in its physiology that may affect sleep and alertness of aged individuals (Duffy et al., 2015). Advanced circadian rhythms of CBT, melatonin, and cortisol have been reported in older compared to younger adults and are consistent with their early morning awakenings and earlier sleep schedules (Duffy et al., 2015). A few studies have specifically investigated circadian rhythms in postmenopausal women (Toffol et al., 2014; Walters et al., 2005), but the contribution of the circadian timing system to the regulation of sleep and its circadian variation has not yet been studied in this population. The aim of the present study is to better understand the role circadian factors play in the sleep changes occurring after menopause, as possible contributors to the increased risk of sleep disturbances in this population.

2.6 Materials and Methods

2.6.1 Participants

Eight healthy postmenopausal women (mean age ± SD: 54.80 ± 3.37 y, range 50 – 61 y) with no sleep complaints were enrolled and their results compared with previously-collected data from 12 healthy naturally ovulating young women in their mid-follicular

phase (age: 25.83 ± 3.35 y, range 20 – 30 y; days 5-9 after menses; see Supplementary Table 2.1 for demographic information). At the time of study, the time elapsed since the last menstrual period of postmenopausal women was between 2 and 11 years and one blood sample was taken on the first morning of the laboratory phase to measure serum levels of FSH, LH, estradiol, and progesterone. Participants' hormone levels were consistent with their menopausal status (Supplementary Table 2.2). Young women confirmed ovulation via plasma progesterone levels on day 21 of their menstrual cycle preceding experimental procedures (Shechter et al., 2010). Postmenopausal women were free of medications, except for one woman who was using estradiol transdermal patches of 25 micrograms every 3 days and micronized progesterone pills 100 mg once a day. Details from 11 of the 12 young women have previously been published (Boivin et al., 2016; Shechter et al., 2010). All young women were physically healthy, had regular menstrual cycles of 26 to 32 days, showed no evidence of psychiatric or gynecological diseases, and were not using any medications or contraceptives.

2.6.2 Recruitment and screening

Eligible postmenopausal women needed to be 45-65 years old and were at least 2 years past their last menstrual period, as described by the Stages of Reproductive Aging Workshop (STRAW) classification (Harlow et al., 2012). Exclusion criteria included shift work, transmeridian travel of two or more time zones in the past 2 months, and chronic pathologies and medications that might affect sleep and circadian rhythms. Participants were healthy physically and mentally as documented by psychological and medical evaluations. All participants were healthy sleepers as verified by a polysomnographic screening night in the laboratory. Postmenopausal women with Apnea-Hypopnea Index (AHI) ≥ 15/h of sleep, and young women with AHI ≥ 5/h of sleep were excluded (Arnardottir et al., 2016; Gabbay & Lavie, 2012). Women with periodic leg movements during sleep (PLMS; ≥ 15/h of sleep) were also excluded (Pennestri et al., 2006; Sateia, 2014). Respiratory events and PLMS were identified according to AASM criteria (Berry et al., 2020; Iber, 2007), and PLMS in young women were scored in accordance with Coleman's criteria (Coleman, 1982), as described previously (Boivin et al., 2016). This project was approved by the Douglas Mental Health University Institute Research Ethics Board (2018-175). Each participant provided informed consent prior to study initiation.

2.6.3 Study design

For at least 2 weeks prior to laboratory entry, participants maintained a regular 8-h nocturnal sleep schedule, as verified by actigraphy for one week prior to laboratory entry. Additionally, participant's compliance to their sleep schedule was visually verified on actigraphy recordings and sleep logs. Bedtime and rise-time for the baseline sleep period at the laboratory was calculated using individual's bedtimes and rise-times of Actiwatch and sleep logs. Upon arrival to the laboratory, participants underwent toxicological screening of urine and training of experimental tasks. Participants were scheduled for one 8-h sleep opportunity at their habitual bedtime to monitor their baseline sleep (see Figure 2.1 and Supplementary Figure 2.1). This was followed by a 48-h (postmenopausal women) or 72-h (young women) ultradian sleep-wake cycle (USW) procedure in constant conditions (described below). This USW procedure consisted of alternating 60-minute wake episodes in dim light (< 10 lux) and 60-minute nap opportunities in complete darkness. In total, postmenopausal women had 24 wake-nap periods whereas young women had 36 wake-nap periods. All participants were allowed to sleep *ad libitum* during the last nap opportunity of the USW procedure.

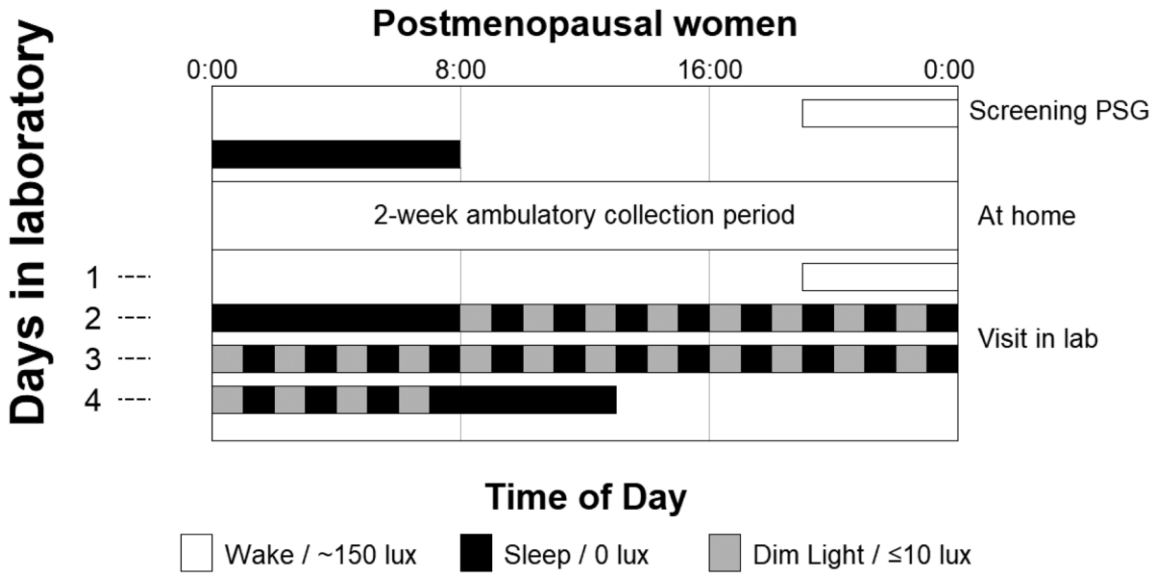


Figure 2. 1. Ultradian sleep–wake cycle (USW) procedure used in postmenopausal women.

PSG screening for sleep disorders was performed prior to laboratory entry. For the laboratory phase, recording equipment were installed upon arrival to the laboratory on the evening of Day 1 (light levels: ~150 lux). Participants slept for an 8-h sleep period (light levels: ~0 lux), based on their habitual bedtimes and rise-times for the past 2 weeks. Upon awakening, participants either began a 48-h (postmenopausal women) or 72-h (young women) USW procedure, consisting of alternating 60-min wake periods and 60-min nap opportunities in constant conditions. All participants were allowed to sleep *ad libitum* during the last nap opportunity of the USW procedure. The figure illustrates a hypothetical participant with a sleep schedule from 00:00 to 08:00 h. More details of the USW procedure of young women are provided in Supplementary Figure 2.1.

2.6.4 Laboratory conditions

While in the laboratory, participants stayed in a windowless time-isolation suite. During wake periods, participants answered pen-and-paper questionnaires and saliva samples were collected. Urine and blood were also collected, but results are not reported in this paper. Constant conditions during the USW were as follows: light exposure was maintained at < 10 lux during wake periods and at ~0 lux during nap opportunities (use

of phone and tablets were not allowed); ambient temperature was maintained at 22.0 ± 2.0°C; food intake comprised isocaloric snacks provided half-way through each wake period. Daily caloric intake was calculated using the Harris–Benedict formula and divided in isocaloric snacks, one per every wake period of the USW procedure. A semi-recumbent position was maintained throughout this procedure in young women. In postmenopausal women, a semi-recumbent position was maintained throughout wake periods, while a supine position was allowed during nap opportunities. To decrease the risk of thrombophlebitis, postmenopausal women were required to use the ensuite bathroom every wake period and to walk around their room every alternate wake period. The period walking around the room started 10 min after lights were turned on and was planned every other wake period. Ambulation lasted less than 10 min and included the time permitted to use the ensuite bathroom. Young women were required to always remain in bed and use a bedpan. Additionally, three 2-mL doses of tinzaparin sodium pre-filled injection (Innohep), an anticoagulant, were administered to the postmenopausal woman taking hormonal replacement therapy (wake periods 1, 13, and 24) to decrease her risk of thrombophlebitis.

2.6.5 Measures and data processing

Sleep was recorded during the baseline night and during all naps using polysomnography (Harmonie, Natus Medical Incorporated, Montreal, QC, Canada), which included electroencephalogram (EEG; C3/A2, C4/A1, O1/A2, O2/A1 leads for all participants, and additionally F3/A2 and F4/A1 for postmenopausal women), electrooculogram, electromyogram, and electrocardiogram. Since the frontal leads of young women were not available, only central and occipital EEG leads were used for scoring. Sleep was visually scored in 30-s epochs according to the American Academy of Sleep Medicine guidelines (Berry et al., 2020). Sleep parameters included: SOL, ROL, TST, SE, awakenings, number of arousals, arousal index, and stages N1, N2, N3, and REM sleep. SOL was the time from lights-off to the first appearance of any sleep stage; a value of 60 min was allocated when participants did not sleep during a nap opportunity. ROL was defined as the time from SOL to the first epoch scored as REM sleep. TST was defined as the sum of stages N1, N2, N3, and REM sleep. SE was calculated by dividing TST over the time in bed and expressed as a percentage. Awakenings were defined as

time spent in ≥ 2 epochs scored as wake (i.e., for ≥ 1 min). Arousals were defined as abrupt shifts of a sleep stage to theta, alpha, or beta frequencies, lasting 3–15 s. Arousal index was calculated as number of arousals per hour of sleep. CBT was recorded every 15 s using a rectal probe, inserted 10-cm into the rectum (DeRoyal General Purpose Temperature Probe, Powell, TN). After visual inspection of CBT recordings, declines in temperature > 0.1°C within 15 s associated with artifacts and probe "slips" were removed by an automatic in-house program and manually confirmed. CBT was not collected in one postmenopausal woman. Melatonin was assayed from saliva samples collected twice every wake period (upon awakening and before lights-off). Saliva was collected using passive drooling technique in young women and Salivettes in postmenopausal women. Melatonin was assayed in duplicate using a specific radioimmunoassay in young women (Stockgrand Ltd, Guilford, UK, coefficient of variation: 8.5%, lower limit of detection: 0.2 pg/ml; Buhlman Alpco Diagnostics, Windham, NH, mean intra and interassay coefficient of variation: 7.9% and 9.8%, respectively, lower limit of detection: 0.2 pg/mL) (Boivin et al., 2016), and ELISA in postmenopausal women (Salimetrics, coefficient of variation: 7.27%, lower limit of detection: 0.78 pg/mL). Self-reported alertness was measured twice per wake period, using a 10-cm bipolar visual analog scale (VAS), anchored by the statements "sleepy" (0 cm) and "alert" (10 cm). Post-nap alertness tests were completed upon awakening, and mid-wake alertness tests were completed 20 (postmenopausal women) or 30 (young women) min after awakening. Post-nap alertness was not available for one young woman. Self-reported sleep quality was assessed upon awakening using a Likert scale from 0 to 6 (0 = poor sleep; 6 = good sleep). Mood and stress levels were also evaluated by 10-cm VAS twice every wake period (mood: happy—sad; stress: relaxed—stressed). Chronotype was assessed once with the Morningness–Eveningness Questionnaire (Horne & Östberg, 1976), which was administered before starting the ambulatory phase.

2.6.6 Statistical analyses

Statistical analyses were performed in R version 4.0.0 (R Core Team, 2020). Normality of all data were verified with the Shapiro-Wilk test. To be able to compare data between postmenopausal women and young women, only the first 48 hours of the USW procedure

were used for all analyses. The package “lme4” (Bates et al., 2015) was used for linear mixed-effect models.

For the baseline sleep period preceding the USW procedure, each sleep parameter was compared between postmenopausal women and young women with a two-sided t-test or Mann-Whitney U tests, when appropriate. Bedtimes and rise-times were compared between groups using circular statistics in the package “circular” (Agostinelli, 2017). To further observe the variation of sleep parameters across the baseline sleep episode, sleep parameters were additionally calculated for each third of the time spent in bed. Since number and duration of sleep cycles displays a high inter-individual variability, the baseline sleep period was analyzed by thirds of the time-in-bed period. This was selected for a consistent comparison between our groups (Svetnik et al., 2017). Baseline sleep parameters were then compared between groups using linear mixed-effects models. Participant parameters were considered as random effects. “Group” and “time” within the sleep period were considered as fixed effects as sleep architecture changes across the night (Shechter et al., 2010). Using a forward method to build the model, likelihood-ratio tests were used to test the significance of the fixed effects.

In the present study, CBT and melatonin were used as circadian markers. Circadian parameters of the CBT and melatonin rhythms were obtained using a cosinor analysis based on time of day as well as time elapsed into the USW procedure. Similarly, parameters for the circadian variation of sleep and alertness were calculated based on time of day as well as time elapsed into the USW procedure. Cosinor analysis was performed with a linear mixed-effects model with a modified version of the package “cosinor” (Michael Sachs, 2014) to include random effects. Circadian rhythm parameters included mesor, amplitude, phase, and phase angle. The mesor was defined as the average value of the fitted rhythm and amplitude corresponded to the difference between mesor and trough values. The acrophase, defined as the peak time of a circadian rhythm, was used as a phase marker for sleep parameters and self-reported alertness. The nadir, defined as the time of minimum, was used as the phase marker for the rhythm of CBT. Phase angle was calculated by subtracting the time of the phase marker from the habitual rise-time. Therefore, a positive phase angle represents a phase occurring before habitual

rise-time, whereas a negative phase angle represents one occurring after habitual rise-time. Mesor, amplitude, phase, and phase angle were compared between groups using two-sided t-test.

To compare the within- and between-group circadian variations of sleep parameters, self-reported measures (alertness, mood, stress), CBT, and melatonin during the USW procedure, data were first aligned by the time elapsed into the USW (i.e., the habitual rise-time). Data were then collapsed into 2-h bins and compared using linear mixed-effects models with factors “group” and “time” elapsed into the USW, since sleep, alertness, CBT, and melatonin were shown to follow a circadian variation (Boivin et al., 2016). Participant parameters were considered as random effects. Using a forward method to build the model, likelihood-ratio tests were used to test the significance of the fixed effects. Tukey’s *post hoc* tests were performed when group × time interactions were significant.

To exclude a possible bias of ambulation on mid-wake alertness levels in postmenopausal women, further analyses were performed excluding wake periods in which walking around the bedroom was required.

Since we included a participant using hormone replacement therapy in the postmenopausal women group, we evaluated her sleep parameters, alertness, and CBT data for outliers. Using the packages “lme4” and “merTools” (Bates et al., 2015; Knowles, 2020), random effect estimates from these parameters were extracted for each participant. The random effect estimates of the postmenopausal woman using hormone replacement therapy remained within 2 SD for all parameters, and her data were thus considered adequate to be included in the analyses.

2.6.7 Melatonin correction

Since melatonin was collected and assayed differently between young (n = 11: RIA, passive drooling, n = 1: ELISA, passive drooling) and postmenopausal women (n = 8: ELISA, Salivette), two corrections were calculated to compare melatonin data between groups. A first correction was calculated to convert melatonin assayed with RIA to ELISA equivalent using data provided by Salimetrics on a sample size of n = 10. ELISA melatonin value = RIA value * 1.21837 + 3.8767. A second correction was calculated to

transform data collected with Salivette to an equivalent of passive drooling. The correction factor was based on data obtained from 8 young and 5 postmenopausal women whose saliva was collected three times for one night, using both passive drooling and Salivette. Passive drooling value = Salivette value * 0.8657 + 0.7814. As a result, melatonin data were transformed to ELISA and passive drooling equivalent for statistical analyses.

2.7 Results

The 8 postmenopausal women were older, showed higher body mass index, and earlier habitual bedtimes and rise-times compared to the 12 young women (Supplementary Table 2.1). Chronotype did not differ between groups.

2.7.1 Sleep parameters during baseline sleep

The results from the PSG recordings obtained during the 8-h baseline sleep period are summarized in Supplementary Table 2.3 and 2.4. During this nocturnal sleep period, awakenings, number of arousals, arousal index, and time spent in stage N1 sleep were higher in postmenopausal compared to young women. The remaining sleep parameters did not differ between groups. When analyzed by thirds of the period spent in bed, a significant main effect of time was observed in TST, stage N1, N3, and REM sleep, whereas a main effect of group yielded more arousals and more stage N1 sleep in postmenopausal women when compared to young women (Figure 2.2).

2.7.2 Sleep parameters during the USW procedure

The variation of SOL, ROL, TST, arousals, and sleep stages based on time elapsed into the USW procedure is depicted in Figure 2.3, and statistical results are provided in Supplementary Table 2.5. A linear-mixed effects model showed a significant main effect of time on SOL, ROL, TST, number of arousals and stages N1, N2, N3, and REM sleep. A significant main effect of group showed shorter SOL, longer ROL, more TST, a higher number of arousals, more stage N1 sleep and more stage N2 sleep in postmenopausal women than in young women. Group × time interactions were observed for SOL, ROL, TST, and arousals count. Tukey's *post hoc* test on significant interactions revealed that postmenopausal women showed shorter SOL, longer ROL, and more TST during the biological day (i.e., the time between the habitual rise-time and bedtime of each

individual) as well as a greater number of arousals during the biological day and surrounding the habitual bedtime and rise-time.

Cosinor analysis based on time elapsed into the USW yielded significant circadian variation of SOL, ROL, TST, arousals count, stages N1, N2, N3, and REM sleep, and are evident in Figure 2.3. The circadian parameters of these sleep measures are reported in Table 2.1. In comparison to young women, we observed a higher mesor in the rhythms of ROL, TST, arousals count and index, stage N1 sleep, and stage N2 sleep, and a lower mesor in the rhythm of SOL in postmenopausal women. Postmenopausal women also presented a lower amplitude in the rhythms of SOL, TST, and stage N3 sleep, whereas a higher amplitude was observed in the rhythm of arousals count and arousal index. Compared to young women, the phase of SOL, ROL, TST, and stage N1 occurred later in the sleep–wake cycle in postmenopausal women (as reflected by smaller positive or larger negative phase angles). The phase angle of ROL shows that its acrophase occurred after and before habitual rise-time in postmenopausal and young women, respectively (Table 2.1). When looking at the time-of-day at which REM sleep acrophase occurred, it was earlier in postmenopausal women compared to young women (Supplementary Table 2.6.). The reverse was observed for ROL. No statistically significant difference was observed for the time-of-day occurrence of other sleep parameters.

The circadian variation of self-reported sleep quality across the USW procedure is depicted on Supplementary Figure 2.2 and statistical results are provided in Supplementary Table 2.5. A significant main effect of time was observed in self-reported sleep quality with no significant effect of group and group × time interactions. Cosinor analysis showed a significant circadian variation with no significant between-group differences in mesor, amplitude, or phase.

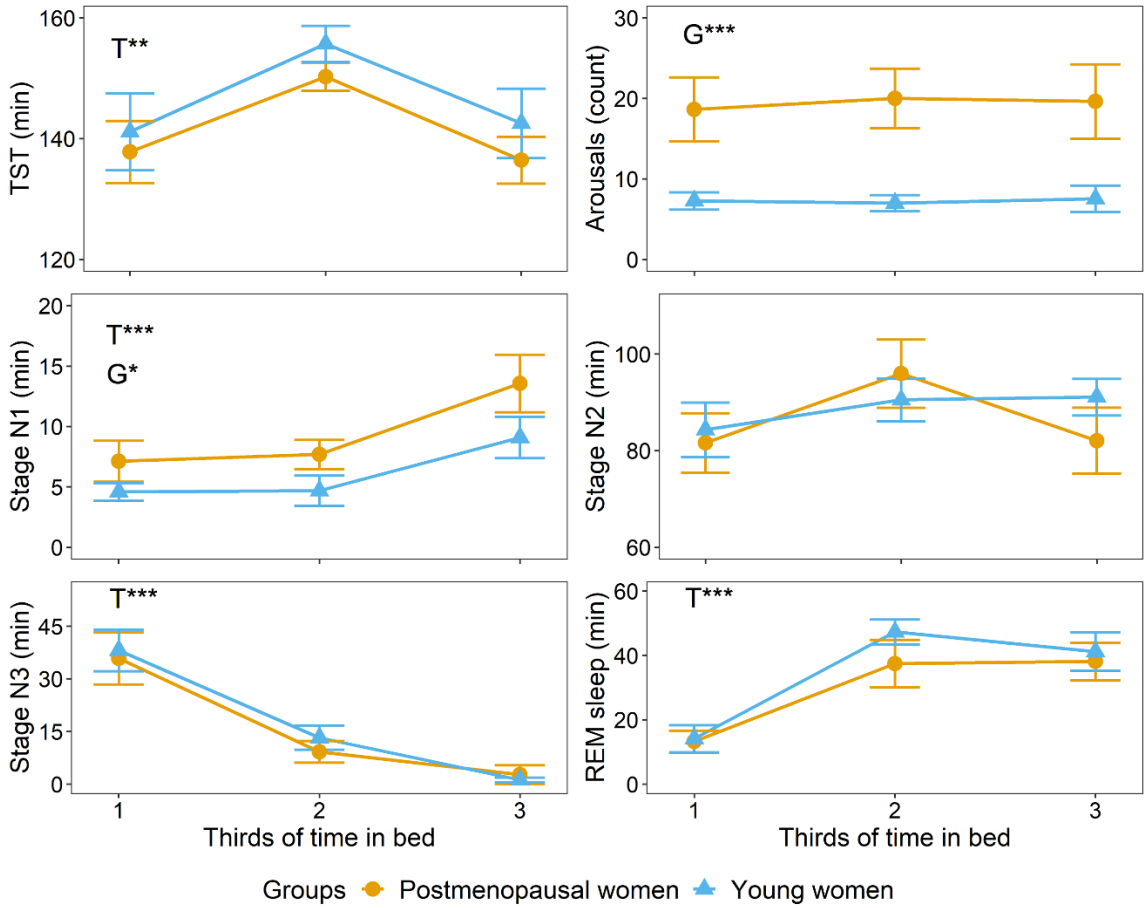


Figure 2. 2. Variation of TST, arousals count, stage N1, stage N2, stage N3, and REM sleep duration during the 8-h baseline sleep period of postmenopausal women and young women.

All baseline sleep parameters were divided into thirds of time spent in bed. Data are presented as mean ± SEM. T = significant main effect of time. G = significant main effect of group. *P < 0.05, *P < 0.01, ***P < 0.001. Statistics provided in Supplementary Table 2.4.

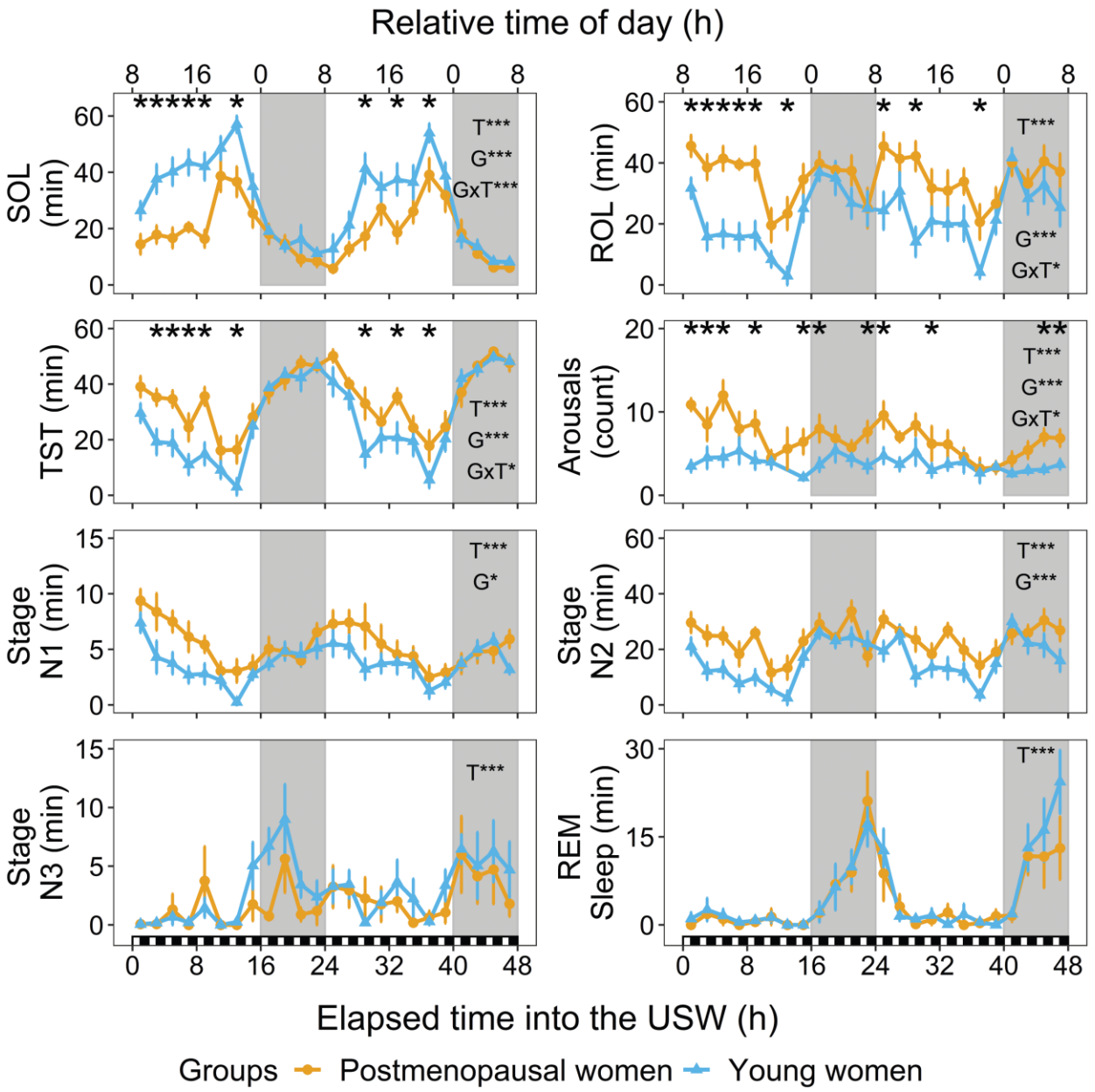


Figure 2. 3. Variation of SOL, ROL, TST, arousals, stage N1, stage N2, stage N3, and REM sleep, in postmenopausal women and young women.

Data were aligned based on the time elapsed into the ultradian sleep-–wake cycle procedure (USW, bottom × axis). Black (~0 lux) and white (~10 lux) small squares above the bottom X axis represent the nap and wake periods across the first 48 h of the USW procedure. The top X axis depicts the corresponding time of day for a participant with a bedtime of 00:00 to 08:00 h. As reported in Supplementary Table 2.1, habitual sleep times are not the same for both study groups. Large gray rectangles depict the projected time

of the habitual nocturnal sleep period, which corresponds to the time between habitual bedtime and rise-time. The asterisks (*) along the top × axis of SOL, ROL, TST, and arousals indicate significant group differences by time ($P < 0.05$). T = significant main effect of time. G = significant main effect of group. G × T = significant group-by-time interaction. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Values are presented as mean ± SEM. Statistics provided in Supplementary Table 2.5.

	Circadian parameters	PMW **Mean** ± SEM [95% CI]	YW **Mean** ± SEM [95% CI]	*p-values*
SOL	Mesor (minutes)	19.15±1.59 [16.03, 22.28]	29.88±2.07 [25.83, 33.93]	**<0.001**
	Amplitude (minutes)	11.71±1.80 [8.17, 15.25]	18.79±1.46 [15.92, 21.66]	**0.002**
	Phase angle (h)	11.80±0.52 [12.82, 10.79]	13.72±0.27 [14.25, 13.18]	**0.001**
ROL	Mesor (minutes)	35.21±1.85 [31.58, 38.83]	22.29±2.40 [17.58, 26.99]	**<0.001**
	Amplitude (minutes)	6.43±1.58 [3.33, 9.52]	9.65±1.32 [7.06, 12.23]	0.12
	Phase angle (h)	-1.67±0.95 [0.20, -3.54]	2.57±0.52 [3.60, 1.54]	**<0.001**
TST	Mesor (minutes)	34.81±1.37 [32.12, 37.49]	27.65±1.78 [24.17, 31.13]	**<0.001**
	Amplitude (minutes)	12.83±1.68 [9.55, 16.12]	18.79±1.43 [15.98, 21.60]	**0.007**
	Phase angle (h)	0.61±0.56 [1.71, -0.49]	2.04±0.31 [2.64, 1.43]	**0.026**
Arousals count	Mesor (count)	6.87±0.53 [5.82, 7.92]	3.62±0.71 [2.23, 5.01]	**<0.001**
	Amplitude (count)	2.28±0.34 [1.61, 2.95]	0.51±0.35 [-0.16, 1.19]	**0.001**
	Phase angle (h)	-3.00±0.55 [-1.91, -4.08]	-4.24±2.54 [0.74, -9.21]	0.52
Arousal index	Mesor (count/h)	4.32±0.37 [3.59, 5.05]	2.11±0.49 [1.15, 3.08]	**<0.001**
	Amplitude (count/h)	2.10±0.21 [1.68, 2.52]	0.70±0.23 [0.26, 1.14]	**<0.001**
	Phase angle (h)	-1.42±0.38 [-0.68, -2.16]	0.58±0.36 [2.85, -1.69]	0.35
Stage N1	Mesor (minutes)	5.35±0.50 [4.36, 6.34]	3.66±0.65 [2.39, 4.94]	**0.010**
	Amplitude (minutes)	2.33±0.36 [1.62, 3.04]	1.66±0.28 [1.12, 2.21]	0.15
	Phase angle (h)	-2.87±0.61 [-1.68, -4.06]	-0.30±0.75 [1.16, -1.76]	**0.007**
Stage N2	Mesor (minutes)	23.55±1.24 [21.12, 25.97]	16.01±1.60 [12.86, 19.15]	**<0.001**
	Amplitude (minutes)	5.23±1.27 [2.74, 7.72]	8.20±1.15 [5.95, 10.45]	0.08
	Phase angle (h)	0.47±1.17 [2.77, -1.83]	2.34±0.58 [3.48, 1.21]	0.15
Stage N3	Mesor (minutes)	1.87±0.59 [0.71, 3.02]	2.96±0.76 [1.47, 4.46]	0.15
	Amplitude (minutes)	0.95±0.53 [-0.09, 1.99]	2.67±0.46 [1.78, 3.56]	**0.014**
	Phase angle (h)	3.25±2.07 [7.30, -0.80]	4.59±0.58 [5.73, 3.44]	0.53
REM sleep	Mesor (minutes)	4.08±0.61 [2.89, 5.28]	4.95±0.79 [3.40, 6.50]	0.27
	Amplitude (minutes)	5.69±0.86 [4.00, 7.39]	7.19±0.72 [5.78, 8.60]	0.18
	Phase angle (h)	1.62±0.58 [2.74, 0.49]	1.31±0.38 [2.05, 0.57]	0.66
Self-reported sleep quality	Mesor (Likert)	3.70±0.32 [3.07, 4.33]	3.09±0.42 [-1.43, 0.23]	0.15
	Amplitude (Likert)	0.77±0.26 [0.26, 1.28]	0.77±0.22 [0.34, 1.21]	0.98
	Phase angle (h)	0.11±0.62 [1.32, -1.09]	1.09±0.60 [2.26, -0.08]	0.25

Table 2. 1. Circadian sleep parameters of postmenopausal women (PMW) and young women at mid-follicular phase (YW) based on time elapsed into the ultradian sleep–wake cycle procedure.

Phase angle was calculated by subtracting the acrophase from the habitual rise-time. P-values for were based on two-tailed t-test. Data are expressed as mean ± SEM.

2.7.3 Self-reported measures, CBT, and melatonin during the USW procedure

The circadian variation of post-nap alertness, mid-wake alertness, CBT, and melatonin based on the elapsed time since the start of the USW procedure is depicted in Figure 2.4, and statistical results are provided in Supplementary Table 2.5. Significant main effects of time were observed for post-nap alertness, mid-wake alertness, CBT, and melatonin. A main effect of group revealed higher mid-wake alertness, and lower CBT and melatonin levels in postmenopausal women compared to young women. Importantly, group × time interactions for mid-wake alertness and melatonin were observed. Mid-wake alertness yielded higher levels in postmenopausal women during the first wake episode of the USW procedure, from 16 to 26 h since start of USW (i.e., at the time corresponding to the first habitual nocturnal sleep), and at 41 h after starting the USW. Melatonin was shown to be lower during the first 2 h of USW and during the first 4 h of the habitual night period in postmenopausal vs young women. No significant interaction was obtained in post-nap alertness and CBT.

Results were similar even when excluding wake periods during which walking around the room occurred. The group effect with higher mid-wake alertness in postmenopausal women remained significant, with a trend for a group × time interaction (Supplementary Figure 2.3).

Circadian parameters based on cosinor analysis of post-nap and mid-wake alertness, CBT, and melatonin are shown in Table 2.2. Post-nap and mid-wake alertness, CBT, and melatonin showed a significant circadian variation based on time of day and time elapsed into the USW. Compared to young women, we observed a higher mesor of the mid-wake alertness rhythm, lower amplitude of the post-nap alertness and melatonin rhythms, and lower mesor of the CBT rhythm in postmenopausal. No other significant group difference was observed for the circadian parameters of alertness, CBT, or melatonin.

No significant effect of time was observed for mood and stress levels ($P > 0.33$). Postmenopausal women rated themselves with better mood ($P = 0.04$) and comparable stress levels ($P = 0.08$) than young women.

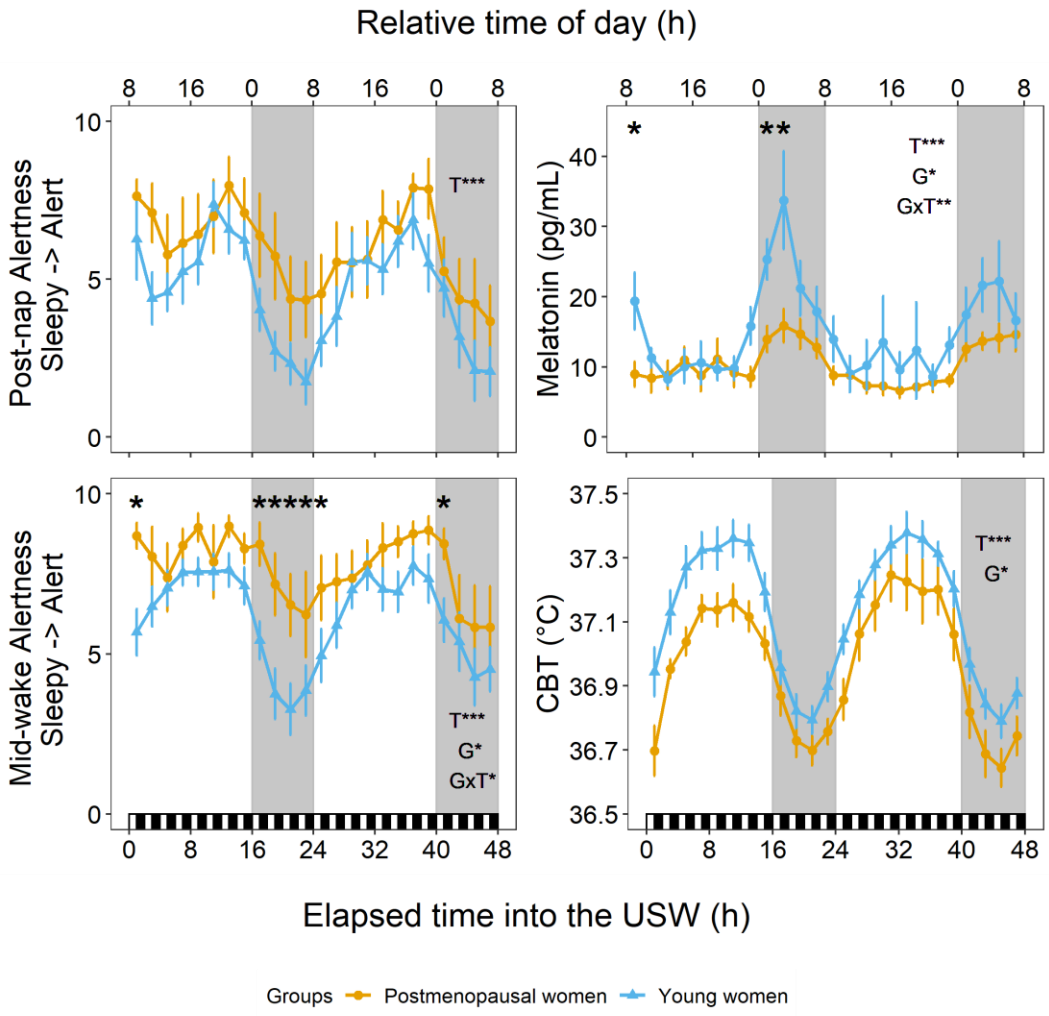


Figure 2. 4. Variation of post-nap alertness, mid-wake alertness, melatonin, and CBT on postmenopausal women and young women.

The top and bottom X axes are as in Figure 2.3. The asterisks (*) along the top × axis of mid-wake alertness, indicates significant group by time differences ($P < 0.05$). Values are presented as mean ± SEM. Statistics provided in Supplementary Table 2.5.

	Circadian parameters	PMW **Mean** ± SEM [95% CI]	YW **Mean** ± SEM [95% CI]	*p-values*
Post-nap alertness[a]	Mesor (cm)	6.01±0.75 [4.54, 7.48]	4.57±0.99 [2.64, 6.50]	0.14
	Amplitude (cm)	1.22±0.28 [0.68, 1.76]	2.08±0.24 [1.60, 2.55]	**0.019**
	Phase angle (h)	-10.25±0.87 [-8.54, -11.95]	-10.30±0.44 [-9.44, -11.17]	0.95
Mid-wake alertness	Mesor (cm)	7.76±0.59 [6.62, 8.91]	6.22±0.76 [4.74, 7.70]	**0.042**
	Amplitude (cm)	0.97±0.30 [0.39, 1.56]	1.68±0.24 [1.21, 2.15]	0.07
	Phase angle (h)	-10.46±1.10 [-8.29, -12.62]	-9.27±0.54 [-8.21, -10.33]	0.33
CBT[b]	Mesor (°C)	36.98±0.05 [36.87, 37.08]	37.12±0.07 [36.99, 37.26]	**0.033**
	Amplitude (°C)	0.28±0.03 [0.22, 0.34]	0.29±0.02 [0.25, 0.34]	0.80
	Phase angle (h)	2.91±0.41 [3.71, 2.10]	2.94±0.30 [3.53, 2.35]	0.95

Table 2. 2. Circadian parameters of post-nap alertness, mid-wake alertness, and CBT of PMW and YW at mid-follicular phase based on time elapsed into the USW procedure. [a]YW: n = 11. [b]PMW n = 7. Phase angle was calculated by subtracting the phase marker from the habitual rise-time. P-values for were based on two-tailed t-test. Data are expressed as mean ± SEM.

2.8 Discussion

Menopause is associated with fluctuations in sex hormones that may affect circadian physiology, but the role of circadian factors in sleep disturbances after menopause is not well-understood. The present study aims to understand the changes occurring after menopause in the circadian variation of sleep and waking. The primary finding in this small group of postmenopausal women with no sleep complaints was a general increase in light sleep and number of arousals during baseline sleep and across circadian phases, as well as a clear dampening of the circadian variation of sleep, alertness, and melatonin rhythms. To our knowledge, our study is the first one to describe the circadian variation of sleep in women after menopause.

2.8.1 Baseline sleep period

Only a few studies have explored the specific changes of polysomnographic sleep after menopause and the changes reported in the architecture of sleep are not homogeneous (Shaver & Woods, 2015).

In our group of postmenopausal women, the habitual timing of sleep was on average 1.1 h earlier than in young women, possibly as a consequence of aging (Duffy et al., 2015). Earlier timing of the sleep schedule has been associated with earlier chronotypes (Randler & Bausback, 2010) which can provide some insight into the circadian timing system (Jones et al., 2019). In the present study, chronotype differences were not observed between postmenopausal and young women, possibly due to the less pronounced changes in women towards morningness with aging (Roenneberg et al., 2007). In a European cross-sectional population-wide study, age-dependent changes in chronotype using the Munich Chronotype Questionnaire showed that women and men both become earlier chronotypes with aging (Roenneberg et al., 2007). Interestingly, this study revealed that the difference in chronotype with aging is less pronounced in women, since they are earlier chronotypes, on average, than men. Sex differences in chronotype disappears after the age 52, around the time of menopause. The less pronounced changes in women towards a morningness preference with aging might explain the absence of significant differences in chronotype between our groups. In the present study, TST and SE were similar for postmenopausal and young women during the baseline nocturnal sleep period, which is consistent with previous research involving premenopausal, perimenopausal and postmenopausal women aged <60 years (de Zambotti et al., 2015; Lampio et al., 2017; Young et al., 2003). Other studies that specifically focused on age-related changes in nocturnal polysomnographic sleep have shown decreased TST and SE with aging, including middle-aged women (Carrier et al., 2001; Mitterling et al., 2015; Ohayon et al., 2004; Schwarz et al., 2017). However, these findings were more pronounced in women over 60 years old, which are older than all but one of the postmenopausal women in our study group. A recent cross-sectional study examined age-related changes in 69,650 adults of both sexes aged 19-67, using wearable activity trackers (Jonasdottir et al., 2021). Sex differences were observed in night-time awakenings with higher values in women compared to men. The greatest disparities between sex occurred during active-reproductive years of women. Interestingly, when looking at weekends sleep duration and timing (presumably more influenced by biological factors), clear sex differences were observed in younger and middle-aged individuals and tended to disappear in older adults. These observations are

coherent with a role of ovarian hormones in the circadian regulation of sleep (Jonasdottir et al., 2021).

We observed more stage N1 sleep, more awakenings, a greater number of arousals, and a higher arousal index in postmenopausal compared to young women. These results show that menopause is a period of increased sleep fragmentation with lighter sleep stages, which are in line with previous research showing more time awake, arousals, and stage N1 sleep in menopausal women and aged individuals (Hachul et al., 2015; Jonasdottir et al., 2021; Kalleinen et al., 2020; Kalleinen et al., 2008; Lampio et al., 2017; Mitterling et al., 2015; Ohayon et al., 2004; Schwarz et al., 2017; Young et al., 2003). Interestingly, we did not find group differences in stage N3 and REM sleep. Whereas studies including pre-, peri-, and postmenopausal women did not show differences in stage N3 (also called slow wave sleep; SWS) and REM sleep as a function of menopausal status (de Zambotti et al., 2015; Kalleinen et al., 2008; Lampio et al., 2017; Young et al., 2003), aging studies have reported an overall reduction of these sleep stages throughout life in women 20-85 years old (Mitterling et al., 2015; Ohayon et al., 2004; Schwarz et al., 2017). It is well known that SWS architecture and REM sleep are influenced by sleep disorders and rebound sleep (Mokhlesi et al., 2014; Schwarz et al., 2017), which we controlled for with our screening procedures and pre-study instructions. Indeed, since REM sleep and SWS decrease as a function of aging, the lack of group differences in REM sleep and SWS in the present study might be explained by the younger and limited age range of our group of postmenopausal women compared to these aging studies.

2.8.2 Circadian rhythms

For the present study, we used CBT and melatonin rhythms as reliable circadian markers. We observed that postmenopausal women had a lower CBT mesor and a dampened circadian amplitude of melatonin compared to young women.

Since CBT is influenced by body metabolism, the reduction of CBT in postmenopausal women has been hypothesized to be a consequence of a reduced metabolic resting expenditure due to aging and/or hormonal changes (Messina et al., 2013; Neff et al., 2016). Consistent with our findings, studies showing age-related changes in circadian rhythms, using either USW or forced desynchrony protocols, also

found a lower mesor of the CBT rhythm in a combined group of older men and women compared to younger participants (Buysse et al., 2005; Duffy et al., 1998; Yoon et al., 2003). On average, mesor differences in CBT between young and older individuals ranged from 0.14–0.30 °C, of comparable magnitude to the 0.15 °C temperature difference between groups in the present study. Since estrogen and progesterone have hypothermic and hyperthermic effects, respectively, hormonal changes occurring at menopause might be another contributing mechanism to the declining CBT. In naturally cycling women aged 19-29 years, the fluctuation of the progesterone/estradiol ratio across the menstrual cycle has been associated with changes in body temperature. Specifically, women in the luteal phase presented an average progesterone/estradiol ratio of ~120, whereas this ratio was ~30 in the follicular phase, coincidental with lower CBT levels during the night in follicular vs luteal phase (Grant et al., 2020). Progesterone and estradiol levels decline during menopause and a progesterone/estradiol ratio of 6.24 was calculated for our group of postmenopausal women. As such, hormonal changes following menopause may additionally contribute to the age-related reduction of CBT.

Group differences were not observed in circadian phase and amplitude of CBT, although an advanced circadian phase and dampened amplitude of CBT with aging have been described in the literature (Duffy et al., 1998; Kim et al., 2014). The earlier sleep schedule combined with a similar phase angle between CBT minimum and the habitual rise-time observed in postmenopausal women led us to hypothesize that the lack of group differences in CBT phase might be due to our small sample size. Since CBT declines during sleep (Duffy et al., 1998), the scheduling of naps during our USW might have exerted some masking effect on this rhythm, although it probably had minimal effects on our ability to assess circadian phase since naps were equally spaced across the USW.

The similar phase angle between our groups suggests that postmenopausal and young women slept at similar circadian phases. These results are consistent with prior studies that found no phase angle differences between young and older adults (Carrier et al., 2002; Munch et al., 2005), as well as with those comparing pre- and postmenopausal women (Walters et al., 2005), although shorter phase angles have also been reported with aging (Duffy et al., 2002). Furthermore, a stable phase angle of

entrainment is coherent with the stable circadian period reported with aging (Duffy et al., 2011).

In the present study, the circadian rhythm of melatonin was dampened in postmenopausal compared to young women, with no phase or phase angle differences. The reduced amplitude of melatonin is consistent with prior studies comparing young and older adults, and of comparable magnitude (Kim et al., 2014; Yoon et al., 2003). Prior studies reported an advanced melatonin rhythm with aging, but no between-sex comparisons were performed (Yoon et al., 2003; Youngstedt et al., 2019). In a large study of 99 participants using an USW protocol, older adults had earlier melatonin rhythms than younger ones, and women displayed earlier melatonin rhythms than men (Youngstedt et al., 2019). Another study employing an USW procedure (Yoon et al., 2003) found that older adults presented an earlier acrophase of urinary 6-sulfatoxymelatonin than younger adults, although no sex differences were observed. In these two studies, aged individuals were older than our group of postmenopausal women (mean ± SD: 66.1 ± 4.7 y; 66.2 ± 4.9 y vs 54.8 ± 3.37 y in our study), and age-related differences by sex were not analyzed. It thus remains difficult to compare our results with these previous studies to address the effects of hormonal changes after menopause.

Interestingly, Walters and colleagues (Walters et al., 2005) showed, in a constant routine protocol, that postmenopausal women aged between 50 and 60 years presented earlier melatonin rhythms of similar amplitude than those of younger premenopausal women aged between 35 and 50. Compared to our study, the study of Walters and colleagues (Walters et al., 2005) had an older group of premenopausal women, whereas postmenopausal women were of similar age. It is thus probable that the dampening in melatonin circadian amplitude in our postmenopausal women might be attributed to an age effect other than hormonal changes alone. The dampened amplitude of melatonin observed in postmenopausal women has been described with aging as a consequence of decreased beta-adrenergic receptors and gene expression of Serotonin N-acetyltransferase in the pineal gland, necessary for melatonin synthesis (Tan et al., 2018). However, hormonal changes at menopause may also influence melatonin levels since sex steroid receptors have been found in the pineal gland (Luboshitzky et al., 1997), but

the effects of sex steroids on melatonin secretion is complex and requires further experimentation (Cipolla-Neto et al., 2022).

2.8.3 Diurnal variation of sleep

We observed a significant diurnal variation of SOL, ROL, TST, arousals, sleep stages N1, N2, N3, REM sleep, and self-reported sleep quality throughout the USW procedure in both postmenopausal and young women.

The rhythm of TST and SOL provided an insight into the diurnal variation of sleep propensity. Contrary to our expectations, postmenopausal women, on average, fell asleep more rapidly and slept more across all circadian phases than young women. These group differences can be attributed to shorter SOL and more TST during the biological day for postmenopausal women, as no group differences were observed during the biological night. These differences resulted in lower circadian amplitudes for these sleep parameters in postmenopausal compared to young women.

Our findings might initially seem contradictory of the current literature describing longer SOL and less TST with aging in USW protocols (Buysse et al., 2005; Munch et al., 2005; Yoon et al., 2003), and during the night in forced desynchrony protocols (Dijk et al., 1999). There are some factors that may account for these discrepancies. First, previous aging studies regarding the circadian variation of sleep did not address sex differences (Buysse et al., 2005; Dijk et al., 2001; Dijk et al., 1999; Munch et al., 2005; Yoon et al., 2003) and therefore, did not specifically explore the age-related changes in women. Geisler and colleagues (Geisler et al., 2006) used a multiple sleep latency test with naps scheduled between 9:00 h and 17:00 h to show that sleep latency was shorter for middle-aged women than for younger and older women. Our results showing shorter SOL for postmenopausal women are in line with these results considering that our participants were all middle-aged, except for one who was 61 years old. Second, the group differences in TST and SOL were only observed for nap opportunities occurring during the day and resulted in a lower amplitude of the diurnal rhythm of these sleep parameters. These results in postmenopausal women are consistent with those of other investigations of aging and additionally supported by the observed reduced amplitude of the circadian variation of stage N3 sleep in postmenopausal women. Other studies employing USW or

forced desynchrony procedures have shown older adults with smaller circadian amplitudes of sleep latency, time spent asleep, and slow wave sleep than younger adults (Buysse et al., 2005; Dijk et al., 1999; Munch et al., 2005), while one study found the opposite (Yoon et al., 2003). Altogether, these findings support the hypothesized impaired output of the circadian pacemaker in older adults (Dijk & Duffy, 2020). However, sex differences were not analyzed and disentangling age-related sleep differences by sex remains difficult.

The diurnal variation of number of arousals indicated that sleep was more disrupted for postmenopausal women than young women. Postmenopausal women consistently presented more arousals per nap, whether scheduled in the day or night, which is consistent with aging studies performed in USW procedures and forced desynchrony protocols (Buysse et al., 2005; Dijk et al., 2010; Klerman et al., 2004). In young women, arousals count was very low throughout circadian phases, thus leading to a smaller rhythm than that of postmenopausal women. In terms of sleep stages, subtle differences were observed between our study groups in the diurnal variation of stages N1, N2, N3, and REM sleep. Postmenopausal women presented, on average, more stage N1 and N2 sleep (i.e., a higher mesor) than young women but no group differences in stage N3 and REM sleep, similar to our observations during the baseline sleep period. The increased duration of lighter stage N1 sleep throughout the USW may reflect the increased frequency of arousals and subsequent transitions from wake to light sleep in postmenopausal women. Despite these observations, self-reported sleep quality remained similar between groups across the USW procedure. This confirmed that both groups reported themselves as good sleepers, but that unperceived changes in the macrostructure of sleep occur after menopause. As for phase angle results, we observed that the phase of SOL, ROL, TST, and N1 sleep occurred later in the sleep-wake cycle of postmenopausal than young women. These show a delay shift in the timing of sleep propensity, with the highest values of TST slightly closer to the habitual time of awakening in postmenopausal compared to young women. Although subtle changes were observed in the timing of sleep propensity at menopause, the clinical implication of these observations remains unclear. When results were analyzed based on time of day, the circadian variation of REM sleep propensity occurred earlier in postmenopausal women.

This observation is consistent with their earlier habitual sleep period and the very sharp pattern in the circadian variation of REM sleep.

2.8.4 Alertness across the USW procedure

Alertness measured upon awakening and at 20-30 minutes after waking exhibited significant diurnal variations for both postmenopausal and young women, and both rhythms followed a similar pattern throughout the USW procedure, with a peak and nadir observed in the early evening and late night, respectively. As SOL, the lowest alertness values occurred late at night and close to the habitual time of awakening in postmenopausal and young women. In comparison to young women, postmenopausal women were significantly more alert at mid-wake (i.e., a higher mesor across the USW procedure), with no differences in post-nap alertness. Based on the mid-wake alertness questionnaire, postmenopausal women were more alert than young women during the biological night and early morning, whereas alertness levels were comparable between groups during daytime hours. These results are in line with previous research performed in constant routine and forced desynchrony protocols indicating that older adults are more resilient to the effects of exposure to adverse circadian phases on alertness and performance than younger adults (Blatter et al., 2006; Duffy et al., 2009; Sagaspe et al., 2012; Silva et al., 2010; Zitting et al., 2018). Controversial results have been observed in USW protocols (Buysse et al., 2005; Munch et al., 2005), in which sleep inertia may possibly have impaired the ability to detect age-related differences in the circadian variation of alertness since alertness was measured only upon awakening. In the present study, group differences were only observed in mid-wake alertness levels and these differences persisted even when removing the wake periods during which postmenopausal women were allowed to walk around the room. Although higher alertness levels at night in postmenopausal women are consistent with reports in the literature, we cannot completely exclude an effect of postural changes on the mid-wake alertness of postmenopausal women. However, it is unlikely that such postural effect accounts for between-group differences at specific times of day. These differences are also not related to between group differences in perceived stress levels and hardly explained by better perceived mood in postmenopausal women. Importantly, most of the prior studies on alertness did not address sex differences or age-specific differences in

women (Buysse et al., 2005; Munch et al., 2005; Sagaspe et al., 2012; Silva et al., 2010; Zitting et al., 2018). In the present study, we did not find differences in the timing of alertness acrophase or phase angle. This is consistent with a prior study that compared premenopausal and postmenopausal women under constant routine conditions and found no differences in the timing of alertness offset (Walters et al., 2005).

In the present study, the amplitude of the circadian rhythm of alertness was lower in postmenopausal women than in young women, but only reached significance when measured upon awakening. It remains unclear why the between-age difference in amplitude we observed are limited to the post-nap assessment. These lower amplitudes of melatonin, post-nap alertness, and sleep parameters in postmenopausal women support the hypothesis of a weakening of the circadian signal promoting sleep and wakefulness with aging (Buysse et al., 2005; Dijk & Duffy, 2020). Interestingly, between-age differences were more consistent at night for alertness and during the day for sleep. These times of day correspond to the habitual sleep and wake periods, respectively. Higher alertness at night and higher sleep propensity during the daytime in postmenopausal women is also suggestive of a disruption in the temporal organization of the sleep-wake cycle with aging. Overall, the higher mesor of alertness, stage N1 sleep, and number of awakenings during sleep in older women suggest an increase in the strength of the arousal signal and a reduction in the strength of the sleep signal that represent physiological differences potentially contributing to the higher prevalence of insomnia complaints in postmenopausal women.

It is well known that the circadian variation of alertness and neurobehavioral performance parallels the rhythm of CBT, with observed crests and nadirs close to those of the CBT rhythm (Grant et al., 2020). Interestingly, constant routine studies in young adults have shown that women in their follicular phase have poorer performance and lower CBT at night than both men (Vidafar et al., 2018) and women in luteal phase (Grant et al., 2020; Vidafar et al., 2018; Wright Jr & Badia, 1999). It was proposed that lower CBT could impair neurobehavioral performance, but differences in self-reported alertness were not observed (Grant et al., 2020; Shechter et al., 2010; Vidafar et al., 2018). In the present study, postmenopausal women reported higher alertness at night despite having lower CBT compared to young women. As previously discussed, CBT declines with aging,

and research has shown that older individuals present higher performances than younger ones in psychomotor vigilance test, and less sleepiness measured by Karolinska Sleepiness Scale (Duffy et al., 2009; Silva et al., 2010). Although performance was not measured in the present study, the relationship between CBT and alertness levels remains unclear, even if CBT and alertness follow a similar diurnal variation.

2.8.5 Physiological mechanisms

The mechanisms by which the circadian variation of sleep and alertness differ between postmenopausal and young women may include the effect of hormonal changes associated with aging and other aging processes. In both sexes, a drop in gonadal hormones is part of the normal aging process. These hormonal changes in women are concentrated across the menopausal transition, whereas in men a gradual drop of testosterone levels occurs progressively starting in their middle age (Feldman et al., 2002). It remains difficult to disentangle the effects of hormonal changes from other aging processes, although there is increasing evidence that the drop of ovarian hormones after menopause can have an important effect on sleep-wake organization.

In nocturnal rodents, estradiol administration to ovariectomized animals have been shown to promote wakefulness and sleep during the active and rest phases, respectively, thus consolidating the sleep-wake cycle (Dorsey et al., 2020; Mong & Cusmano, 2016). It was proposed that estradiol could promote wakefulness by inhibiting somnogens such as adenosine and lipocalin-type prostaglandin D in the VLPO, a sleep-promoting nucleus (Dorsey et al., 2020). Since menopause is characterized by declining levels of estradiol and progesterone, it is plausible that the reduction of these hormones in postmenopausal women contributes to their decreased wake propensity during the day and fragmented sleep. This could account for the therapeutic effects of hormonal replacement therapy on sleep disturbances of postmenopausal women, although it remains unclear whether these drugs exert a direct effect on sleep, or improve it by reducing VMS, or both (Cintron et al., 2017; Proserpio et al., 2020). The mechanisms by which reduced levels of sex hormones in postmenopausal women might produce higher alertness at night remains unclear. Besides hormonal changes, other aging processes could be involved. With aging, the number of neurons in the POA and the LHA declines (Kessler et al., 2011; Miller et al., 1989). In postmenopausal women, it is thus possible that age-related changes occurring

in the POA promoting sleep and the LHA promoting wakefulness may also contribute to the reduced amplitude of sleep and alertness propensity, respectively. This hypothesis is consistent with the reduced circadian amplitudes of TST, SOL, and self-reported alertness of postmenopausal women in the current study. Further experimentation will be necessary to clarify the effects of ovarian hormone changes on sleep and circadian rhythms after menopause.

2.9 Strengths and limitations

The small sample size in the present study is an important limitation that may have not allowed us to observe group differences in circadian parameters of CBT. Additionally, we must acknowledge that we used a USW procedure, and not a constant routine protocol, in which the sleep opportunities may mask the CBT. However, naps were scheduled regularly at 2-h intervals, minimizing their masking effect on CBT. On the other hand, the USW procedure allowed us to minimize the effects of sleep deprivation by maintaining constant wake periods lasting 1 h each. Our results need to be taken with caution, since postmenopausal women included in this study were thoroughly screened and thus only represent a minor healthy proportion of this population. However, this also represents a strength of the study as it allowed us to study the specific effect of menopause rather than comorbidities associated with it.

We cannot completely exclude the possibility that slight differences in the scheduling of the baseline sleep period could have affected between-group comparisons. However, the absence of group differences in N3 sleep as well as similar TST and SE suggest its effect is minimal.

In the current study, we accounted for menopausal status, screened for sleep disorders, asked participants to maintain regular nocturnal sleep/darkness at night for 2 weeks prior to entering the laboratory, and confirmed that participants did not nap via actigraphy, thus reducing the possible confound of an irregular sleep schedule.

The group of postmenopausal women also included one participant taking hormone replacement therapy. Nevertheless, her results were within 2 SD of the group data and were deemed adequate to be included in the analyses.

Due to the exploratory design of our study, adjustment for false discovery rate was not performed. Therefore, subsequent studies will be necessary to further explore the role of menopause on sleep and circadian rhythms.

2.10 Conclusion

In the present study, we observed significant changes in the sleep of postmenopausal women at baseline and during the USW procedure. During the baseline sleep, postmenopausal women presented shallower and more fragmented sleep than young women. In the USW procedure, postmenopausal women presented more sleep propensity during the day and higher alertness levels at night, which resulted in a dampening of their circadian variation along with a dampened rhythm of melatonin. It remains difficult to disentangle whether the observed effects in sleep and alertness are due to hormonal changes occurring after menopause, other aging processes, or a combination of both. The described disruption of the temporal organization of the sleep–wake cycle evident at menopause might be one of the many factors contributing to the sleep disturbances occurring at menopause.

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2.12 Data and Material Availability

The data underlying this article cannot be shared publicly because participants did not agree that their data be placed in a publicly accessible database. Therefore, for ethical and confidentiality reasons, the authors cannot provide public access to these data. Nevertheless, materials, data, and protocols will be made available for investigation of scientific integrity if necessary. Readers are free to contact the principal investigator if they wish to initiate discussions regarding research collaborations to build on these published data. The data will be shared on reasonable request to the corresponding author.

2.13 Disclosure Statement

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2.14 References

Agostinelli, C., and Lund, U. (2017). Circular Statistics (version 0.4-93). In https://r-forge.r-project.org/projects/circular

Arnardottir, E. S., Bjornsdottir, E., Olafsdottir, K. A., Benediktsdottir, B., & Gislason, T. (2016). Obstructive sleep apnoea in the general population: highly prevalent but minimal symptoms. Eur Respir J, 47(1), 194-202. https://doi.org/10.1183/13993003.01148-2015

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Usinglme4. J. Stat. Softw., 67(1), 1-48. https://doi.org/10.18637/jss.v067.i01

Berry, R. B., Quan, S. F., Abreu, A. R., Bibbs, M. L., DelRosso, L., Harding, S. M., Mao, M.-M., Plante, D. T., Pressman, M. R., Troester, M. M., & Vaughn, B. V. (2020). The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.6.

Blatter, K., Graw, P., Munch, M., Knoblauch, V., Wirz-Justice, A., & Cajochen, C. (2006). Gender and age differences in psychomotor vigilance performance under differential sleep pressure conditions. Behav Brain Res, 168(2), 312-317. https://doi.org/10.1016/j.bbr.2005.11.018

Boivin, D. B., Shechter, A., Boudreau, P., Begum, E. A., & Ng Ying-Kin, N. M. (2016). Diurnal and circadian variation of sleep and alertness in men vs. naturally cycling women. Proc Natl Acad Sci U S A, 113(39), 10980-10985. https://doi.org/10.1073/pnas.1524484113

Buysse, D. J., Monk, T. H., Carrier, J., & Begley, A. (2005). Circadian patterns of sleep, sleepiness, and performance in older and younger adults. Sleep, 28(11), 1365-1376. https://doi.org/10.1093/sleep/28.11.1365

Carrier, J., Land, S., Buysse, D. J., Kupfer, D. J., & Monk, T. H. (2001). The effects of age and gender on sleep EEG power spectral density in the middle years of life (ages

20-60 years old). Psychophysiology, 38(2), 232-242. https://doi.org/10.1111/1469-8986.3820232

Carrier, J., Paquet, J., Morettini, J., & Touchette, E. (2002). Phase advance of sleep and temperature circadian rhythms in the middle years of life in humans. Neurosci Lett, 320(1-2), 1-4. https://doi.org/10.1016/s0304-3940(02)00038-1

Cintron, D., Lipford, M., Larrea-Mantilla, L., Spencer-Bonilla, G., Lloyd, R., Gionfriddo, M. R., Gunjal, S., Farrell, A. M., Miller, V. M., & Murad, M. H. (2017). Efficacy of menopausal hormone therapy on sleep quality: systematic review and meta-analysis. Endocrine, 55(3), 702-711. https://doi.org/10.1007/s12020-016-1072-9

Cipolla-Neto, J., Amaral, F. G., Soares Jr, J. M., Gallo, C. C., Furtado, A., Cavaco, J. E., Gonçalves, I., Santos, C. R. A., & Quintela, T. (2022). The crosstalk between melatonin and sex steroid hormones. Neuroendocrinology, 112(2), 115-129. https://doi.org/10.1159/000516148

Coleman, R. (1982). Periodic movements in sleep (nocturnal myoclonus) and restless legs syndrome. Sleeping and waking disorders: indications and techniques, 265-295.

de Zambotti, M., Colrain, I. M., & Baker, F. C. (2015). Interaction between reproductive hormones and physiological sleep in women. J Clin Endocrinol Metab, 100(4), 1426-1433. https://doi.org/10.1210/jc.2014-3892

Dijk, D.-J., Duffy, J. F., & Czeisler, C. A. (2001). Age-related increase in awakenings: impaired consolidation of nonREM sleep at all circadian phases. Sleep, 24(5), 565-577. https://doi.org/10.1093/sleep/24.5.565

Dijk, D. J., & Duffy, J. F. (2020). Novel Approaches for Assessing Circadian Rhythmicity in Humans: A Review. J Biol Rhythms, 35(5), 421-438. https://doi.org/10.1177/0748730420940483

Dijk, D. J., Duffy, J. F., Riel, E., Shanahan, T. L., & Czeisler, C. A. (1999). Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J Physiol, 516 (Pt 2)(2), 611-627. https://doi.org/10.1111/j.1469-7793.1999.0611v.x

Dijk, D. J., Groeger, J. A., Stanley, N., & Deacon, S. (2010). Age-related reduction in daytime sleep propensity and nocturnal slow wave sleep. Sleep, 33(2), 211-223. https://doi.org/10.1093/sleep/33.2.211

Dorsey, A., de Lecea, L., & Jennings, K. J. (2020). Neurobiological and Hormonal Mechanisms Regulating Women's Sleep. Front Neurosci, 14, 625397. https://doi.org/10.3389/fnins.2020.625397

Duffy, J. F., Cain, S. W., Chang, A. M., Phillips, A. J., Munch, M. Y., Gronfier, C., Wyatt, J. K., Dijk, D. J., Wright, K. P., Jr., & Czeisler, C. A. (2011). Sex difference in the near-24-hour intrinsic period of the human circadian timing system. Proc Natl Acad Sci U S A, 108 Suppl 3, 15602-15608. https://doi.org/10.1073/pnas.1010666108

Duffy, J. F., Dijk, D. J., Klerman, E. B., & Czeisler, C. A. (1998). Later endogenous circadian temperature nadir relative to an earlier wake time in older people. Am J Physiol, 275(5 Pt 2), R1478-1487. https://doi.org/10.1152/ajpregu.1998.275.5.r1478

Duffy, J. F., Willson, H. J., Wang, W., & Czeisler, C. A. (2009). Healthy older adults better tolerate sleep deprivation than young adults. J Am Geriatr Soc, 57(7), 1245-1251. https://doi.org/10.1111/j.1532-5415.2009.02303.x

Duffy, J. F., Zeitzer, J. M., Rimmer, D. W., Klerman, E. B., Dijk, D.-J., & Czeisler, C. A. (2002). Peak of circadian melatonin rhythm occurs later within the sleep of older subjects. Am J Physiol Endocrinol Metab, 282(2), E297-E303. https://doi.org/10.1152/ajpendo.00268.2001

Duffy, J. F., Zitting, K. M., & Chinoy, E. D. (2015). Aging and Circadian Rhythms. Sleep Med Clin, 10(4), 423-434. https://doi.org/10.1016/j.jsmc.2015.08.002

Feldman, H. A., Longcope, C., Derby, C. A., Johannes, C. B., Araujo, A. B., Coviello, A. D., Bremner, W. J., & McKinlay, J. B. (2002). Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J Clin Endocrinol Metab, 87(2), 589-598. https://doi.org/10.1210/jcem.87.2.8201

Gabbay, I. E., & Lavie, P. (2012). Age- and gender-related characteristics of obstructive sleep apnea. Sleep Breath, 16(2), 453-460. https://doi.org/10.1007/s11325-011-0523-z

Geisler, P., Tracik, F., Crönlein, T., Fulda, S., Wichniak, A., Popp, R., Zulley, J., & Hajak, G. (2006). The influence of age and sex on sleep latency in the MSLT-30—a normative study. Sleep, 29(5), 687-692. https://doi.org/10.1093/sleep/29.5.687

Grant, L. K., Gooley, J. J., St Hilaire, M. A., Rajaratnam, S. M. W., Brainard, G. C., Czeisler, C. A., Lockley, S. W., & Rahman, S. A. (2020). Menstrual phase-dependent differences in neurobehavioral performance: the role of temperature and the progesterone/estradiol ratio. Sleep, 43(2), zsz227. https://doi.org/10.1093/sleep/zsz227

Hachul, H., Frange, C., Bezerra, A. G., Hirotsu, C., Pires, G. N., Andersen, M. L., Bittencourt, L., & Tufik, S. (2015). The effect of menopause on objective sleep parameters: data from an epidemiologic study in Sao Paulo, Brazil. Maturitas, 80(2), 170-178. https://doi.org/10.1016/j.maturitas.2014.11.002

Harlow, S. D., Gass, M., Hall, J. E., Lobo, R., Maki, P., Rebar, R. W., Sherman, S., Sluss, P. M., de Villiers, T. J., & Group, S. C. (2012). Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. J Clin Endocrinol Metab, 97(4), 1159-1168. https://doi.org/10.1210/jc.2011-3362

Horne, J. A., & Östberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol, 4(2), 97-110.

Iber, C. (2007). The AASM manual for the scoring of sleep and associated events: Rules. Terminology and Technical Specification.

Jonasdottir, S. S., Minor, K., & Lehmann, S. (2021). Gender differences in nighttime sleep patterns and variability across the adult lifespan: a global-scale wearables study. Sleep, 44(2), zsaa169. https://doi.org/10.1093/sleep/zsaa169

Jones, S. E., Lane, J. M., Wood, A. R., van Hees, V. T., Tyrrell, J., Beaumont, R. N., Jeffries, A. R., Dashti, H. S., Hillsdon, M., Ruth, K. S., Tuke, M. A., Yaghootkar, H., Sharp, S. A., Jie, Y., Thompson, W. D., Harrison, J. W., Dawes, A., Byrne, E. M., Tiemeier, H., . . . Weedon, M. N. (2019). Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms. Nat Commun, 10(1), 343. https://doi.org/10.1038/s41467-018-08259-7

Kalleinen, N., Aittokallio, J., Lampio, L., Kaisti, M., Polo-Kantola, P., Polo, O., Heinonen, O. J., & Saaresranta, T. (2020). Sleep during menopausal transition: a 10-year follow-up. Sleep. https://doi.org/10.1093/sleep/zsaa283

Kalleinen, N., Polo-Kantola, P., Himanen, S.-L., Alhola, P., Joutsen, A., Urrila, A. S., & Polo, O. (2008). Sleep and the menopause–do postmenopausal women experience worse sleep than premenopausal women? Menopause Int, 14(3), 97-104. https://doi.org/10.1258/mi.2008.008013

Kessler, B. A., Stanley, E. M., Frederick-Duus, D., & Fadel, J. (2011). Age-related loss of orexin/hypocretin neurons. Neuroscience, 178, 82-88. https://doi.org/10.1016/j.neuroscience.2011.01.031

Kim, S. J., Benloucif, S., Reid, K. J., Weintraub, S., Kennedy, N., Wolfe, L. F., & Zee, P. C. (2014). Phase-shifting response to light in older adults. J Physiol, 592(1), 189-202. https://doi.org/10.1113/jphysiol.2013.262899

Klerman, E. B., Davis, J. B., Duffy, J. F., Dijk, D.-J., & Kronauer, R. E. (2004). Older people awaken more frequently but fall back asleep at the same rate as younger people. Sleep, 27(4), 793-798. https://doi.org/10.1093/sleep/27.4.793

Knowles, J. E. F., Carl. (2020). merTools: Tools for Analyzing Mixed Effect Regression Models. In (Version R package version 0.5.2) https://CRAN.R-project.org/package=merTools

Kravitz, H. M., Zhao, X., Bromberger, J. T., Gold, E. B., Hall, M. H., Matthews, K. A., & Sowers, M. R. (2008). Sleep disturbance during the menopausal transition in a multi-ethnic community sample of women. Sleep, 31(7), 979-990. https://doi.org/10.5665/sleep/31.7.979

Lampio, L., Polo-Kantola, P., Himanen, S. L., Kurki, S., Huupponen, E., Engblom, J., Heinonen, O. J., Polo, O., & Saaresranta, T. (2017). Sleep During Menopausal Transition: A 6-Year Follow-Up. Sleep, 40(7), zsx090. https://doi.org/10.1093/sleep/zsx090

Luboshitzky, R., Dharan, M., Goldman, D., Herer, P., Hiss, Y., & Lavie, P. (1997). Seasonal variation of gonadotropins and gonadal steroids receptors in the human pineal gland. Brain Res Bull, 44(6), 665-670. https://doi.org/10.1016/s0361-9230(97)00106-8

Messina, G., Viggiano, A., De Luca, V., Messina, A., Chieffi, S., & Monda, M. (2013). Hormonal changes in menopause and orexin-a action. Obstet Gynecol Int, 2013, 209812. https://doi.org/10.1155/2013/209812

Michael Sachs. (2014). cosinor: Tools for estimating and predicting the cosinor model. In https://CRAN.R-project.org/package=cosinor

Miller, M. M., Gould, B. E., & Nelson, J. F. (1989). Aging and long-term ovariectomy alter the cytoarchitecture of the hypothalamic-preoptic area of the C57BL/6J mouse. Neurobiol Aging, 10(6), 683-690. https://doi.org/10.1016/0197-4580(89)90005-5

Mitterling, T., Högl, B., Schönwald, S. V., Hackner, H., Gabelia, D., Biermayr, M., & Frauscher, B. (2015). Sleep and respiration in 100 healthy caucasian sleepers—a polysomnographic study according to American Academy of Sleep Medicine standards. Sleep, 38(6), 867-875. https://doi.org/10.5665/sleep.4730

Mokhlesi, B., Finn, L. A., Hagen, E. W., Young, T., Hla, K. M., Van Cauter, E., & Peppard, P. E. (2014). Obstructive sleep apnea during REM sleep and hypertension. results of the Wisconsin Sleep Cohort. Am J Respir Crit Care Med, 190(10), 1158-1167. https://doi.org/10.1164/rccm.201406-1136OC

Mong, J. A., & Cusmano, D. M. (2016). Sex differences in sleep: impact of biological sex and sex steroids. Philos Trans R Soc Lond B Biol Sci, 371(1688), 20150110. https://doi.org/10.1098/rstb.2015.0110

Munch, M., Knoblauch, V., Blatter, K., Schroder, C., Schnitzler, C., Krauchi, K., Wirz-Justice, A., & Cajochen, C. (2005). Age-related attenuation of the evening circadian arousal signal in humans. Neurobiol Aging, 26(9), 1307-1319. https://doi.org/10.1016/j.neurobiolaging.2005.03.004

Neff, L. M., Hoffmann, M. E., Zeiss, D. M., Lowry, K., Edwards, M., Rodriguez, S. M., Wachsberg, K. N., Kushner, R., & Landsberg, L. (2016). Core body temperature is lower in postmenopausal women than premenopausal women: potential implications for energy metabolism and midlife weight gain. Cardiovasc Endocrinol, 5(4), 151-154. https://doi.org/10.1097/XCE.0000000000000078

Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy

individuals: developing normative sleep values across the human lifespan. Sleep, 27(7), 1255-1273. https://doi.org/10.1093/sleep/27.7.1255

Pennestri, M.-H., Whittom, S., Adam, B., Petit, D., Carrier, J., & Montplaisir, J. (2006). PLMS and PLMW in healthy subjects as a function of age: prevalence and interval distribution. Sleep, 29(9), 1183-1187. https://doi.org/10.1093/sleep/29.9.1183

Proserpio, P., Marra, S., Campana, C., Agostoni, E. C., Palagini, L., Nobili, L., & Nappi, R. E. (2020). Insomnia and menopause: a narrative review on mechanisms and treatments. Climacteric, 23(6), 539-549. https://doi.org/10.1080/13697137.2020.1799973

R Core Team. (2020). R: A Language and Environment for Statistical Computing. In R Foundation for Statistical Computing. https://www.R-project.org/

Randler, C., & Bausback, V. (2010). Morningness-eveningness in women around the transition through menopause and its relationship with climacteric complaints. Biol. Rhythm Res., 41(6), 415-431. https://doi.org/10.1080/09291010903407631

Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M., & Merrow, M. (2007). Epidemiology of the human circadian clock. Sleep Med Rev, 11(6), 429-438. https://doi.org/10.1016/j.smrv.2007.07.005

Sagaspe, P., Taillard, J., Amieva, H., Beck, A., Rascol, O., Dartigues, J. F., Capelli, A., & Philip, P. (2012). Influence of age, circadian and homeostatic processes on inhibitory motor control: a Go/Nogo task study. PloS one, 7(6), e39410. https://doi.org/10.1371/journal.pone.0039410

Sateia, M. J. (2014). International classification of sleep disorders-third edition: highlights and modifications. Chest, 146(5), 1387-1394. https://doi.org/10.1378/chest.14-0970

Schwarz, J. F. A., Akerstedt, T., Lindberg, E., Gruber, G., Fischer, H., & Theorell-Haglow, J. (2017). Age affects sleep microstructure more than sleep macrostructure. J Sleep Res, 26(3), 277-287. https://doi.org/10.1111/jsr.12478

Shaver, J. L., & Woods, N. F. (2015). Sleep and menopause: a narrative review. Menopause, 22(8), 899-915. https://doi.org/10.1097/GME.0000000000000499

Shechter, A., Varin, F., & Boivin, D. B. (2010). Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. Sleep, 33(5), 647-656. https://doi.org/10.1093/sleep/33.5.647

Silva, E. J., Wang, W., Ronda, J. M., Wyatt, J. K., & Duffy, J. F. (2010). Circadian and wake-dependent influences on subjective sleepiness, cognitive throughput, and reaction time performance in older and young adults. Sleep, 33(4), 481-490. https://doi.org/10.1093/sleep/33.4.481

Svetnik, V., Snyder, E. S., Ma, J., Tao, P., Lines, C., & Herring, W. J. (2017). EEG spectral analysis of NREM sleep in a large sample of patients with insomnia and good sleepers: effects of age, sex and part of the night. J Sleep Res, 26(1), 92-104. https://doi.org/10.1111/jsr.12448

Tan, D. X., Xu, B., Zhou, X., & Reiter, R. J. (2018). Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and Rejuvenation of the Pineal Gland. Molecules, 23(2), 301. https://doi.org/10.3390/molecules23020301

Toffol, E., Kalleinen, N., Haukka, J., Vakkuri, O., Partonen, T., & Polo-Kantola, P. (2014). The effect of hormone therapy on serum melatonin concentrations in premenopausal and postmenopausal women: a randomized, double-blind, placebo-controlled study. Maturitas, 77(4), 361-369. https://doi.org/10.1016/j.maturitas.2014.01.015

Vidafar, P., Gooley, J. J., Burns, A. C., Rajaratnam, S. M. W., Rueger, M., Van Reen, E., Czeisler, C. A., Lockley, S. W., & Cain, S. W. (2018). Increased vulnerability to attentional failure during acute sleep deprivation in women depends on menstrual phase. Sleep, 41(8), zsy098. https://doi.org/10.1093/sleep/zsy098

Walters, J. F., Hampton, S. M., Ferns, G. A., & Skene, D. J. (2005). Effect of menopause on melatonin and alertness rhythms investigated in constant routine conditions. Chronobiol Int, 22(5), 859-872. https://doi.org/10.1080/07420520500263193

Woods, N. F., & Mitchell, E. S. (2010). Sleep symptoms during the menopausal transition and early postmenopause: observations from the Seattle Midlife Women's Health Study. Sleep, 33(4), 539-549. https://doi.org/10.1093/sleep/33.4.539

Wright Jr, K. P., & Badia, P. (1999). Effects of menstrual cycle phase and oral contraceptives on alertness, cognitive performance, and circadian rhythms during

sleep deprivation. Behav Brain Res, 103(2), 185-194. https://doi.org/10.1016/s0166-4328(99)00042-x

Xu, Q., & Lang, C. P. (2014). Examining the relationship between subjective sleep disturbance and menopause: a systematic review and meta-analysis. Menopause, 21(12), 1301-1318. https://doi.org/10.1097/GME.0000000000000240

Yoon, I. Y., Kripke, D. F., Elliott, J. A., Youngstedt, S. D., Rex, K. M., & Hauger, R. L. (2003). Age-related changes of circadian rhythms and sleep-wake cycles. J Am Geriatr Soc, 51(8), 1085-1091. https://doi.org/10.1046/j.1532-5415.2003.51356.x

Young, T., Rabago, D., Zgierska, A., Austin, D., & Laurel, F. (2003). Objective and subjective sleep quality in premenopausal, perimenopausal, and postmenopausal women in the Wisconsin Sleep Cohort Study. Sleep, 26(6), 667-672. https://doi.org/10.1093/sleep/26.6.667

Youngstedt, S. D., Elliott, J. A., & Kripke, D. F. (2019). Human circadian phase-response curves for exercise. J Physiol, 597(8), 2253-2268. https://doi.org/10.1113/JP276943

Zitting, K.-M., Münch, M. Y., Cain, S. W., Wang, W., Wong, A., Ronda, J. M., Aeschbach, D., Czeisler, C. A., & Duffy, J. F. (2018). Young adults are more vulnerable to chronic sleep deficiency and recurrent circadian disruption than older adults. Sci Rep, 8(1), 1-14. https://doi.org/10.1038/s41598-018-29358-x

Zolfaghari, S., Yao, C., Thompson, C., Gosselin, N., Desautels, A., Dang-Vu, T. T., Postuma, R. B., & Carrier, J. (2020). Effects of menopause on sleep quality and sleep disorders: Canadian Longitudinal Study on Aging. Menopause, 27(3), 295-304. https://doi.org/10.1097/GME.0000000000001462

2.15 Supplementary material

Group	Participant	Age	BMI	Bedtime (hh:mm)	Rise-time (hh:mm)	Chronotype
YW	**1**	22.70	23.09	23:50	07:50	66 (moderate morning)
	2	25.81	N/A	23:29	07:29	N/A
	3	23.70	22.41	23:28	07:28	55 (intermediate)
	4	27.57	23.38	01:19	09:19	32 (moderate evening)
	5	27.77	26.49	23:29	07:29	50 (intermediate)
	6	30.77	19.60	00:48	08:48	38 (moderate evening)
	7	23.97	20.03	00:55	08:55	56 (intermediate)
	8	25.21	20.70	23:59	07:59	57 (intermediate)
	9	29.58	17.63	23:17	07:17	70 (definite morning)
	10	30.21	17.78	00:30	08:30	46 (intermediate)
	11	21.92	23.46	11:58	07:58	16 (definite evening)
	12	20.78	21.20	01:30	09:30	57 (intermediate)
	Mean	**25.83***	**21.43***	**00:13** *	**08:13** *	**49.36**
	SD	**3.35**	**2.66**	**00:12**	**00:12**	**15.65**
PMW	**1**	53.83	25.50	22:00	06:00	72 (definite morning)
	2	53.99	25.50	22:30	06:30	69 (moderate morning)
	3	55.20	19.40	23:01	07:01	73 (definite morning)
	4	50.84	27.90	22:32	06:32	48 (intermediate)
	5[a]	61.52	23.00	00:00	08:00	42 (intermediate)
	6	54.19	26.20	23:30	07:30	56 (intermediate)
	7	51.58	23.40	23:20	07:20	52 (intermediate)
	8	57.28	24.60	00:01	08:01	46 (intermediate)
	Mean	**54.80***	**24.44***	**23:07** *	**07:07** *	**57.25**
	SD	**3.37**	**2.56**	**00:11**	**00:11**	**12.41**

Supplementary Table 2. 1. Demographic information of postmenopausal women (PMW) and young women in mid-follicular phase (YW).

[a]PMW taking HRT. Age and body mass index (BMI) were compared between PMW and YW with independent t-tests (one-tailed), and bedtimes and rise-times were compared using circular analysis of variance. *p<0.05.

Group	Participant	Time since LMP (years)	AHI	Respiratory-related arousal index	FSH	LH	E2	P4	P4:E2 ratio
PMW	**1**	2.08	2.05	0.15	114.8	72.72	48.53	0.478	9.85
	2	3.13	8.16	2.86	62.08	47.2	37.58	0.161	4.28
	3	6.31	6.02	1.36	104.6	65.72	36.45	0.08	2.19
	4	6.04	0.85	0.14	64.61	35.9	68.11	0.11	1.62
	5[a]	11.28	2.96	0.28	73.02	56.86	78.9	1.47	18.63
	6	6.67	3.42	1.49	85.77	53.94	88.08	0.4	4.54
	7	2.31	5.36	1.07	103	67.16	36.66	0.118	3.22
	8	7.25	3.3	1.05	102.9	54.36	18.35	0.103	5.61
	Mean	**5.63**	**4.02**	**1.05**	**88.85**	**56.73**	**51.58**	**0.37**	**6.24**
	SD	**3.07**	**2.36**	**0.91**	**20.30**	**11.85**	**24.24**	**0.47**	**5.61**

Supplementary Table 2. 2. Supplementary information of postmenopausal women (PMW).

AHI = Apnea-hypopnea index. FSH = Follicle stimulating hormone. LH = luteinizing hormone. E2 = estradiol. P4 = progesterone. P4:E2 ratio = (P4 * 1000) / E2. [a]PMW taking hormone replacement therapy.

Sleep parameter	PMW		YW		*p-value*
	Mean	SEM	Mean	SEM	
SOL (minutes)	13.94	3.11	13.22	1.66	0.84
ROL (minutes)	79.81	4.90	93.00	9.61	0.80
TST (minutes)	424.00	8.19	435.11	4.01	0.25
SE (%)	88.82	1.54	90.81	0.93	0.20
WASO (min)	37.56	6.20	27.28	3.79	0.18
Awakenings ≥1 min (count)	12.13	2.18	6.22	0.83	**0.032**
Arousals (count)	56.50	8.66	14.61	3.26	**<0.001**
Arousal index	8.04	1.25	2.04	0.45	**<0.001**
Stage N1 (minutes)	29.56	3.79	16.31	1.95	**0.016**
Stage N2 (minutes)	257.63	11.35	266.58	6.08	0.50
Stage N3 (minutes)	48.00	6.09	50.69	5.34	0.74
REM sleep (minutes)	88.81	5.77	101.47	4.63	0.11

Supplementary Table 2. 3. Baseline sleep parameters of postmenopausal women (PMW) and young women (YW).

SOL = sleep onset latency. ROL = REM sleep latency. TST = total sleep time. SE = sleep efficiency. WASO = Wake after sleep onset. REM = rapid eye movements. SE was calculated based on time in bed. Arousal index defined as number of arousals per hour of sleep. Sleep parameters are expressed as mean ± SEM. P-values for sleep parameters were based on two-tailed t-test or Mann-Whitney U test when appropriate.

Baseline sleep parameter	Mixed-model term					
	Time effect		Group effect (postmenopausal vs young women)		Group × Time interaction	
	X^2	p value	X^2	p value	X^2	p value
TST	**10.09**	**0.006**	1.64	0.20	0.10	0.95
Arousals	0.08	0.96	**14.65**	**<0.001**	0.15	0.93
Stage N1	**18.87**	**<0.001**	**4.39**	**0.036**	0.69	0.71
Stage N2	3.20	0.20	0.23	0.63	1.86	0.40
Stage N3	**48.86**	**<0.001**	0.22	0.64	0.46	0.80
REM sleep	**31.18**	**<0.001**	1.25	0.26	0.88	0.65

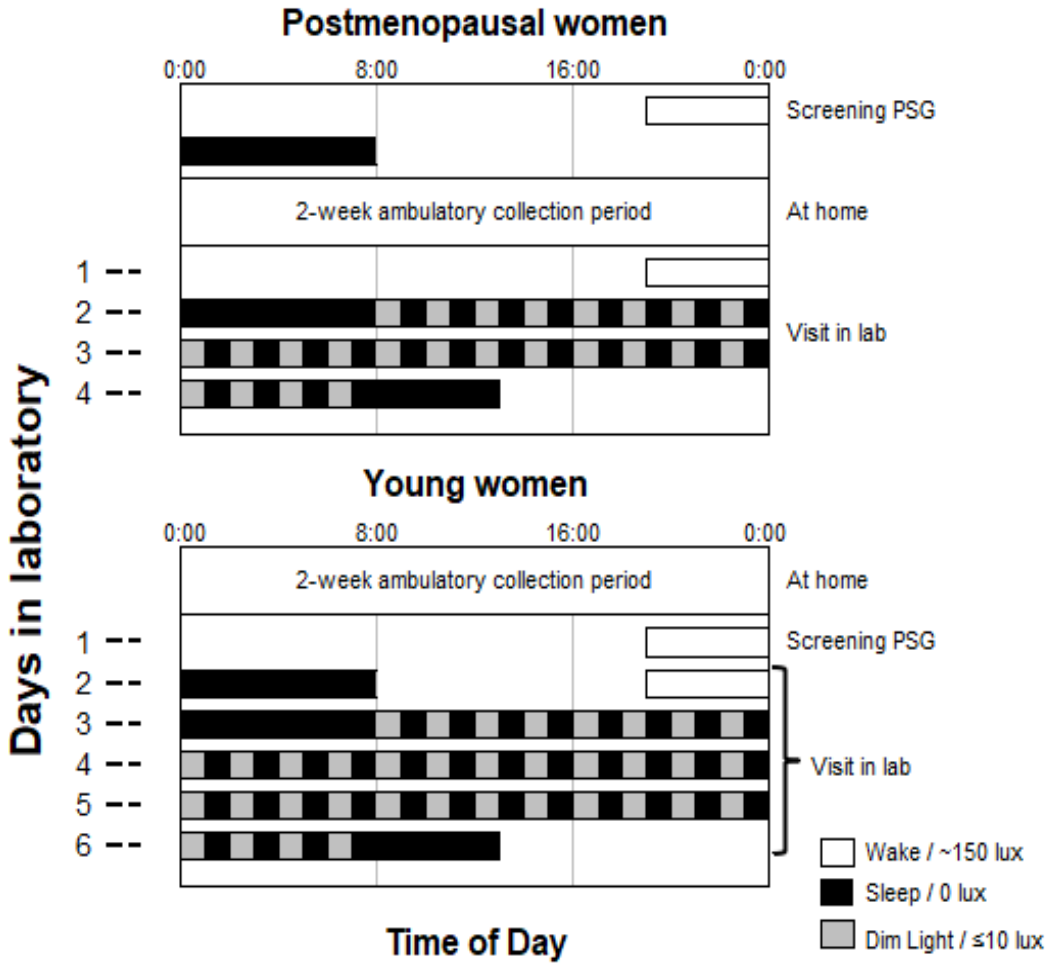
Supplementary Table 2. 4. Summary of linear mixed-effects model results of baseline sleep parameters divided by thirds.

USW parameter	Mixed-model term					
	Time effect		Group effect (postmenopausal vs young women)		Group × Time interaction	
	X^2	p value	X^2	p value	X^2	p value
SOL	**314.70**	**< 0.001**	**18.24**	**< 0.001**	**56.49**	**< 0.001**
ROL	**118.00**	**< 0.001**	**19.31**	**< 0.001**	**37.85**	**0.026**
TST	**343.41**	**< 0.001**	**12.83**	**< 0.001**	**41.12**	**0.012**
Arousals	**64.25**	**< 0.001**	**16.92**	**< 0.001**	**36.45**	**0.027**
Stage N1	**124.19**	**< 0.001**	**6.16**	**0.013**	30.88	0.13
Stage N2	**147.07**	**< 0.001**	**15.94**	**< 0.001**	32.24	0.10
Stage N3	**89.72**	**< 0.001**	2.07	0.15	19.00	0.70
REM sleep	**236.53**	**< 0.001**	1.12	0.29	15.05	0.89
Self-reported sleep quality	**115.38**	**<0.001**	1.79	0.18	30.36	0.14
Post-nap alertness	**185.75**	**< 0.001**	2.08	0.15	25.37	0.39
Mid-wake alertness	**171.75**	**< 0.001**	**4.14**	**0.042**	**35.88**	**0.042**
CBT	**620.24**	**< 0.001**	**4.96**	**0.026**	13.73	0.93
Melatonin	**126.38**	**< 0.001**	**4.19**	**0.041**	**37.71**	**0.027**

Supplementary Table 2. 5. Summary of linear mixed-effects model results of USW parameters.

Circadian parameters		PMW **Mean** ± SEM [95% CI]	YW **Mean** ± SEM [95% CI]	*p-values*
SOL	Mesor (minutes)	19.10±1.59 [15.98, 22.22]	29.91±2.06 [25.87, 33.96]	**<0.001**
	Amplitude (minutes)	11.76±1.76 [8.30, 15.21]	18.15±1.43 [15.36, 20.95]	**0.005**
	Acrophase (clock time, h)	19.17±0.70 [17.79, 20.55]	18.53±0.38 [17.78, 19.28]	0.43
ROL	Mesor (minutes)	35.25±1.85 [31.62, 38.88]	22.25±2.40 [17.54, 26.96]	**<0.001**
	Amplitude (minutes)	6.50±1.64 [3.30, 9.71]	9.38±1.31 [6.81, 11.96]	0.17
	Acrophase (clock time, h)	8.54±0.99 [6.61, 10.47]	5.65±0.58 [4.51, 6.80]	**0.012**
TST	Mesor (minutes)	34.83±1.37 [32.15, 37.5]	27.62±1.77 [24.15, 31.09]	**<0.001**
	Amplitude (minutes)	12.81±1.64 [9.61, 16.02]	18.02±1.34 [15.39, 20.66]	**0.038**
	Acrophase (clock time, h)	6.43±0.72 [5.01, 7.85]	6.22±0.43 [5.39, 7.06]	0.80
Arousals count	Mesor (count)	6.89±0.53 [5.86, 7.93]	3.60±0.71 [2.21, 4.98]	**<0.001**
	Amplitude (count)	2.29±0.39 [1.53, 3.04]	0.48±0.39 [-0.29, 1.25]	**0.001**
	Acrophase (clock time, h)	10.21±0.55 [9.13, 11.30]	11.93±2.61 [6.81, 17.04]	0.52
Arousal index	Mesor (count/h)	4.34±0.37 [3.61, 5.07]	2.11±0.49 [1.14, 3.08]	**<0.001**
	Amplitude (count /h)	2.15±0.21 [1.73, 2.57]	0.66±0.22 [0.22, 1.10]	**<0.001**
	Acrophase (clock time, h)	8.60±0.37 [7.88, 9.32]	7.41±1.23 [5.01, 9.82]	0.35
Stage N1	Mesor (minutes)	5.35±0.50 [4.36, 6.34]	3.66±0.65 [2.98, 4.94]	**0.010**
	Amplitude (minutes)	2.37±0.38 [1.63, 3.11]	1.70±0.28 [1.16, 2.24]	0.15
	Acrophase (clock time, h)	9.91±0.53 [8.88, 10.94]	8.38±0.69 [7.03, 9.72]	0.08
Stage N2	Mesor (minutes)	23.55±1.24 [21.12, 25.98]	15.99±1.61 [12.85, 19.14]	**<0.001**
	Amplitude (minutes)	5.32±1.25 [2.88, 7.77]	7.69±1.02 [5.70, 9.69]	0.14
	Acrophase (clock time, h)	6.56±1.27 [4.16, 9.06]	5.93±0.73 [4.49, 7.36]	0.67
Stage N3	Mesor (minutes)	1.87±0.59 [0.72, 3.02]	2.95±0.76 [1.46, 4.44]	0.15
	Amplitude (minutes)	0.89±0.50 [-0.09, 1.87]	2.58±0.41 [1.77, 3.39]	**0.009**
	Acrophase (clock time, h)	3.57±2.20 [23.26, 7.87]	3.61±0.63 [2.37, 4.85]	0.98
REM sleep	Mesor (minutes)	4.09±0.61 [2.89, 5.30]	4.93±0.80 [3.37, 6.50]	0.29
	Amplitude (minutes)	5.63±0.87 [3.92, 7.34]	6.93±0.72 [5.52, 8.35]	0.25
	Acrophase (clock time, h)	5.42±0.59 [4.27, 6.58]	6.94±0.40 [6.16, 7.71]	**0.032**
Self-reported sleep quality	Mesor (Likert)	3.69±0.32 [3.06, 4.33]	3.10±0.43 [-1.43, 0.24]	0.16
	Amplitude (Likert)	0.76±0.25 [0.27, 1.26]	0.72±0.22 [0.29, 1.14]	0.89
	Phase angle (h)	6.70±0.77 [5.19, 8.20]	7.18±0.75 [5.72, 8.64]	0.65
Post-nap alertness[a]	Mesor (cm)	6.01±0.75 [4.54, 7.48]	4.57±0.99 [2.64, 6.50]	0.14
	Amplitude (cm)	1.17±0.31 [0.57, 1.77]	2.02±0.27 [1.50, 2.54]	**0.034**
	Acrophase (clock time, h)	17.44±0.96 [15.57, 19.31]	18.80±0.48 [17.87, 19.73]	0.20
Mid-wake alertness	Mesor (cm)	7.77±0.64 [6.62, 8.91]	6.23±0.76 [4.75, 7.71]	**0.042**
	Amplitude (cm)	0.92±0.37 [0.19, 1.65]	1.62±0.31 [1.02, 2.22]	0.15
	Acrophase (clock time, h)	17.91±0.92 [16.10, 19.71]	17.67±0.44 [16.81, 18.53]	0.82
CBT[b]	Mesor (°C)	36.98±0.05 [36.87, 37.08]	37.13±0.07 [36.99, 37.26]	**0.033**
	Amplitude (°C)	0.28±0.03 [0.22, 0.33]	0.29±0.02 [0.25, 0.33]	0.80
	Nadir (clock time, h)	4.23±0.51 [3.23, 5.23]	5.27±0.40 [4.50, 6.05]	0.11
Melatonin[a]	Mesor (pg/ml)	10.38±1.71 [7.03, 13.73]	14.78±2.26 [-0.02, 8.83]	0.051
	Amplitude (pg/ml)	3.08±1.17 [0.78, 5.37]	7.11±1.10 [4.95, 9.27]	**0.012**
	Acrophase (clock time, h)	3.05±1.43 [0.25, 5.85]	3.32±0.53 [2.28, 4.36]	0.86

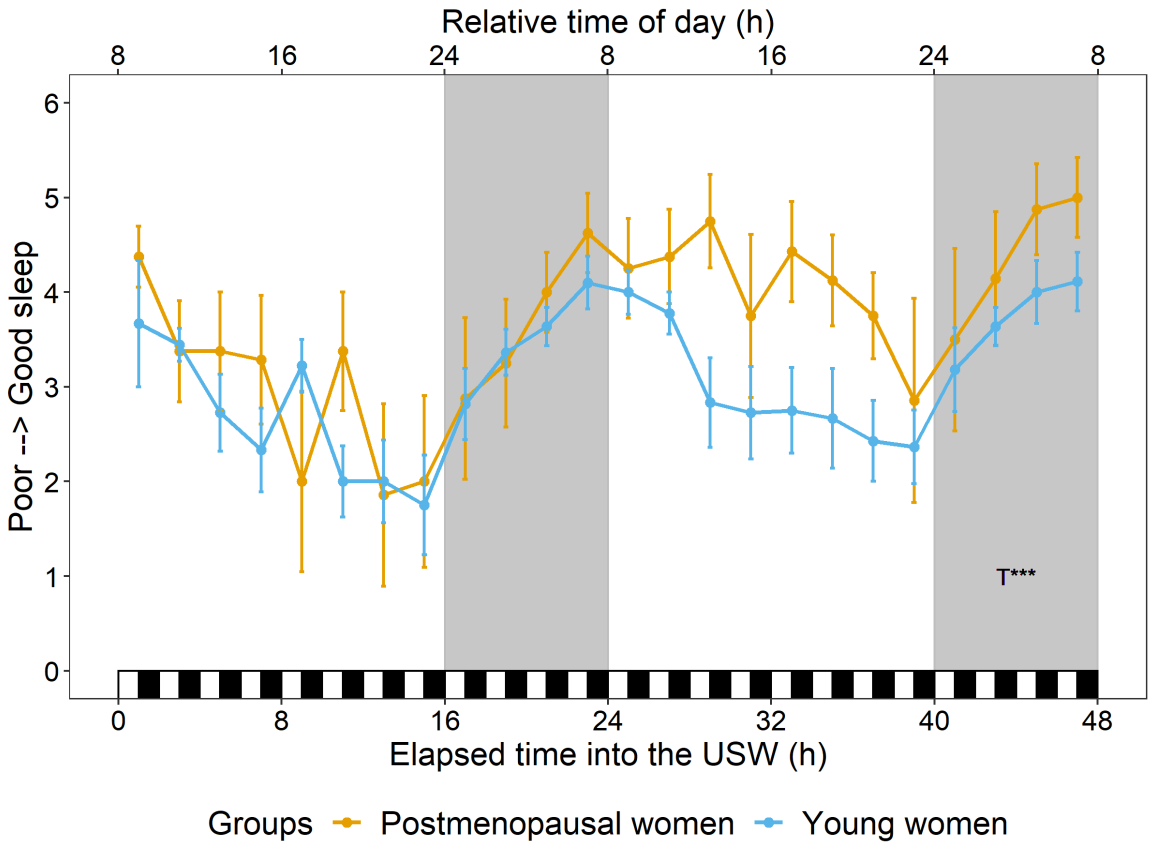
Supplementary Table 2. 6. Circadian parameters of postmenopausal women (PMW) and young women at mid-follicular phase (YW) based on time of day.
SOL = sleep onset latency. ROL = REM sleep latency, TST = total sleep time, CBT = core body temperature. YW: n=12; PMW n=8. aYW: n=11. bPMW n=7. The time of the minimum was used as a circadian phase marker for CBT, whereas the time of the peak was used for the rest of the parameters. P-values for were based on two-tailed t-test. Data are expressed as mean ± SEM.



Supplementary Figure 2. 1. Ultradian sleep-wake cycle (USW) procedure used in postmenopausal and young women.

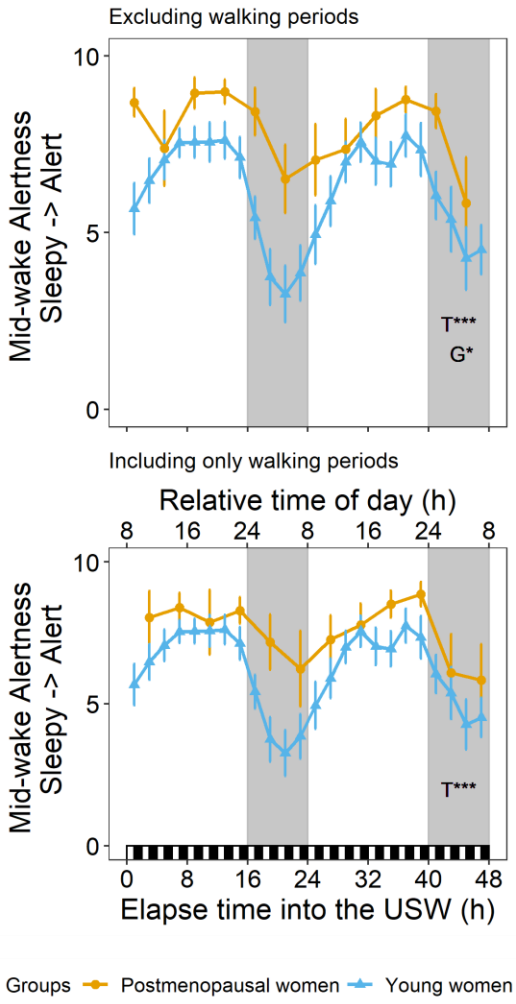
For the 2-week ambulatory collection, participants maintained a regular 8-h nocturnal sleep schedule, at their habitual sleep time, as verified by actigraphy the week prior to laboratory entry (postmenopausal women: Actiwatch Spectrum, Phillips Respironics, PA, USA; young women: Actiwatch 64, Phillips Respironics, PA, USA), sleep logs on a cellphone, and by calling the laboratory at bedtime and rise-time. Participants were also instructed to avoid drugs, caffeinated products, alcohol, and tobacco for at least 1 week. Eight of the young women participated in the laboratory study on 2 occasions, once at their mid-follicular phase and once at their mid-luteal phase, whereas the other four young women were studied only during their mid-follicular phase (Boivin et al., 2016; Shechter et al., 2010). PSG screening for sleep disorders in postmenopausal women was performed separately from the experimental phase of study, prior to the 2-week

ambulatory phase. In young women, the screening PSG was performed at the first laboratory visit and was followed by a baseline sleep period preceding the USW procedure. Eight young women returned to the laboratory at the other phase of their menstrual cycle and thus screening PSG was not needed at the second visit (Shechter et al., 2010). As such, all postmenopausal and 4 young women had a first screening night in another session than the experimental laboratory visit, whereas in 8 young women the PSG screening was planned on night 1 and baseline sleep on night 2. For every laboratory visit, recording equipment was installed upon arrival to the laboratory on the evening of Day 1 (light levels: ~150 lux). Participants slept for an 8-h sleep period, based on their habitual bedtimes and rise-times for the past 2 weeks (light levels: ~0 lux). Upon awakening, participants began a 48-h (postmenopausal) or 72-h (young women) USW procedure, consisting of alternating 60-minute wake periods and 60-minute nap opportunities in constant conditions (light levels: ~0 lux during naps, <10 lux during wake periods). All participants were allowed to sleep *ad libitum* during the last nap opportunity of the USW procedure. One young woman (young women subject 12) was part of another unpublished study involving postural changes during the last 24 hours of USW procedure. The figure illustrates a hypothetical participant with a sleep schedule from 0:00 h to 8:00 h.



Supplementary Figure 2. 2. Variation of self-reported sleep quality in postmenopausal and young women in mid-follicular phase.

Orange and blue lines represent postmenopausal and young women, respectively. Data were aligned based on the time elapsed into the ultradian sleep-wake cycle procedure (USW, bottom x axis). Black (~0 lux) and white (~10 lux) small squares above the bottom X axis represent the nap and wake periods across the first 48 h of the USW procedure. The top X axis depicts the corresponding time of day for a participant with a bedtime of 0:00 h to 8:00 h. Large grey rectangles depict the projected time of the habitual nocturnal sleep period, which corresponds to the time between habitual bedtime and rise-time. T*** = significant main effect of time, $p < 0.001$. Values are presented as mean ± SEM.



Supplementary Figure 2. 3. Variation of mid-wake alertness in postmenopausal and young women in mid-follicular phase.

Orange and blue lines represent postmenopausal and young women, respectively. Data were aligned based on the time elapsed into the ultradian sleep-wake cycle procedure (USW, bottom x axis). Black (~0 lux) and white (~10 lux) small squares above the bottom X axis represent the nap and wake periods across the first 48 h of the USW procedure. The top X axis depicts the corresponding time of day for a participant with a bedtime of 0:00 h to 8:00 h. Large grey rectangles depict the projected time of the habitual nocturnal sleep period, which corresponds to the time between habitual bedtime and rise-time. Top figure: T*** = significant main effect of time, $p < 0.001$; G* = significant main effect of group, $p = 0.025$. Bottom figure: T*** = significant main effect of time, $p < 0.001$. All values are presented as mean ± SEM.

2.16 References for supplementary material

Boivin, D. B., Shechter, A., Boudreau, P., Begum, E. A., & Ng Ying-Kin, N. M. (2016). Diurnal and circadian variation of sleep and alertness in men vs. naturally cycling women. Proc Natl Acad Sci U S A, 113(39), 10980-10985. https://doi.org/10.1073/pnas.1524484113

Shechter, A., Varin, F., & Boivin, D. B. (2010). Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. Sleep, 33(5), 647-656. https://doi.org/10.1093/sleep/33.5.647

Chapter 3.

Dampened circadian amplitude of EEG power in women after menopause

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3.1 Preface

The previous chapter presented findings indicating a dampened circadian amplitude of sleep architecture in postmenopausal women compared to young women, including TST, SOL, and stage N3. To gain a more refined understanding of sleep, we must analyze the EEG activity obtained from EEG recordings. Although the circadian variation of EEG activity have been previously reported in studies with aged individuals (Dijk et al., 1999; Munch et al., 2005), no information is available on menopausal status. Thus, the objective of this study is to characterize the EEG power spectra of postmenopausal women and its circadian variation. Along with the results of Chapter 2, this research will offer valuable insights into sleep changes experienced by postmenopausal women that may increase their risk of developing sleep disturbances.

3.2 Abstract

Postmenopausal women are at high risk of developing sleep-wake disturbances. We previously reported dampened circadian rhythms of melatonin, alertness, and sleep in postmenopausal compared to young women. The present study aims to further explore EEG power spectral changes in the sleep of postmenopausal women.

Eight healthy postmenopausal women were compared to 12 healthy, naturally ovulating, young women in their mid-follicular phase. Participants followed a regular 8-h sleep schedule for ≥2 weeks prior to laboratory entry. The laboratory visit included an 8-h baseline sleep period followed by an USW procedure, consisting of alternating 1-h wake periods and nap opportunities. EEG power spectral analysis was performed on NREM sleep obtained over a 48-hour period.

The baseline nocturnal sleep of postmenopausal women comprised lower power within delta and sigma, and higher power within alpha bands compared to that of younger women. During nighttime naps of the USW, decreased power within delta and sigma, and increased power within beta bands were observed in postmenopausal women. During the USW procedure, postmenopausal women presented lower power of delta and theta, undetectable rhythms of delta, theta, and sigma (12-13 Hz), and a dampened amplitude of sigma power (13-14 Hz) compared to younger women.

Our results support the hypothesis of a dampened circadian variation of sleep in aged women. These findings in the sleep microstructure of healthy-sleeping postmenopausal women suggest a potential mechanism for increased susceptibility to develop sleep disturbances; however, further research is needed to clarify their clinical implications and contribution to insomnia.

3.3 Introduction

Women are at a greater risk than men of developing sleep disturbances both during and after the menopausal transition (Zolfaghari et al., 2020). Various factors contribute to these sleep disturbances at this time, including an increased prevalence of vasomotor symptoms, sleep-disordered breathing, chronic health conditions, and mental health disorders (Arnardottir et al., 2016; Pennestri et al., 2006; Xu & Lang, 2014). We previously investigated changes in the circadian variation of sleep and alertness as

contributing factors to the sleep disturbances of postmenopausal women (Perez-Medina-Carballo et al., 2023). We found that postmenopausal women had more disrupted sleep, slept more during daytime, and were more alert at night compared to younger women. We also found dampened amplitudes of the circadian rhythms of TST, SOL, stage N3 sleep, alertness, and melatonin levels in postmenopausal women. These findings are consistent with a weakened circadian signal regulating sleep, alertness, and melatonin secretion (Dijk & Duffy, 2020; Perez-Medina-Carballo et al., 2023).

In addition to the conventional sleep stage scoring analysis, studying the microstructure of sleep measured by EEG power provides a more refined analysis of sleep (Feige et al., 2013). Existing research about the microstructure of sleep after menopause is scarce (Campbell et al., 2011; Nea Kalleinen et al., 2008; Matthews et al., 2021). Consequently, comprehension of changes in the microstructure of sleep associated with menopause becomes imperative given their potential contribution to the sleep disturbances commonly developed during this period of life. One longitudinal study showed that women who transitioned to a postmenopausal stage had increased beta power during NREM sleep, compared to women who remained pre- or early perimenopausal (Matthews et al., 2021). No postmenopausal changes were observed in delta power, sleep duration, or WASO. Campbell et al, in a cross-sectional study, found higher beta power in NREM and REM sleep in late perimenopausal and postmenopausal women compared to premenopausal and early perimenopausal women, but no differences in delta power as a result of menopausal status (Campbell et al., 2011). In another cross-sectional study, Kalleinen et al, found that pre- and postmenopausal women had less slow wave activity (SWA; or delta power) than younger women (beta power not reported) (Nea Kalleinen et al., 2008). While two studies found higher beta power, results for delta power were inconsistent and other EEG frequency bands such as theta, alpha, or sigma power were not measured. A cross-sectional study including women and men (aged between 18-64 years), found that women had greater delta, theta, and sigma power than men at all ages with a similar age decline in both genders. In women, an age-related decrease in delta, theta, and sigma with no differences in alpha or beta power have been described, but menopausal status was not considered (Schwarz et al., 2017; Svetnik et al., 2017).

Although increased beta and decreased delta power have been described at menopause, a definitive consensus remains elusive since menopausal status has not been consistently considered in aging studies. The aim of the present study is to better understand the changes occurring after menopause in EEG power frequency bands and their circadian variations as these might predispose women to experience sleep disturbances.

3.4 Methods

3.4.1 Participants

Participant demographic, recruitment and screening methods, study design, and laboratory conditions were previously described in Pérez-Medina-Carballo et al (Perez-Medina-Carballo et al., 2023). Briefly, participants included 8 healthy postmenopausal women (mean age ± SD: 54.80 ± 3.37 y, range 50 – 61 y, at least 2 years since their last menstrual cycle) and 12 healthy young women from a prior study, in their mid-follicular phase (age: 25.83 ± 3.35 y, range 20 – 30 y; days 5-9 after menses) (Shechter et al., 2010). All women were good sleepers based on a clinical evaluation by a sleep disorder physician (DBB) and verified by a polysomnographic screening night at the laboratory. During screening procedures, postmenopausal women also reported good sleep quality based on the Insomnia Severity Index (Mean ± SD: 3.75 ± 2.96) and the Pittsburgh Sleep Quality Index (3.25 ± 2.60). As reported in Pérez-Medina-Carballo et al (Perez-Medina-Carballo et al., 2023), we previously showed that this group of postmenopausal women had comparable self-reported sleep quality to young women throughout the USW procedure (described below). All participants were physically and psychologically healthy. Participants were not using medications or hormonal therapy apart from one postmenopausal woman who was using estradiol transdermal patches and micronized progesterone pills daily. Both groups were screened for sleep apnea (AHI <15/hour of sleep in postmenopausal women; <5 in young women; Supplementary Table 3.1) and periodic leg movements during sleep (PLMS index<15/hour of sleep). All procedures were approved by the Research Ethics Board of the Montreal West Island IUHSSC (no. 2018-175), and each participant provided informed consent.

3.4.2 Design

Prior to laboratory entry, participants maintained a regular 8-h sleep schedule for ≥2 weeks, confirmed by wrist actigraphy. This preparatory phase was planned to reduce variability in circadian entrainment between participants. Sleep schedules were discussed with each participant during the screening phase and were selected based on their regular sleep habits. Participants were admitted to the laboratory for an 8-h baseline sleep period at their habitual sleeping time. This was immediately followed by a USW procedure lasting 48 h (postmenopausal women) or 72 h (young women). The USW procedure consisted of alternating 1-h wake periods and 1-h sleep opportunities in constant conditions. This procedure ended with an *ad libitum* nap.

During the laboratory visit, participants remained in a time-isolation room without windows or time cues. Participants were exposed to dim light (< 10 lux) during wake periods of the USW procedure and complete darkness (~0 lux) during sleep opportunities. Food intake was divided into isocaloric snacks throughout wake periods of the USW procedure. Ambient temperature was maintained at 22.0 ± 2.0 °C. Young women maintained a semi-recumbent position throughout the USW procedure and were required to use a bed pan. Due to the risk of thrombophlebitis, postmenopausal women stayed in a semi-recumbent position throughout the USW procedure but were permitted to use the toilet in an ensuite bathroom every wake period and walk around the bedroom for 10 minutes every other wake period. Additionally, an anticoagulant was administered three times in the postmenopausal woman taking hormonal replacement therapy (2-ml tinzaparin sodium – Innohep, once per morning).

3.4.3 Measures

Sleep was polysomnographically recorded (Harmonie, Stellate Systems, Canada) throughout baseline sleep and naps of the USW procedure. Polysomnography (PSG) recordings included EEG (F3/A2, F4/A1, C3/A2, C4/A1, O1/A2, O2/A1 for postmenopausal women; C3/A2, C4/A1, O1/A2, O2/A1 for young women), electrooculogram, electromyogram, and electrocardiogram. PSG data was sampled with a frequency of 250 Hz in 11 young women and 512 Hz in postmenopausal women and one young woman. In order to compare that was collected with 250 Hz and 512 Hz, a resampling method based on linear interpolation was used (see details in statistical

analysis). Sleep was visually scored in 30-second epochs based on the American Academy of Sleep Medicine scoring guidelines (Berry et al., 2020) using central and occipital leads. For the baseline sleep period, sleep parameters included TST, SOL, SE, NREM sleep, and WASO. These sleep parameters were calculated based on time in bed and were previously reported (Perez-Medina-Carballo et al., 2023).

For the power spectral analysis, EEG power was obtained from Harmonie, Stellate Systems. Automatic artifact removal were performed using HarmAct, an ad-hoc software developed by “Sacré-Cœur-de-Montréal” Hospital (Montreal, QC, Canada) (Provencher et al., 2020). Spectral band power was calculated using Fast Fourier Transform with a 4-sec Hamming window with 50% overlap between two adjacent windows. Artifact removal was confirmed by visual inspection of each recording. EEG spectral analysis was performed on non-REM sleep stages of central derivation C3. EEG frequency bands were categorized as follows: delta or SWA (0.5-4.5 Hz), theta (4.75-7.5 Hz), alpha (8-12 Hz), sigma or spindle frequency activity (SFA; 12-16 Hz), and beta (16-24.5 Hz). Some of the sigma power bands correspond to a range of low SFA (LSFA; 12.25-13.75 Hz) and others correspond to a range of high SFA (HSFA; 14-15.5 Hz) as defined by Munch and colleagues (Munch et al., 2010). As spindle frequencies were suggested to differ between young and older women (Purcell et al., 2017), additional analyses were conducted with sigma power (12-16 Hz) divided into 1-Hz bands. This allowed us to observe clearer variations in sigma power.

3.4.4 Statistical analyses

All statistical analyses were performed in R version 4.2.1. Absolute spectral power was calculated for the baseline sleep period and USW naps. Only the 24 naps from the first 48 hours of the USW were compared between groups. To analyze data collected at different sampling rates (i.e., 250 Hz or 512 Hz), data from young women (250 Hz) were interpolated using a linear method to estimate their value at 512 Hz using the “prospectr” package (Stevens & Ramirez-Lopez, 2022). After resampling, young women’s data were expressed at a sampling frequency of 512 Hz, comparable to those of postmenopausal women. Using the package “lme4” (Bates et al., 2015), linear mixed-effects models were used to compare EEG spectral power within and between groups. EEG power spectral

analysis was performed using bins of 0.25 Hz as well as standard frequency bands (delta, theta, alpha, sigma, beta).

EEG power of the baseline sleep was analyzed as a full night and divided into thirds based on the duration of the sleep period (from sleep onset to final awakening) to observe time-dependent changes (Svetnik et al., 2017). The baseline sleep period of one young woman was excluded due to technical problems. USW recordings were categorised into daytime (nap episodes 1-8 and 13-20) and nighttime (nap episodes 9-12 and 21-24) naps, corresponding to each participant's projected habitual sleep schedule.

Since data were not normally distributed, EEG power for the various frequency bands were log-transformed for statistical analyses and used as dependent variables in separate linear mixed-effect models: baseline sleep, daytime naps, and nighttime naps. EEG power was binned in 0.25 Hz. The 0.25 Hz intervals are labelled based on the lower end of the range. For example, the frequency bin named 0.75 Hz includes the power between ≥0.75 to <1 Hz. Participant "group" (postmenopausal women vs young women) and "frequency bin" (in 0.25 Hz) were used as fixed effects. Participant numbers were included as a random effect. A forward stepwise method (Olive, 2017) was used to build the model and likelihood-ratio tests were used to determine significance of the fixed effect when added to the model, as previously used in Pérez-Medina-Carballo et al. (Perez-Medina-Carballo et al., 2023). Tukey's *post hoc* tests were performed when group × frequency interactions were significant. Additionally, to investigate the relationship between daytime and nighttime power spectra of the USW, nighttime data were transformed as percentage of daytime power as follows: [(nighttime EEG power / daytime EEG power) * 100].

Circadian parameters (mesor, amplitude, and acrophase) were calculated for each frequency band (delta, theta, alpha, sigma, beta) to analyze their circadian variation throughout the naps of the USW. Circadian parameters were calculated based on the time elapsed into the USW procedure, which was aligned to each participant's habitual sleep period. The mesor was defined as the average value of the fitted or unfitted rhythm. Because we added a time elapsed into the USW linear effect into the model (explained below), the average values changed with time into the USW. Therefore, the mesor

corresponded to the intercept value in the middle of the USW procedure. The acrophase was defined as the peak time of the fitted rhythm. The amplitude was defined as half the difference between the peak and through levels. To analyze the circadian variation of brain activity in each EEG frequency band, data were collapsed into 2-h bins and then Z-scored using the mean and standard deviation of the baseline sleep period. Cosinor analysis was then performed with a linear mixed-effects model using a modified version of the package “cosinor” (Michael Sachs, 2014) to include participant’s number as random effects. “Group”, “frequency bands” (in Hz), and “time elapsed into the USW procedure” were used as fixed effects. A forward stepwise method (Olive, 2017) was utilized to build the model and likelihood-ratio tests were used to determine significance of the fixed effects added to the model. Tukey’s *post hoc* tests were performed when group × frequency band interactions were significant.

Since we included one postmenopausal woman taking hormone replacement therapy, we evaluated her EEG power spectral results for outliers. Random effects estimates were extracted for each participant using the package “merTools” (Knowles, 2020). The data of this postmenopausal woman remained within 2 SD for all parameters, and therefore her data were included in the analyses.

3.5 Results

3.5.1 Baseline sleep period

Table 1 summarizes the parameters of the baseline sleep period. As reported previously, no significant group differences were observed in TST, SOL, SE, NREM sleep and WASO (Perez-Medina-Carballo et al., 2023). Postmenopausal women had earlier bedtimes (23:07±00:11 vs 00:13±12; p=0.005) and rise-times (07:07±00:11 vs 08:13±12; p=0.005) than young women (Perez-Medina-Carballo et al., 2023).

Baseline EEG power density during NREM sleep is presented in Supplementary Figure 3.1 and results of the linear mixed-effects model are summarized in Supplementary Table 3.2. Across the entire nighttime baseline sleep, a significant main effect of frequency bin (0.25 Hz bin) was observed ($p<0.001$), but no main group effect ($p=0.54$). The group by frequency bin interaction was significant ($p<0.001$), but Tukey’s *post hoc* tests revealed no significant differences between groups ($p\geq0.060$).

The baseline sleep period was further divided into thirds and results are presented in Figure 3.1 – panels B-D and Supplementary Table 3.2. In each third of the night, the main effect of frequency bin (0.25 Hz bin; $p<0.001$) and the group by frequency bin interaction were significant ($p<0.001$), while the main effect of group was not ($p\geq0.42$). Tukey's *post hoc* tests revealed that, during the first part of the night (Figure 3.1B), postmenopausal women showed significantly lower power for frequencies within the delta range (1-1.5 Hz; $p\leq0.049$) and higher power within the alpha range (10-10.5 Hz; $p\leq0.040$) than young women. During the second and last thirds of the night (Figure 3.1C and 3.1D), postmenopausal women showed significantly lower power within the sigma range (13-14 Hz; $p\leq0.049$) than young women.

	Young women (mean ± SEM)	Postmenopausal women (mean ± SEM)	*p-value*
Age (years)	25.83 ± 0.97	54.80 ± 1.19	**<0.001**
TST (min)	435.11 ± 4.01	424.00 ± 8.19	0.25
SOL (min)	13.22 ± 1.66	13.94 ± 3.11	0.84
SE (%)	90.81 ± 0.93	88.82 ± 1.54	0.20
NREM (min)	336.27 ± 4.71	335.19 ± 8.93	0.92
WASO (min)	27.28 ± 3.79	37.56 ± 6.20	0.18

Table 3. 1. Baseline sleep parameters of postmenopausal and young women studied in their mid-follicular phase.
TST = total sleep time. SOL = Sleep onset latency. SE = Sleep efficiency. NREM = non-REM sleep. WASO = Wake after sleep onset. All values are expressed as mean ± SEM. P-values were based on two-tailed t-test or Mann-Whitney U test when appropriate. Bold values denote statistical significance.

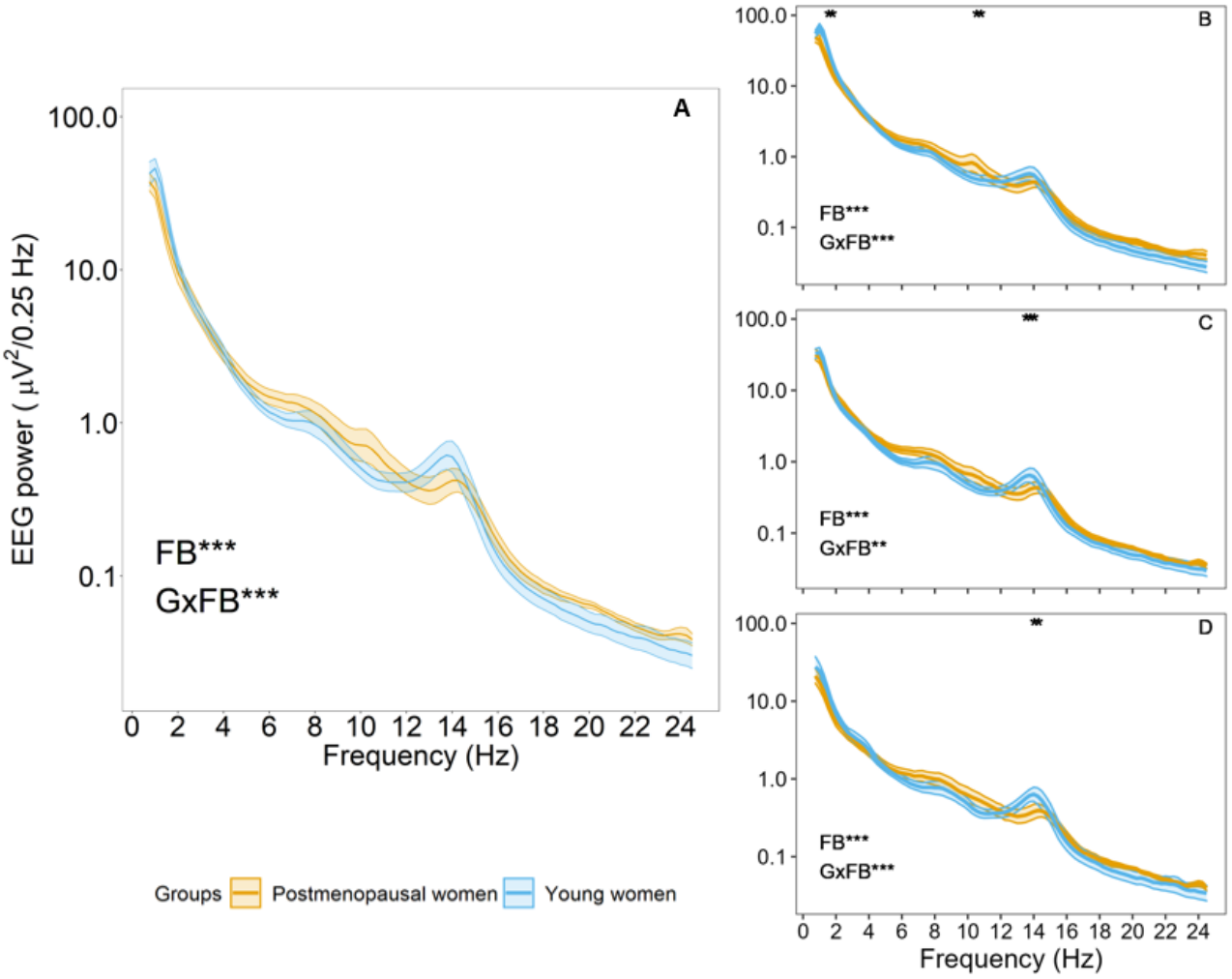

Figure 3. 1. EEG power of the baseline night sleep period for the full night (panel A) and divided into thirds of the sleep period (panels B-D).

Panel B: 1st part of the night. Panel C: 2nd part of the night. Panel D: 3rd part of the night. FB = significant main effect of frequency band. GxFB = significant group by frequency band interaction. The asterisks (*) along the top X-axis indicate significant group differences by frequency band ($p<0.05$). *** $p<0.001$, ** $p<0.01$. Lines represent mean EEG power and shaded areas represent SEM.

EEG power values of standard frequency bands during the full baseline sleep period and divided by thirds are presented in Supplementary Table 3.3. When baseline sleep was binned in standard frequency bands, only sigma power in the range of 13-14 Hz was significantly lower in postmenopausal compared to young women.

3.5.2 EEG power density during the USW procedure

Results of EEG power density during NREM sleep for the USW procedure are presented in Figure 3.2. During daytime naps (Figure 3.2A), a main effect of frequency bin ($p<0.001$) and a group by frequency bin interaction ($p<0.001$) were observed, but no main effect of group ($p=0.62$). Tukey's *post hoc* tests did not reveal significant differences during the daytime naps ($p\geq0.062$). During nighttime naps (Figure 3.2B), a main effect of frequency bin ($p<0.001$) and a group by frequency bin interaction ($p<0.001$) were observed, but no main effect of group ($p=0.58$). For nighttime naps, tukey's *post hoc* tests revealed a significantly reduced power for frequencies within the delta (0.75–1.5 Hz; $p\leq0.047$) and sigma (13.25–14 Hz; $p\leq0.048$) ranges, as well as increased power for frequencies within the beta range (23.75–24.5 Hz; $p\leq0.042$) in postmenopausal compared to young women.

The EEG power of nighttime relative to daytime naps was calculated to better depict the diurnal changes of spectral power by frequency band (Figure 3.2C). A main effect of frequency bin ($p<0.001$) and a group by frequency bin interaction ($p=0.004$) were observed, but no main effect of group. *Post hoc* tests revealed significantly lower power within the sigma range (12.00-13.5 Hz; $p\leq0.030$) and higher power within the beta range at 22.25-22.5 Hz in postmenopausal compared to young women, in the nighttime relative to daytime. The nocturnal peak of LSFA occurred at higher frequencies in postmenopausal (~14 Hz) compared to young women (~13 Hz) (Figure 3.2). As such, sigma power divided in 1-Hz bins further allowed us to observe clearer variations of these frequency bands in both groups.

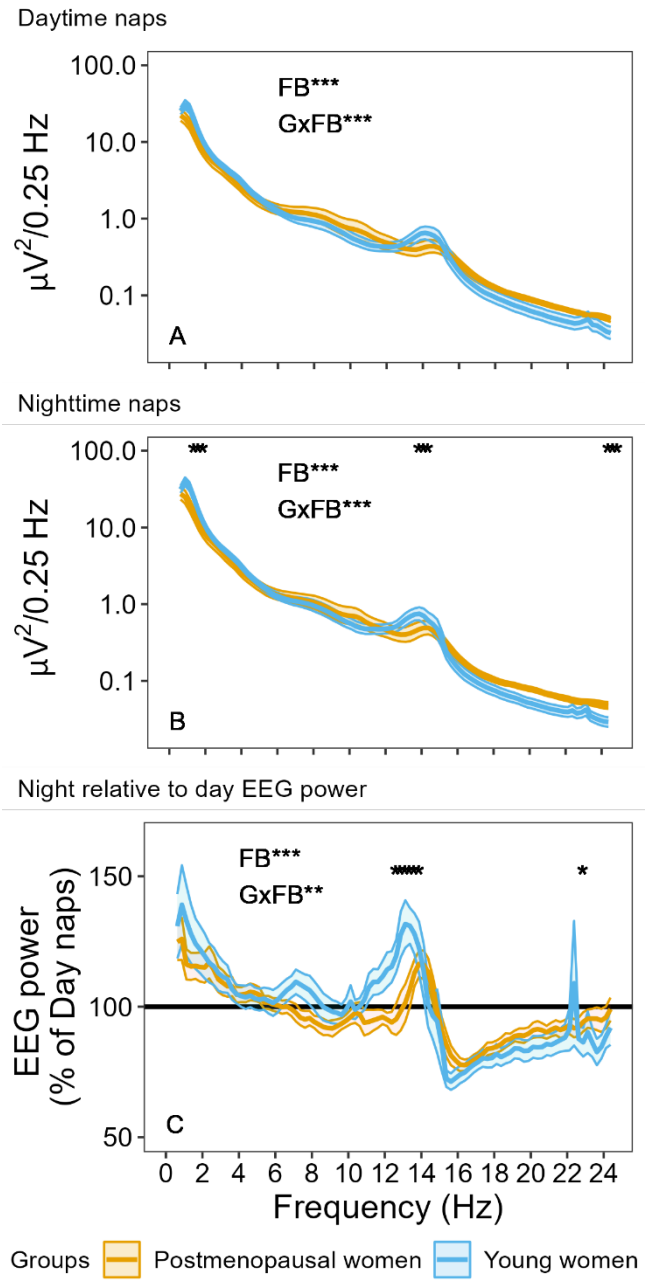

Figure 3. 2. EEG power during daytime and nighttime naps of the USW procedure. Panel A: EEG power during nighttime naps. Panel B: EEG power during daytime naps. Panel C: EEG power of nighttime naps relative to daytime naps. FB = significant main effect of frequency band. GxFB = significant group by frequency band interaction. The asterisks (*) along the top X-axis indicate significant group differences by frequency band ($p<0.05$). *** $p<0.001$, ** $p<0.01$. Lines represent mean EEG power whereas shaded areas represent SEM.

3.5.3 Circadian variation of frequency bands during the USW procedure

The circadian variation of delta, theta, alpha, sigma, and beta power across the USW procedure are presented in Figure 3.3. In both groups, a significant circadian variation was observed in alpha, sigma at (13-14 Hz and 15-16 Hz bins), and beta power. Young women additionally presented a significant circadian variation of delta, theta, and sigma power (12-13 Hz bin). A lower mesor in delta, theta, and sigma (14-15 Hz bin) power was observed in postmenopausal compared to young women. A lower amplitude in sigma (13-14 Hz bin) power was observed in postmenopausal women, whereas the power of other frequency bands did not show amplitude differences between groups. Relative to rise-time, postmenopausal women presented an earlier peak of the alpha power rhythm compared to young women. No other phase difference was observed in other frequency bands. The circadian parameters of frequency bands power are reported in Table 3.3. Finally, an effect of time elapsed into the USW was observed for delta, theta, and alpha power, with increasing values across the USW procedure ($p \leq 0.016$) and without group differences ($p \geq 0.19$). Results of the linear mixed-effects model and cosinor regressions of delta, theta, alpha, sigma, and beta power are reported in Tables 3.2 and 3.3, respectively.

The circadian variation of LSFA (12.25-13.75 Hz) and HSFA (14-15.5 Hz) across the USW procedure is presented in Supplementary Figure 3.1. In LSFA, a significant circadian variation was observed in young women but not in postmenopausal women. HSFA showed no significant circadian variation in either group. The results of their linear mixed-effects model and circadian parameters are summarized on Supplementary Table 3.4 and 3.5.

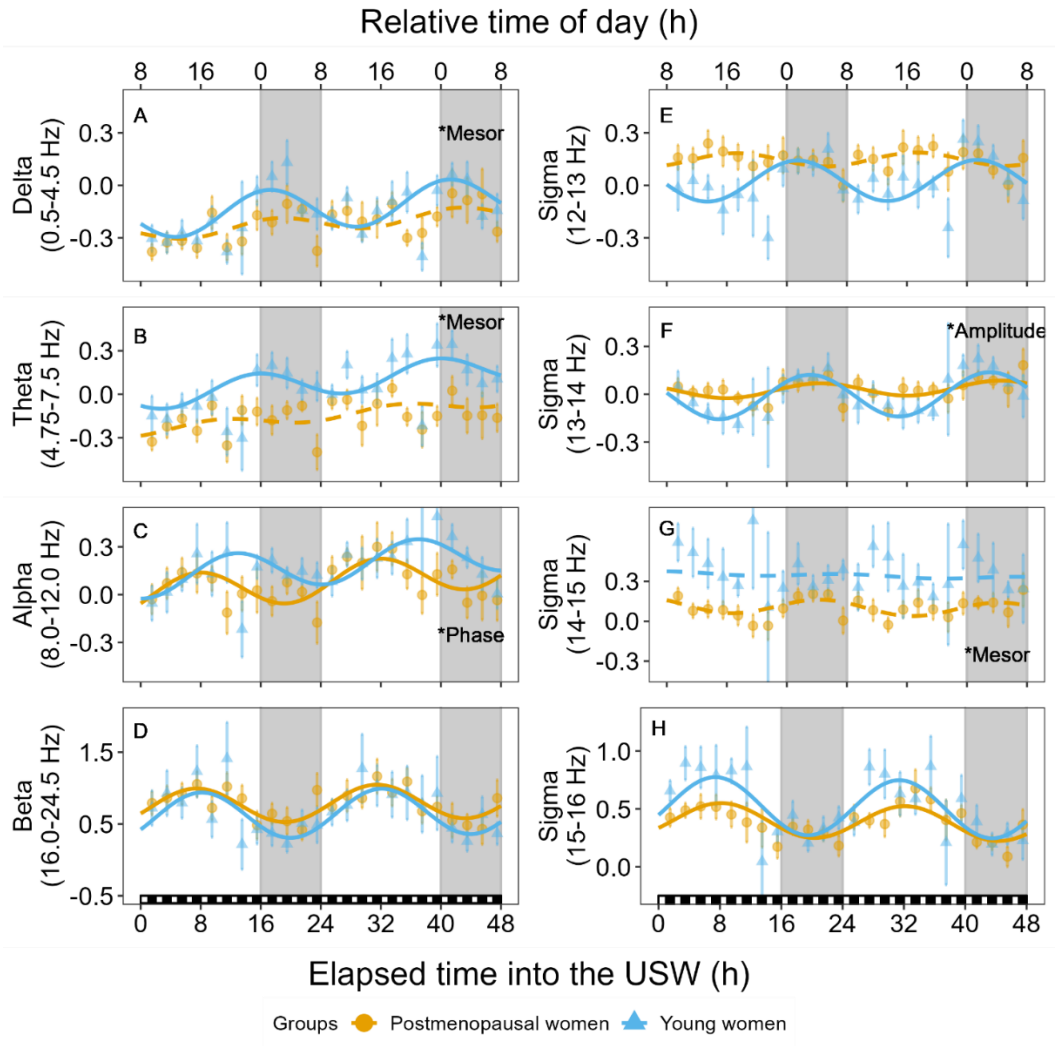

Figure 3. 3. Circadian variation of EEG spectral power per frequency band throughout the USW procedure.

Data were aligned on the time elapsed into the USW procedure (USW, bottom X axis). Solid lines represent significant cosinor regressions, whereas dashed lines depict non-significant regressions. Black (~0 lux) and white (~10 lux) small squares above the bottom X-axis represent the nap and wake periods across the first 48 h of the USW procedure. The top X-axis depicts the corresponding time of day for a participant with a hypothetical bedtime of 00:00 h to 08:00 h. The Y axis illustrates Z-scores. Large grey rectangles depict the projected time of the habitual nocturnal sleep period, which corresponds to the time between habitual bedtime and rise-time. *Mesor = group differences in mesor, *Amplitude = group differences in amplitude, *Phase = group differences in phase. Values are presented as mean ± SEM.

Parameter	Group (p-value)	Time into USW (p-value)	Time into USW x group (p-value)	Circadian variation (p-value)	Circadian x Group Interaction (p-value)
Delta power (0.5-4.5 Hz)	**0.045**	**0.002**	0.31	**0.011**	0.23
Theta power (4.75-7.5 Hz)	**0.008**	**<0.001**	0.22	**0.017**	0.21
Alpha power (8-12 Hz)	0.30	**<0.001**	0.92	**<0.001**	**0.004**
Beta power (16-24.5 Hz)	0.51	0.56	0.88	**<0.001**	0.43
Sigma power:					
12-13 Hz	0.16	0.60	0.67	**0.023**	**<0.001**
13-14 Hz	0.60	0.10	0.61	**<0.001**	**0.003**
14-15 Hz	**0.051**	0.85	0.70	0.63	0.79
15-16 Hz	0.26	0.18	0.19	**<0.001**	0.25

Table 3. 2. Results of linear mixed-effects model of EEG power frequency bands during the USW procedure. Bold values denote statistical significance.

Parameter	Mesor (z-score)					Amplitude (z-score)							Acrophase (elapsed time into the USW in h)				
	PMW		YW			PMW			YW				PMW		YW		
	Mean	SEM	Mean	SEM	p-value	Mean	SEM	95% CI	Mean	SEM	95% CI	p-value	Mean	SEM	Mean	SEM	p-value
Delta power	-0.216	0.032	-0.129	0.029	**0. 045**	0.044	0.039	-0.033, 0.121	0.120	0.034	**0.052, 0.187**	0.14	-	-	16.976	0.817	-
Theta power	-0.149	0.063	0.085	0.054	**0.008**	0.035	0.028	-0.020, 0.090	0.094	0.033	**0.029, 0.159**	0.18	-	-	15.387	1.245	-
Alpha power	0.080	0.070	0.182	0.060	0.30	0.118	0.033	**0.053, 0.183**	0.119	0.025	**0.070, 0.169**	0.97	7.562	0.868	12.545	0.999	**<0.001**
Beta power	0.784	0.182	0.644	0.156	0.51	0.247	0.064	**0.121, 0.372**	0.329	0.057	**0.216, 0.441**	0.33	7.337	0.887	8.040	0.643	0.52
Sigma power:																	
12-13 Hz	0.147	0.068	0.026	0.058	0.16	0.039	0.024	-0.008, 0.085	0.116	0.019	**0.079, 0.154**	0.012	-	-	17.420	0.798	-
13-14 Hz	0.029	0.052	-0.010	0.045	0.60	0.043	0.021	**0.002, 0.083**	0.135	0.018	**0.099, 0.171**	**<0.001**	20.246	2.016	19.016	0.627	0.56
14-15 Hz	0.103	0.094	0.343	0.081	**0.051**	-	-	-	-	-	-	-	-	-	-	-	-
15-16 Hz	0.389	0.078	0.512	0.067	0.26	0.144	0.045	**0.055, 0.233**	0.244	0.043	**0.159, 0.329**	0.11	8.232	1.038	7.452	0.551	0.51

Table 3. 3. Circadian parameters of EEG power frequency bands in postmenopausal women (PMW) and young women studied at mid-follicular phase (YW) based on time elapsed into the USW procedure.

Circadian parameters were Z-scored based on the mean and SD of the baseline sleep period. Positive mean values represent increased values in the USW relative to baseline sleep. Negative mean values represent decreased values in the USW relative to baseline sleep. Amplitude and phase were calculated only when the circadian variation was significant. *P-values* for mesor are equivalent to the group effect on Table 3.2. *P*-values for amplitude and acrophase comparisons were based on two-tailed t-test. Bold values denote statistical significance.

3.6 Discussion

The objective of this study was to investigate postmenopausal changes in sleep microstructure and its circadian variation. During the baseline sleep period, we found that postmenopausal women had a lower power within the delta and sigma ranges, and increased power within alpha at specific thirds of the night. During the USW procedure, postmenopausal women had a dampened or undetectable circadian amplitude of delta (or SWA), theta, and bins within the sigma power (12-15 Hz). Additionally, we found that postmenopausal women had lower levels of delta, theta, and bins within the sigma power (14-15 Hz). The increased in sigma band bins was also lower during nighttime relative to daytime naps in postmenopausal women.

3.6.1 Sigma power

Main findings in sigma power of postmenopausal women include the absence of rhythm in the 12-13 Hz bin, a dampened amplitude of the 13-14 Hz bin, and a decreased power in the 14-15 Hz bin during the USW procedure, as well as decreased power within sigma bins during the final two thirds of the baseline night compared to younger women. In prior studies, SFA has been classified into LSFA and HSFA which follow different circadian rhythms: LSFA peaking at night and HSFA peaking during the day (Dijk et al., 1997; Munch et al., 2010).

In the current study, we observed a clear circadian variation of LSFA with a nocturnal peak in young women (Figure A1), whereas no rhythm was observed in postmenopausal women. This is consistent with others reports with similar LSFA definitions (Munch et al: 12.25-13.75 Hz (Munch et al., 2010); Dijk et al: 12.25-13 Hz (Dijk et al., 1997)). In a forced desynchrony protocol of seven young men, Dijk and colleagues found a circadian variation of LSFA peaking along with melatonin secretion. Munch and colleagues studied 24 men and women aged 55-78 years in a forced desynchrony protocol and did not observe a significant circadian modulation of LSFA in their aged population (Munch et al., 2010). Although somewhat consistent with our LSFA results in postmenopausal women, their study included both older men and women and did not consider menopausal status, thus limiting comparison. Since the rhythms of LSFA was absent in postmenopausal women, LSFA was further divided into 1-Hz bins (12-13 and 13-14 Hz), allowing us to observe a more detailed variation of these frequency bands. As

expected, we observed that young women had significant rhythms in both the 12-13 Hz and 13-14 Hz ranges, with peak power density at night. Interestingly, postmenopausal women showed no circadian rhythm observed at 12-13 Hz but there was a significant rhythm at 13-14 Hz of decreased amplitude and peak power density at night. It has been previously described that frequency within sleep spindles increases with aging (Purcell et al., 2017), which may explain the significant rhythm of 13-14 Hz in postmenopausal women, and the absence of one at 12-13 Hz. These LSFA observations are also present when night EEG power was plotted relative to day (Figure 2), where the peak of LSFA in postmenopausal women occurs at a higher frequency than in younger women (Dijk et al., 1997; Munch et al., 2005; Munch et al., 2010).

The absence of HSFA circadian variation in our study contrasts with previous findings. Dijk et al and Munch et al reported a circadian modulation of HSFA in young men (13.75-15.5 Hz) and older men and women (14-15.5 Hz) (Munch et al., 2010), with the peak occurring during the day. While 14-15 Hz showed no circadian modulation in either group, 15-16 Hz presented a significant circadian variation in both postmenopausal and young women with a daytime peak. This 15-16 Hz variation, similar to the HSFA variation described in the literature, appeared at higher frequencies than prior studies (Dijk et al: men aged 21-25 y, 13.75-15.5 Hz (Dijk et al., 1997); Munch et al: men and women aged 55-78 y, 14-15.5 Hz (Munch et al., 2010)) and may indicate age and sex differences in this frequency band. However, more studies are needed to confirm this statement.

The absent and dampened circadian variation of low-frequency sigma power (12-14 Hz) in postmenopausal women may represent an impaired output of the circadian pacemaker regulating spindle activity during sleep (Dijk & Duffy, 2020). Sleep spindles are part of sigma power and are considered a hallmark of NREM sleep as a protective factor to external stimuli and memory consolidation contributor (Fernandez & Luthi, 2020). They are generated in the reticular nucleus of the thalamus and integrated in the thalamocortical loop to reach the cortex (Fernandez & Luthi, 2020). Although it remains unknown whether the SCN of the hypothalamus directly regulates the thalamocortical network, neuronal projections from the SCN to the thalamus have been previously

described (Novak et al., 2000) and the presence of a circadian variation of SFA further supports this connection.

Different processes may contribute to the lower levels and dampened circadian variation of SFA in postmenopausal women in the current study. Brain atrophy due to aging has been correlated with reduced sigma power (Guazzelli et al., 1986). Animal studies and clinical trials have observed enhanced sleep spindles with progesterone administration, possibly by stimulating the reticular nucleus of the thalamus (Belelli & Lambert, 2005; Fernandez & Luthi, 2020). We previously reported reduced melatonin in postmenopausal women (Perez-Medina-Carballo et al., 2023). Melatonin administration has also been shown to increase LSFA compared to placebo during daytime naps (Dijk et al., 1995). Consequently, brain atrophy, the declining levels of progesterone and melatonin, may contribute to the decreases SFA and dampened rhythms of postmenopausal women, but the specific effect and contribution of these factors remain to be elucidated.

3.6.2 Delta power

Another interesting observation was the lower levels of SWA during the USW procedure and its blunted circadian variation in postmenopausal women compared to younger women. Although there were no differences between groups across the full delta frequency band (0.5-4.5 Hz), a lower power within the delta range was observed for postmenopausal women from 0.75 to 1.25 Hz during the first third of the baseline night, as well as the nighttime naps of the USW procedure. Given that SWA is understood to reflect the homeostatic sleep process (Borbely et al., 2016), the lower levels observed for postmenopausal women indicate a reduced accumulation of sleep pressure during time awake. To mitigate the confounding effect of sleep deprivation on SWA levels, both groups in this study followed a regular sleep-wake schedule with a similar duration of time in bed for two weeks prior to laboratory entry. In fact, they presented comparable TST, time in NREM sleep, and WASO during the baseline sleep period, and thus sleep deprivation prior to laboratory entry likely does not explain the group differences in SWA during the baseline sleep.

Although SWA is mainly controlled by the homeostatic process regulating sleep propensity, circadian modulation of SWA has also been demonstrated in both young (Dijk et al., 1997; Lazar et al., 2015; Santhi et al., 2016) and older (Munch et al., 2010) men and women. While the circadian variation of SWA seems to be reduced in older populations, we are not aware of prior studies that performed statistical comparisons between older and younger women. In addition to reduced production of SWA, consistent with a reduced sleep homeostatic process, the dampened rhythm of SWA in postmenopausal women during the USW suggest a weakened output of the circadian pacemaker.

SWA is generated in the basal forebrain and distributed throughout the cortex, and accordingly, brain atrophy has been associated with decreased SWA with aging (Jones, 2020; Liu et al., 2017; Niethard et al., 2018). Estrogen administration has also been shown to decrease SWA in animal models and in one randomized clinical trial in humans (N. Kalleinen et al., 2008; Smith et al., 2022). In the present study, young women had higher levels of SWA despite their higher levels of estrogen compared to postmenopausal women (Perez-Medina-Carballo et al., 2023). Although not measured in the current study, it is more likely that brain atrophy is the main contributor to the declined levels of SWA in this group of postmenopausal women based on the interpretation of the literature.

3.6.3 Theta power

Theta power also presented a significant circadian modulation in young women only. As a low frequency EEG wave, average levels of theta were also reduced during the USW procedure in postmenopausal women. It is clear that theta power is mainly modulated by homeostatic and affected by sleep deprivation, thus is expected to progressively increase in both groups during the USW procedure due to the slight built-up sleep restriction (Cajochen et al., 1999). Although the literature on the circadian variation of theta power in NREM sleep is limited, our results are consistent with those showing a circadian variation in young (Dijk et al., 1997) but not in aged populations (Munch et al., 2010). During wakefulness and REM sleep, theta power has been associated with learning and memory consolidation, but its function during NREM sleep remains unclear (Karakas, 2020).

3.6.4 Alpha and beta power

Power in the high frequency bands, such as alpha and beta, has been associated with cortical arousal, sleep disruption, and non-restorative sleep (Ehrhart et al., 2000; Feige et al., 2013; Stone et al., 2008). Research on menopausal women found increased beta power linked to disrupted sleep, while alpha power has been less explored (Campbell et al., 2011; Matthews et al., 2021; Schwarz et al., 2017). In the current study, we found increased power within the alpha frequency band in postmenopausal women during the first third of the baseline sleep period. Likewise, during nighttime naps of the USW, increased power in frequencies of the beta range was observed at 24-24.5 Hz in postmenopausal compared to young women. Although the function of alpha power during NREM sleep remains unclear, it is possible that the increased alpha in postmenopausal women during the 1st part of the baseline sleep period relate to the increased number of arousals as reported in Pérez-Medina-Carballo et al (Perez-Medina-Carballo et al., 2023). In the current study, the increase in beta power during nighttime naps may reflect a relative state of hyperarousal at night in the postmenopausal group (Shi et al., 2022; Zhao et al., 2021), even though these women considered themselves to be healthy sleepers. Interestingly, EEG power was comparable between groups during daytime naps, suggesting a possible influence of the endogenous circadian system.

In terms of circadian phase, we only observed differences between groups in the alpha rhythm, with postmenopausal women having an acrophase advanced by ~5 hours compared to young women. The significance of this finding remains to be elucidated. Although this advanced alpha rhythm may contribute to reinforce earlier sleep times in older women, the sleep schedule was only advanced by ~1 hour in postmenopausal compared to younger women. It also remains intriguing that no other EEG frequency bands showed phase differences. In a previous analysis of the current study, we did not observe phase differences in either melatonin, core body temperature, and alertness rhythms between postmenopausal and young women (Perez-Medina-Carballo et al., 2023).

3.6.5 Strengths and limitations

Our study has limitations, including the small sample size, which increases the possibility of type 2 errors and limits the clinical implications of the study results. As such,

during baseline sleep we were not able to observe group differences between standard frequency bands and group differences were only evident in the frequency bin analysis (by 0.25 Hz). However, our results are still consistent with an increased sleep fragility in postmenopausal women. We also included a postmenopausal woman taking hormone replacement therapy. However, since her results were within 2 SD of the group data, her data were deemed adequate to be included in the analyses. Additionally, women recruited in this study represent only a healthy minority of postmenopausal women which may affect the generalizability of our results to the broader population at this stage of life.

Our SWA analysis presents two main limitations. First, only central channels (C3) were analyzed, since frontal channels were not recorded in young women. The literature reports that the highest SWA activity is recorded in the frontal EEG channels (Lazar et al., 2015), which might influence our ability to detect between group differences in the present study. Second, we cannot completely exclude that the maintenance of an 8-hour sleep schedule by participants prior to entry into the laboratory may have induced some sleep restriction. However, the groups were comparable at baseline in terms of several sleep parameters (TST, SE and stages N2, N3, and REM sleep) (Perez-Medina-Carballo et al., 2023), which suggests this risk is minimal. Moreover, the maintenance of a regular sleep schedule during the preparatory phase minimized the risk that the group differences observed in the circadian variation of sleep could be explained by differences in the regularity of their sleep-wake cycle.

Despite these limitations, our rigorous screening procedures allowed us to exclude several conditions that may affect the sleep and circadian rhythms of postmenopausal women such as depression, sleep apnea, chronic conditions, use of medications, etc. Indeed, all our participants were free of medication apart from one postmenopausal woman taking hormone replacement therapy. Another strength of our study is the specialized protocol that allowed us to record sleep throughout day and night for 48 hours, while minimizing masking factors that may affect our dependent variables. To the best of our knowledge, this is the first study to examine changes in the circadian variation of EEG power in postmenopausal women, adding important insights into the understanding of menopause-related sleep changes.

3.7 Conclusion

In this small group of healthy-sleeping postmenopausal women, we observed changes in the microstructure of sleep and its circadian variation. SFA differences may reflect aging processes such as hormonal changes at menopause, brain atrophy, and declining melatonin levels, whereas SWA differences may largely reflect the effect of brain atrophy rather than that of ovarian hormones. Increased power within beta and alpha bands may reflect increased sleep disruption in this group of postmenopausal women. Furthermore, the dampened circadian variation of delta, theta, and sigma power along with previous findings (Perez-Medina-Carballo et al., 2023) support the hypothesized weakened circadian signal promoting sleep in aged women. Although our group of postmenopausal women were healthy and reported no sleep complaints, changes in their sleep microstructure underline the greater fragility of sleep at this vulnerable time of life, akin to that reported in patients with insomnia (Zhao et al., 2021).It is reasonable to hypothesize that the changes in EEG power frequency bands changes observed after menopause may contribute to the increased risk of developing sleep disturbances, although the observational design of our study does not allow us to assume causal relationships. Nevertheless, the present study clarifies the changes in EEG parameters that occur in women after menopause across circadian phases. These results may serve as a basis for the design of future epidemiological and clinical studies.

3.8 Acknowledgments

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3.9 Data sharing and data availability

The data underlying this article cannot be shared publicly because participants did not agree that their data be placed in a publicly accessible database. Therefore, for ethical and confidentiality reasons, the authors cannot provide public access to these data. Nevertheless, materials, data, and protocols will be made available for investigation of

scientific integrity if necessary. Readers are free to contact the principal investigator if they wish to discuss collaborations to build on these published data.

3.10 Ethics approval and patient consent statement

All procedures were approved by the Research Ethics Board of the Montreal West Island IUHSSC – Douglas Research Centre (no. 2018-175), and each participant provided informed consent.

3.11 References

Arnardottir, E. S., Bjornsdottir, E., Olafsdottir, K. A., Benediktsdottir, B., & Gislason, T. (2016). Obstructive sleep apnoea in the general population: highly prevalent but minimal symptoms. Eur Respir J, 47(1), 194-202. https://doi.org/10.1183/13993003.01148-2015

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Usinglme4. J. Stat. Softw., 67(1), 1-48. https://doi.org/10.18637/jss.v067.i01

Belelli, D., & Lambert, J. J. (2005). Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci, 6(7), 565-575. https://doi.org/10.1038/nrn1703

Berry, R. B., Quan, S. F., Abreu, A. R., Bibbs, M. L., DelRosso, L., Harding, S. M., Mao, M.-M., Plante, D. T., Pressman, M. R., Troester, M. M., & Vaughn, B. V. (2020). The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.6.

Borbely, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: a reappraisal. J Sleep Res, 25(2), 131-143. https://doi.org/10.1111/jsr.12371

Cajochen, C., Foy, R., & Dijk, D. J. (1999). Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. Sleep Res Online, 2(3), 65-69. https://www.ncbi.nlm.nih.gov/pubmed/11382884

Campbell, I. G., Bromberger, J. T., Buysse, D. J., Hall, M. H., Hardin, K. A., Kravitz, H. M., Matthews, K. A., Rasor, M. O., Utts, J., & Gold, E. (2011). Evaluation of the association of menopausal status with delta and beta EEG activity during sleep. Sleep, 34(11), 1561-1568. https://doi.org/10.5665/sleep.1398

Dijk, D. J., & Duffy, J. F. (2020). Novel Approaches for Assessing Circadian Rhythmicity in Humans: A Review. J Biol Rhythms, 35(5), 421-438. https://doi.org/10.1177/0748730420940483

Dijk, D. J., Roth, C., Landolt, H. P., Werth, E., Aeppli, M., Achermann, P., & Borbely, A. A. (1995). Melatonin effect on daytime sleep in men: suppression of EEG low frequency activity and enhancement of spindle frequency activity. Neurosci Lett, 201(1), 13-16. https://doi.org/10.1016/0304-3940(95)12118-n

Dijk, D. J., Shanahan, T. L., Duffy, J. F., Ronda, J. M., & Czeisler, C. A. (1997). Variation of electroencephalographic activity during non-rapid eye movement and rapid eye movement sleep with phase of circadian melatonin rhythm in humans. J Physiol, 505 (Pt 3)(Pt 3), 851-858. https://doi.org/10.1111/j.1469-7793.1997.851ba.x

Ehrhart, J., Toussaint, M., Simon, C., Gronfier, C., Luthringer, R., & Brandenberger, G. (2000). Alpha activity and cardiac correlates: three types of relationships during nocturnal sleep. Clin Neurophysiol, 111(5), 940-946. https://doi.org/10.1016/s1388-2457(00)00247-9

Feige, B., Baglioni, C., Spiegelhalder, K., Hirscher, V., Nissen, C., & Riemann, D. (2013). The microstructure of sleep in primary insomnia: an overview and extension. Int J Psychophysiol, 89(2), 171-180. https://doi.org/10.1016/j.ijpsycho.2013.04.002

Fernandez, L. M. J., & Luthi, A. (2020). Sleep Spindles: Mechanisms and Functions. Physiol Rev, 100(2), 805-868. https://doi.org/10.1152/physrev.00042.2018

Guazzelli, M., Feinberg, I., Aminoff, M., Fein, G., Floyd, T. C., & Maggini, C. (1986). Sleep spindles in normal elderly: comparison with young adult patterns and relation to nocturnal awakening, cognitive function and brain atrophy. Electroencephalogr Clin Neurophysiol, 63(6), 526-539. https://doi.org/10.1016/0013-4694(86)90140-9

Jones, B. E. (2020). Arousal and sleep circuits. Neuropsychopharmacology, 45(1), 6-20. https://doi.org/10.1038/s41386-019-0444-2

Kalleinen, N., Polo-Kantola, P., Himanen, S.-L., Alhola, P., Joutsen, A., Urrila, A. S., & Polo, O. (2008). Sleep and the menopause–do postmenopausal women experience worse sleep than premenopausal women? Menopause Int, 14(3), 97-104. https://doi.org/10.1258/mi.2008.008013

Kalleinen, N., Polo, O., Himanen, S. L., Joutsen, A., & Polo-Kantola, P. (2008). The effect of estrogen plus progestin treatment on sleep: a randomized, placebo-controlled, double-blind trial in premenopausal and late postmenopausal women. Climacteric, 11(3), 233-243. https://doi.org/10.1080/13697130802112033

Karakas, S. (2020). A review of theta oscillation and its functional correlates. Int J Psychophysiol, 157, 82-99. https://doi.org/10.1016/j.ijpsycho.2020.04.008

Knowles, J. E. F., Carl. (2020). merTools: Tools for Analyzing Mixed Effect Regression Models. In (Version R package version 0.5.2) https://CRAN.R-project.org/package=merTools

Lazar, A. S., Lazar, Z. I., & Dijk, D. J. (2015). Circadian regulation of slow waves in human sleep: Topographical aspects. Neuroimage, 116, 123-134. https://doi.org/10.1016/j.neuroimage.2015.05.012

Liu, H., Yang, Y., Xia, Y., Zhu, W., Leak, R. K., Wei, Z., Wang, J., & Hu, X. (2017). Aging of cerebral white matter. Ageing Res Rev, 34, 64-76. https://doi.org/10.1016/j.arr.2016.11.006

Matthews, K. A., Lee, L., Kravitz, H. M., Joffe, H., Neal-Perry, G., Swanson, L. M., Evans, M. A., & Hall, M. H. (2021). Influence of the menopausal transition on polysomnographic sleep characteristics: a longitudinal analysis. Sleep, 44(11). https://doi.org/10.1093/sleep/zsab139

Michael Sachs. (2014). cosinor: Tools for estimating and predicting the cosinor model. In https://CRAN.R-project.org/package=cosinor

Munch, M., Knoblauch, V., Blatter, K., Schroder, C., Schnitzler, C., Krauchi, K., Wirz-Justice, A., & Cajochen, C. (2005). Age-related attenuation of the evening circadian arousal signal in humans. Neurobiol Aging, 26(9), 1307-1319. https://doi.org/10.1016/j.neurobiolaging.2005.03.004

Munch, M., Silva, E. J., Ronda, J. M., Czeisler, C. A., & Duffy, J. F. (2010). EEG sleep spectra in older adults across all circadian phases during NREM sleep. Sleep, 33(3), 389-401. https://doi.org/10.1093/sleep/33.3.389

Niethard, N., Ngo, H. V., Ehrlich, I., & Born, J. (2018). Cortical circuit activity underlying sleep slow oscillations and spindles. Proc Natl Acad Sci U S A, 115(39), E9220-E9229. https://doi.org/10.1073/pnas.1805517115

Novak, C. M., Harris, J. A., Smale, L., & Nunez, A. A. (2000). Suprachiasmatic nucleus projections to the paraventricular thalamic nucleus in nocturnal rats (Rattus norvegicus) and diurnal nile grass rats (Arviacanthis niloticus). Brain Res, 874(2), 147-157. https://doi.org/10.1016/s0006-8993(00)02572-5

Olive, D. J. (2017). Linear regression. Springer. https://doi.org/10.1007/978-3-319-55252-1

Pennestri, M.-H., Whittom, S., Adam, B., Petit, D., Carrier, J., & Montplaisir, J. (2006). PLMS and PLMW in healthy subjects as a function of age: prevalence and interval distribution. Sleep, 29(9), 1183-1187. https://doi.org/10.1093/sleep/29.9.1183

Perez-Medina-Carballo, R., Kosmadopoulos, A., Boudreau, P., Robert, M., Walker, C. D., & Boivin, D. B. (2023). The circadian variation of sleep and alertness of postmenopausal women. Sleep, 46(2). https://doi.org/10.1093/sleep/zsac272

Provencher, T., Fecteau, S., & Bastien, C. H. (2020). Patterns of Intrahemispheric EEG Asymmetry in Insomnia Sufferers: An Exploratory Study. Brain Sci, 10(12). https://doi.org/10.3390/brainsci10121014

Purcell, S. M., Manoach, D. S., Demanuele, C., Cade, B. E., Mariani, S., Cox, R., Panagiotaropoulou, G., Saxena, R., Pan, J. Q., Smoller, J. W., Redline, S., & Stickgold, R. (2017). Characterizing sleep spindles in 11,630 individuals from the National Sleep Research Resource. Nat Commun, 8, 15930. https://doi.org/10.1038/ncomms15930

Santhi, N., Lazar, A. S., McCabe, P. J., Lo, J. C., Groeger, J. A., & Dijk, D. J. (2016). Sex differences in the circadian regulation of sleep and waking cognition in humans. Proc Natl Acad Sci U S A, 113(19), E2730-2739. https://doi.org/10.1073/pnas.1521637113

Schwarz, J. F. A., Akerstedt, T., Lindberg, E., Gruber, G., Fischer, H., & Theorell-Haglow, J. (2017). Age affects sleep microstructure more than sleep macrostructure. J Sleep Res, 26(3), 277-287. https://doi.org/10.1111/jsr.12478

Shechter, A., Varin, F., & Boivin, D. B. (2010). Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. Sleep, 33(5), 647-656. https://doi.org/10.1093/sleep/33.5.647

Shi, Y., Ren, R., Lei, F., Zhang, Y., Vitiello, M. V., & Tang, X. (2022). Elevated beta activity in the nighttime sleep and multiple sleep latency electroencephalograms of chronic insomnia patients. Front Neurosci, 16, 1045934. https://doi.org/10.3389/fnins.2022.1045934

Smith, P. C., Phillips, D. J., Pocivavsek, A., Byrd, C. A., Viechweg, S. S., Hampton, B., & Mong, J. A. (2022). Estradiol influences adenosinergic signaling and nonrapid eye movement sleep need in adult female rats. Sleep, 45(3). https://doi.org/10.1093/sleep/zsab225

Stevens, A., & Ramirez-Lopez, L. (2022). An introduction to the prospectr package. In R package Vignette.

Stone, K. C., Taylor, D. J., McCrae, C. S., Kalsekar, A., & Lichstein, K. L. (2008). Nonrestorative sleep. Sleep Med Rev, 12(4), 275-288. https://doi.org/10.1016/j.smrv.2007.12.002

Svetnik, V., Snyder, E. S., Ma, J., Tao, P., Lines, C., & Herring, W. J. (2017). EEG spectral analysis of NREM sleep in a large sample of patients with insomnia and good sleepers: effects of age, sex and part of the night. J Sleep Res, 26(1), 92-104. https://doi.org/10.1111/jsr.12448

Xu, Q., & Lang, C. P. (2014). Examining the relationship between subjective sleep disturbance and menopause: a systematic review and meta-analysis. Menopause, 21(12), 1301-1318. https://doi.org/10.1097/GME.0000000000000240

Zhao, W., Van Someren, E. J. W., Li, C., Chen, X., Gui, W., Tian, Y., Liu, Y., & Lei, X. (2021). EEG spectral analysis in insomnia disorder: A systematic review and meta-analysis. Sleep Med Rev, 59, 101457. https://doi.org/10.1016/j.smrv.2021.101457

Zolfaghari, S., Yao, C., Thompson, C., Gosselin, N., Desautels, A., Dang-Vu, T. T., Postuma, R. B., & Carrier, J. (2020). Effects of menopause on sleep quality and sleep disorders: Canadian Longitudinal Study on Aging. Menopause, 27(3), 295-304. https://doi.org/10.1097/GME.0000000000001462

3.12 Supplementary material

Participant	AHI	Participant	AHI
YW-1	0	PMW-1	2.05
YW-2	0.68	PMW-2	8.16
YW-3	0	PMW-3	6.02
YW-4	1.44	PMW-4	0.85
YW-5	0	PMW-5	2.96
YW-6	0.4	PMW-6	3.42
YW-7	3.36	PMW-7	5.36
YW-8	0.95	PMW-8	3.3
YW-9	2.21		
YW-10	0		
YW-11	N/A		
YW-12	0		
Mean	**0.82**		**4.02**
SD	**1.11**		**2.36**

Supplementary Table 3. 1. Apnea-hypopnea index (AHI) of postmenopausal (PMW) and young women (YW).

	Mixed-model term					
	Frequency band effect		Group effect (postmenopausal vs young women)		Group × Frequency band interaction	
	X^2	p value	X^2	p value	X^2	p value
Full night	**6332.2**	**<0.001**	0.38	0.54	**173.03**	**<0.001**
1st part	**6357**	**<0.001**	0.66	0.42	**177.75**	**<0.001**
2nd part	**6152.6**	**<0.001**	0.63	0.43	**150.77**	**<0.001**
3rd part	**5694.2**	**<0.001**	0.13	0.72	**176.92**	**<0.001**

Supplementary Table 3. 2. Summary of linear mixed-effects model on baseline sleep parameters divided by thirds.

X2 and p-values are obtained from likelihood-ratio tests. Bold values denote statistical significance.

	Young women (mean ± SEM)	Postmenopausal women (mean ± SEM)	*p-value*
Delta power (μv^2/0.25 Hz)			
Full night	240.49 ± 28.48	189.15 ± 25.62	0.20
1st part	296.54 ± 25.54	232.90 ± 35.41	0.17
2nd part	140.12 ± 18.10	139.76 ± 25.688	0.84
3rd part	174.13 ± 76.29	92.87 ± 13.48	0.54
Theta power (μv^2/0.25 Hz)			
Full night	13.98 ± 1.31	17.12 ± 1.61	0.15
1st part	15.74 ± 1.51	18.63 ± 1.88	0.25
2nd part	11.29 ± 1.41	14.79 ± 1.35	0.09
3rd part	11.47 ± 1.76	12.06 ± 1.24	0.49
Alpha power (μv^2/0.25 Hz)			
Full night	10.09 ± 1.31	13.13 ± 2.49	0.30
1st part	10.02 ± 1.26	13.61 ± 2.83	0.27
2nd part	8.85 ± 1.35	11.21 ± 2.04	0.35
3rd part	8.48 ± 1.29	10.23 ± 2.03	0.48
Sigma power (μv^2/0.25 Hz)			
Full night	7.98 ± 1.17	6.01 ± 0.84	0.19
1st part	7.12 ± 1.10	5.79 ± 0.80	0.34
2nd part	6.23 ± 0.88	5.20 ± 0.72	0.38
3rd part	6.56 ± 1.03	4.82 ± 0.66	0.18
12-13 Hz			
Full night	2.04 ± 0.24	1.69 ± 0.28	0.35
1st part	2.02 ± 0.27	1.72 ± 0.30	0.46
2nd part	1.59 ± 0.17	1.44 ± 0.22	0.62
3rd part	1.50 ± 0.20	1.32 ± 0.22	0.55
13-14 Hz			
Full night	2.78 ± 0.39	1.79 ± 0.34	0.07
1st part	2.46 ± 0.37	1.78 ± 0.33	0.19
2nd part	2.25 ± 0.33	1.55 ± 0.29	0.13
3rd part	2.06 ± 0.27	1.31 ± 0.21	**0.042**
14-15 Hz			
Full night	2.24 ± 0.49	1.61 ± 0.19	0.25
1st part	1.85 ± 0.43	1.46 ± 0.16	0.09
2nd part	1.67 ± 0.36	1.41 ± 0.18	0.52
3rd part	2.01 ± 0.42	1.31 ± 0.18	0.15
15-16 Hz			
Full night	0.92 ± 0.21	0.92 ± 0.13	0.66
1st part	0.79 ± 0.18	0.83 ± 0.12	0.44
2nd part	0.71 ± 0.14	0.80 ± 0.09	0.62
3rd part	1.00 ± 0.25	0.88 ± 0.13	0.66
Beta power (μv^2/0.25 Hz)			
Full night	2.18 ± 0.34	2.35 ± 0.16	0.65
1st part	2.05 ± 0.30	2.42 ± 0.20	0.32
2nd part	2.24 ± 0.31	2.41 ± 0.16	0.64
3rd part	2.80 ± 0.57	2.64 ± 0.16	0.80

Supplementary Table 3. 3. Frequency bands for the full nocturnal baseline sleep period and divided by thirds.

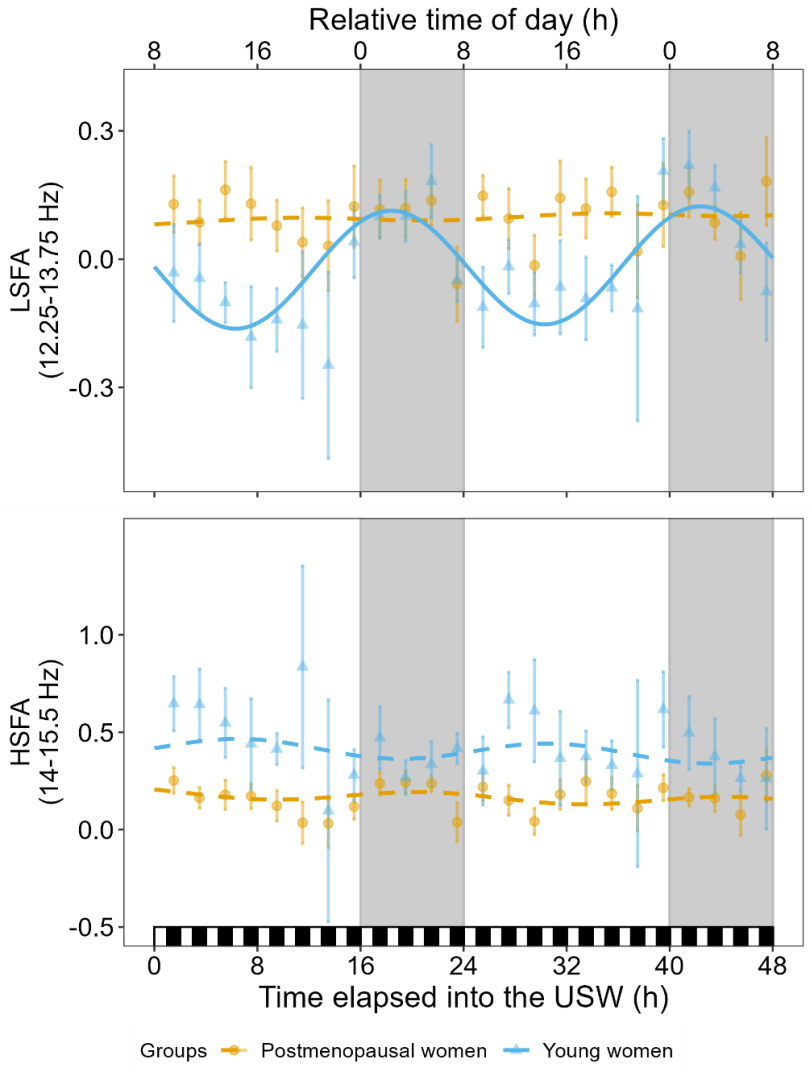
All values are expressed as mean ± SEM. P-values were based on two-tailed t-tests or Mann-Whitney U tests when appropriate. Bold values denote statistical significance.

Parameter	Group (p-value)	Time into USW (p-value)	Time into USW x group (p-value)	Circadian variation (p-value)	Circadian x Group Interaction (p-value)
LSFA	0.10	0.31	0.49	**0.010**	**<0.001**
HSFA	0.056	0.38	0.58	0.91	0.65

Supplementary Table 3. 4. Summary of linear mixed-effects model results of low and high spindle frequency activity (LSFA, HSFA, respectively).
Bold values denote statistical significance.

Parameter	Mesor/Intercept (z-score)					Amplitude (z-score)							Acrophase (elapsed time into the USW in h)				
	PMW		YW			PMW			YW				PMW		YW		
	Mean	SEM	Mean	SEM	p-value	Mean	SEM	95% CI	Mean	SEM	95% CI	p-value	Mean	SEM	Mean	SEM	p-value
LSFA	0.097	0.054	-0.020	0.047	0.10	0.006	0.025	-0.044, 0.055	**0.135**	**0.022**	**0.092, 0.179**	**<0.001**	-	-	18.340	0.682	-
HSFA	0.164	0.095	0.403	0.082	0.06	-	-	-	-	-	-	-	-	-	-	-	-

Supplementary Table 3. 5. Circadian parameters of low and high spindle frequency activity (LSFA, HSFA, respectively) throughout the USW procedure.
Circadian parameters were calculated on Z-scores relative to baseline sleep period. Negative values represent negative changes relative to baseline sleep period. Amplitude and phase were not calculated when the circadian variation was not significant. P-value for mesor is equivalent to the group effect in Supplementary Table 3.2. P-values were based on a two-tailed t-test. Bold values denote statistical significance.



Supplementary Figure 3. 1. Circadian variation of low and high spindle frequency activity (LSFA, HSFA, respectively) throughout the USW procedure.

Data were aligned on the time elapsed into the ultradian sleep-wake cycle procedure (USW, bottom X axis). Solid lines represent significant cosinor regressions, whereas dashed lines depict non-significant regressions. Black (~0 lux) and white (~10 lux) small squares above the bottom X axis represent the nap and wake periods across the first 48 h of the USW procedure. The top X axis depicts the corresponding time of day for a participant with a hypothetical bedtime of 00:00 h to 08:00 h. The Y axis illustrates Z-scores. Large grey rectangles depict the projected time of the habitual nocturnal sleep period, which corresponds to the time between habitual bedtime and rise-time. Values are presented as mean ± SEM.

Chapter 4.

Circadian modulation of heart rate variability in postmenopausal women: implications for cardiovascular health

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4.1 Preface

Since the circadian timing system also regulates the cardiovascular system, we extended our analyses to investigate the changes in heart rate and heart rate variability in healthy postmenopausal women. Our aim was to understand the cardiovascular changes that occur after menopause and how these changes are influenced by sleep stages and the circadian phase.

Most research on HRV is conducted during wakefulness, and only a handful of studies have investigated HRV changes during the sleep of menopausal women (Magri et al., 2006; Virtanen et al., 2023). This aspect is important as specific changes in HRV during sleep have been associated with sleep disturbances such as insomnia (de Zambotti et al., 2018). Our study contributes to the existing literature by analyzing HRV measures specifically during the sleep period of postmenopausal women.

4.2 Abstract

Women are at higher risk of developing cardiovascular diseases (CVD) after the menopausal transition, which has been partially attributed to the declining levels of estrogen and progesterone. Our study investigates the circadian variation of heart rate variability (HRV) during sleep as a potential contributor to the increased CVD risk of postmenopausal women. Eight healthy postmenopausal women and 12 naturally ovulating young women underwent a ≥48-hour ultradian sleep-wake cycle (USW) procedure, consisting of alternating 1-hour nap opportunities and wake periods. Our findings revealed that, compared to younger women, postmenopausal women consistently presented lower HRV (measured by the standard deviation of RR intervals [SDNN]) and parasympathetic activity (measured by the root mean square of successive differences in RR intervals [RMSSD] and the high-frequency [HF] band) during both baseline sleep and the USW procedure. Circadian analyses revealed dampened HR, SDNN, and RMSSD rhythms in postmenopausal women during the USW procedure. These dampened rhythms suggest a weakened output of the circadian timing system regulating the cardiovascular system after menopause. Furthermore, the decreased values of HRV and parasympathetic activity observed in our small group of postmenopausal women suggest an increased CVD compared to young naturally ovulating women. Our work emphasizes the need for further exploration to disentangle the contributions of declining sex hormone levels and aging to cardiovascular risk and circadian variations in postmenopausal women.

4.3 Introduction

After menopause, women present health challenges such as an increased susceptibility to cardiovascular disease (CVD), sleep disturbances, osteoporosis, and genitourinary conditions compared to their younger counterparts (El Khoudary, 2017; El Khoudary et al., 2020). Based on epidemiological studies, the role of hormonal changes and their impact on the cardiovascular system has been a subject of growing interest. More specifically, estrogen in women has been linked to maintaining cardiovascular health, promoting peripheral vasodilation, and promoting parasympathetic activity through its central effects (Aryan et al., 2020; Spary et al., 2009). Therefore, the decreased levels of this hormone may negatively impact the cardiovascular system of postmenopausal women.

Heart rate (HR) and HRV are markers of the cardiovascular system, as they can reflect the adaptability and parasympathetic activity of the autonomic nervous system (ANS). HRV is obtained from the electrocardiogram (EKG) and is a non-invasive, reliable measure of cardiovascular health (Laborde et al., 2017). Different HRV measures have been described in the literature for several years, and recommendations have been made for physiologically relevant HRV variables (Laborde et al., 2017). The variability of heartbeats is measured by the standard deviation of RR intervals (SDNN). The parasympathetic activity of a branch of the vagus nerve can be measured through the high-frequency (HF) band of the RR interval power spectrum and the root mean square of successive differences between RR intervals (RMSSD) (Laborde et al., 2017; Ottaviani et al., 2020). HRV measures may provide valuable insight into the relationship between menopausal changes and cardiovascular health. In fact, lower HRV and parasympathetic activity have been linked to metabolic and CVD such as hypertension, type 2 diabetes, coronary artery disease, and all-time mortality (Hillebrand et al., 2013; Jain et al., 2023; Stein et al., 2008; Wulsin et al., 2015).

The declining levels of estrogen at menopause coincide with reduced HRV and may negatively affect the cardiovascular health of postmenopausal women (El Khoudary et al., 2020; Harlow et al., 2012; von Holzen et al., 2016). As reported in the literature, postmenopausal women present a decline in vagal activity and HRV as compared to their

younger counterparts (Brockbank et al., 2000; Liu et al., 2003; Moodithaya & Avadhany, 2009; Ribeiro et al., 2001; von Holzen et al., 2016), while perimenopausal women exhibit similar HRV values to those of younger women (Brockbank et al., 2000). These observations, in combination with the increased prevalence of CVD after menopause, strongly imply the role of sex hormones in women's cardiovascular health (El Khoudary et al., 2020). Moreover, estrogen therapy might improve HRV values, mainly when administered within a decade of the last menstrual period, but the effectiveness remains debatable (El Khoudary, 2017; von Holzen et al., 2016).

Sleep disturbances become more common after menopause, affecting 40-60% of women (Shaver & Woods, 2015; Woods & Mitchell, 2010; Zolfaghari et al., 2020). These sleep disturbances could be reflected in HRV. For instance, disruptions in the function of the ANS during sleep, characterized by a decrease in variability and parasympathetic activity, have been linked to insomnia symptoms in various studies (Cosgrave et al., 2021), but results are heterogeneous (Zhao & Jiang, 2023). In fact, changes in sleep have been associated with change in cardiovascular function since HRV variables fluctuate across sleep stages (Eddie et al., 2022). Our previous research has discovered postmenopausal changes in the circadian variation of sleep, alertness, and melatonin (Perez-Medina-Carballo et al., 2023). Our group has also previously discovered day-night fluctuations in HR and HRV (Boudreau et al., 2013), indicating that the circadian system also plays an essential role in regulating cardiac ANS.

Altogether, exploring the relationship between sleep and cardiovascular function in postmenopausal women becomes important. Thus, we aim to understand how circadian changes in cardiac ANS function, as reflected in HRV patterns during sleep, may act as potential contributors to the increased susceptibility of postmenopausal women to cardiovascular disorders.

4.4 Methods

4.4.1 Participants

Details of participants, recruitment and screening methods, laboratory conditions and study design have been previously published (Perez-Medina-Carballo et al., 2023). In summary, two groups of women participated in this study: healthy postmenopausal

women (n=8; mean age ± SD: 54.80 ± 3.37 y, age range 50 – 61 y, 2.08 – 11.28 y since their final menstrual period) and healthy naturally ovulating young women in their mid-follicular phase (n=12; 25.83 ± 3.35 y, range 20 – 30 y; days 5-9 after menses) (Boivin et al., 2016). All participants were physically and mentally healthy and reported no sleep disturbances regardless of their menopausal status. A polysomnographic sleep recording was used to screen participants for sleep apnea and periodic leg movements during sleep. All postmenopausal women had an AHI <15/h of sleep, and young women had an AHI <5/h of sleep. PLMS index was <15/h of sleep in both groups (Berry et al., 2020; Iber, 2007). Participants were not using any medications, except for one postmenopausal woman currently using estradiol transdermal patches and micronized progesterone pills daily. Each participant provided informed consent prior to the study, and all our procedures were approved by the Douglas Mental Health University Institute Research Ethics Board (no. 2018-175).

4.4.2 Design

For at least two weeks before entering the laboratory, participants followed a regular 8-hour sleep schedule, as verified by wrist actigraphy. Following admission to the laboratory, they were provided with an 8-hour baseline sleep opportunity aligned with their habitual sleep schedule. Upon awakening, participants underwent an ultradian sleep-wake cycle (USW) protocol lasting 48 hours for postmenopausal women and 72 hours for young women. This USW protocol involved alternating 1-hour wake periods and 1-hour sleep opportunities in a controlled environment. The protocol concluded with an *ad libitum* nap (Perez-Medina-Carballo et al., 2023; Shechter et al., 2010).

Participants spent the entirety of the protocol in a time-isolated room (i.e., windowless and sound attenuated) maintained at a constant ambient temperature (22.0 ± 2.0 °C). Throughout the USW procedure, participants remained in dim light exposure (< 10 lux) during wake periods, and in complete darkness (0 lux) during sleep opportunities. Isocaloric snacks were provided during wake periods of the USW protocol. Young women maintained a semi-recumbent position during the entire USW procedure, using a bedpan as necessary. To decrease the risk of thrombophlebitis, postmenopausal women remained in a semi-recumbent position for the majority of the procedure but were

permitted brief breaks to use an ensuite bathroom every wake period and 10-minute walks within the bedroom every other wake period. Additionally, a thrice-daily anticoagulant, 2-ml tinzaparin sodium (Innohep), was administered to postmenopausal women on hormonal replacement therapy (Perez-Medina-Carballo et al., 2023).

4.4.3 Measures

Sleep was recorded with polysomnography (PSG; Harmonie, Natus Medical Incorporated, Montreal, QC, Canada) during the baseline sleep and naps of the USW procedure. The PSG montage included an electroencephalogram (EEG; C3/A2, C4/A1, O1/A2, O2/A1 for all participants, and additionally F3/A2 and F4/A1 for postmenopausal women), an EKG, an electrooculogram, and an electromyogram. PSG recordings were sampled with a frequency of 250 Hz in 11 young women, and 512 Hz in one young woman and all postmenopausal women. Sleep stages (i.e., wake, N1, N2, N3 and REM sleep) were visually scored in 30-second epochs using the American Academy of Sleep Medicine (AASM) criteria (Berry et al., 2020). All epochs were visually inspected for artifacts, and the EKG channel was used for HRV analyses.

EKG recordings were exported into the HRVanalysis software (Pichot et al., 2016) for artifact removal and calculation of HRV parameters. Heart rate (HR) was calculated using the RR intervals. Other HRV parameters were also calculated, including the standard deviation of RR intervals (SDNN), root mean square of successive differences between RR intervals (RMSSD), and the high-frequency band of the RR interval power spectrum. The SDNN reflects overall HRV in the time domain. Parasympathetic activity is reflected in RMSSD in the time domain, and HF in the frequency domain. The spectral power of the HF frequency band (0.15-0.4 Hz) was calculated using the Fast Fourier Transform algorithm. These measures were selected based on the recommendations made by Laborde et al. (Laborde et al., 2017). HR and HRV parameters were extracted and binned into 5-min epochs using the HRVanalysis software.

In order to associate HRV and sleep, 5-min HRV epochs (HR, SDNN, RMSSD, and HF) were aligned with 5-min sleep epochs (Cosgrave et al., 2021; Laborde et al., 2017). Only epochs which comprised a single sleep stage across their entire 5-minute period were included. This criterion was selected to reduce variability within HRV results

as HRV fluctuates with sleep stages (Eddie et al., 2022; Yeh et al., 2022). As such, 55.10% (baseline) and 60.16% (USW) of the total number of 5-min HRV epochs were matched to a corresponding sleep stage. The total number of valid 5-min epochs derived from the baseline sleep episode was as follows for each sleep stage: N1 (n=0), N2 (n=526), N3 (n=86), REM sleep (n=217), and wake (n=35). The total number of valid 5-min epochs derived from the naps of the USW procedure for each sleep stage was as follows: N1 (n=5), N2 (n=770), N3 (n=74), REM sleep (n=225), and wake (n=1344).

4.4.4 Statistical analysis

Baseline sleep

All statistical analyses were performed in R version 4.3.2 (R Core Team, 2020). For the 8-hour baseline sleep period, data from HRV parameters (HR, SDNN, RMSSD, HF) were divided into thirds of time in bed, which allowed us to observe time-dependent changes. A linear mixed-effects model was used to compare data within and between subjects using the “lme4” package (Bates et al., 2015). “Time” in bed and participant “group” (postmenopausal women vs young women) were included as fixed effects. Participants were included as random effects. The model tests for the main effects of time, group, and their interactions. Likelihood-ratio tests were used to determine significance of the fixed effect when added to the model. Tukey’s *post hoc* tests were performed when the interactions were significant.

Since HRV parameters have been described to change based on sleep stage, HRV measures were additionally matched with their corresponding sleep stage. A linear mixed-effects model was also used for statistical analysis, with sleep stage, participant group, and their interaction included as fixed effects. Participant IDs were entered as random effects. Tukey’s *post hoc* tests were performed when the interactions were significant.

USW Procedure

Statistical analyses regarding the rhythmicity of HRV in postmenopausal and young women only included data from the first 48 hours of the USW procedure. HRV was only analyzed during the nap opportunities of the USW procedure to avoid artifacts caused by movement during wake periods. Based on their alignment with 5-min sleep

stage epochs, HRV parameters were categorized as occurring during either NREM sleep (i.e., N1, N2, or N3) or wake and then grouped into 2-hour bins based on the elapsed time into the procedure. The circadian variation of HRV was evaluated during NREM sleep overall instead of individual N1, N2, and N3 stages because there was insufficient data at each stage to assess it with this specificity. Since insufficient 5-min REM sleep epochs were observed during the USW procedure to evaluate its circadian variation, HRV changes during REM sleep were not included in the USW analyses. The circadian rhythmicity of each parameter was assessed using the “circacompare” package in R (Parsons et al., 2020). Circadian parameters (mesor, amplitude, and acrophase) were calculated based on the time elapsed into the USW procedure (aligned with the participant’s habitual sleep schedule) and time of day. Circadian parameters were assessed and compared between groups using linear mixed-effects models (Bates et al., 2015). Additionally, HR values may have been influenced by the preceding wake period, particularly in postmenopausal women who had a walking period opportunity every other wake period. Therefore, an additional HR analysis was conducted, excluding naps with a preceding walking period in postmenopausal women.

We assessed the HRV measure outcomes for outliers after including a single postmenopausal woman who was undergoing HRT. The “merTools” package (Knowles, 2020) was employed to extract the random effects estimates for each participant. The HRV results of this postmenopausal woman's data remained within 2 SD for all parameters, and thus, we included her data in the analyses.

4.5 Results

4.5.1 Baseline sleep

For the baseline sleep period, postmenopausal women had an earlier habitual sleep schedule, on average, than young women (mean ± SD; 23:07 ± 00:11 h vs 00:13 ± 00:12 h). HR, SDNN, RMSSD, and HF were divided into thirds of time in bed and are depicted in Figure 4.1. The results of the linear mixed-effects model are described in Table 4.1. A significant time effect was observed in HR, SDNN, and HF. A significant group effect was observed in SDNN, RMSSD, and HF, in which postmenopausal women presented a lower SDNN, RMSSD, and HF than younger women. HR, SDNN, RMSSD, and HF

showed significant interactions. In HR, Tukey's *post hoc* test showed a significant time-by-group interaction ($p \leq 0.001$), but the group-by-time interactions were not ($p \geq 0.53$). In particular, HR decreased throughout the sleep period in postmenopausal women. In young women, HR decreased from the first compared to the second and last thirds of time in bed. In SDNN, *post hoc* comparisons yielded significantly lower SDNN in postmenopausal than young women in the second and last thirds of the time in bed ($p \leq 0.023$). Additionally, SDNN increased throughout the sleep period in young women, whereas in postmenopausal women, only the last third presented higher SDNN compared to the first third of the time in bed ($p < 0.001$). In RMSSD and HF, Tukey's *post hoc* test showed that postmenopausal women had consistently lower RMSSD and HF than young women throughout the time in bed ($p \leq 0.028$). In postmenopausal women, RMSSD and HF decreased throughout the sleep period ($p \leq 0.004$), whereas in young women, RMSSD and HF increased ($p \leq 0.031$).

	Mixed-model term					
	Time effect		Group effect		Interaction	
	X^2	p-value	X^2	p-value	X^2	p-value
HR	**106.64**	**<0.001**	0.24	0.62	**20.17**	**<0.001**
SDNN	**128.51**	**<0.001**	**5.04**	**0.025**	**37.67**	**<0.001**
RMSSD	5.91	0.052	**7.55**	**0.006**	**34.65**	**<0.001**
HF	**12.95**	**0.002**	**12.36**	**<0.001**	**23.55**	**<0.001**

Table 4. 1. Results of linear mixed-effects model on baseline sleep parameters divided by thirds of time in bed.

X^2 and p-values are obtained from likelihood-ratio tests. Bold values denote statistical significance.

	Mixed-model term					
	Sleep stage effect		Group effect		Interaction	
	X^2	p-value	X^2	p-value	X^2	p-value
HR	**161.15**	**<0.001**	0.22	0.64	3.64	0.30
SDNN	**126.74**	**<0.001**	**6.15**	**0.013**	**12.28**	**0.006**
RMSSD	**18.38**	**<0.001**	**7.27**	**0.007**	**8.82**	**0.032**
HF	**33.20**	**<0.001**	**12.00**	**<0.001**	**25.76**	**<0.001**

Table 4. 2. Results of linear mixed-effects model on baseline sleep parameters by sleep stages.

X^2 and p-values are obtained from likelihood-ratio tests. Bold values denote statistical significance ($p<0.05$).

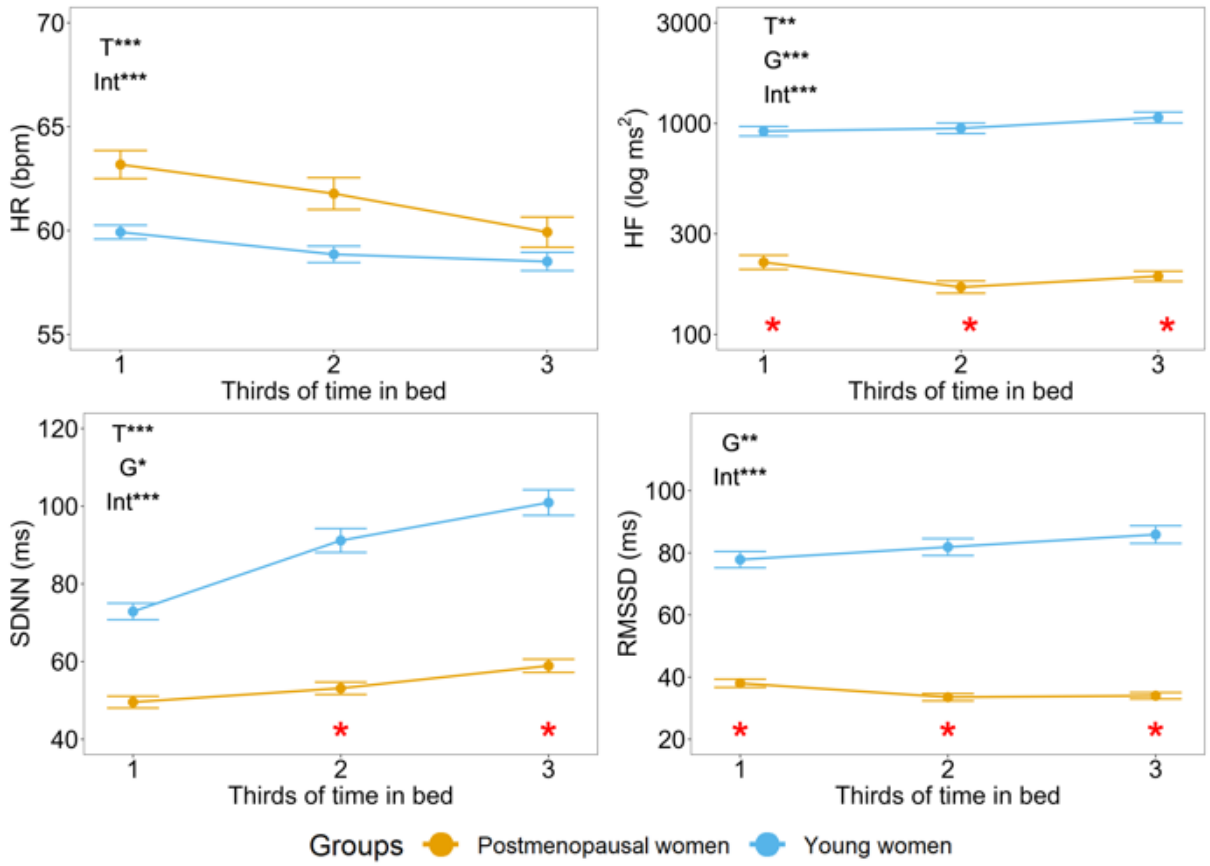

Figure 4. 1. HRV parameters of postmenopausal and young women during the 8-hour baseline sleep period.

HRV parameters were divided into thirds of time spent in bed. T = significant effect of time. G = significant effect of group. Int = significant interaction between factors group and time. *P<0.05, **p<0.01, ***p<0.001. Data are presented as mean ± SEM. Statistics are provided in Table 4.1.

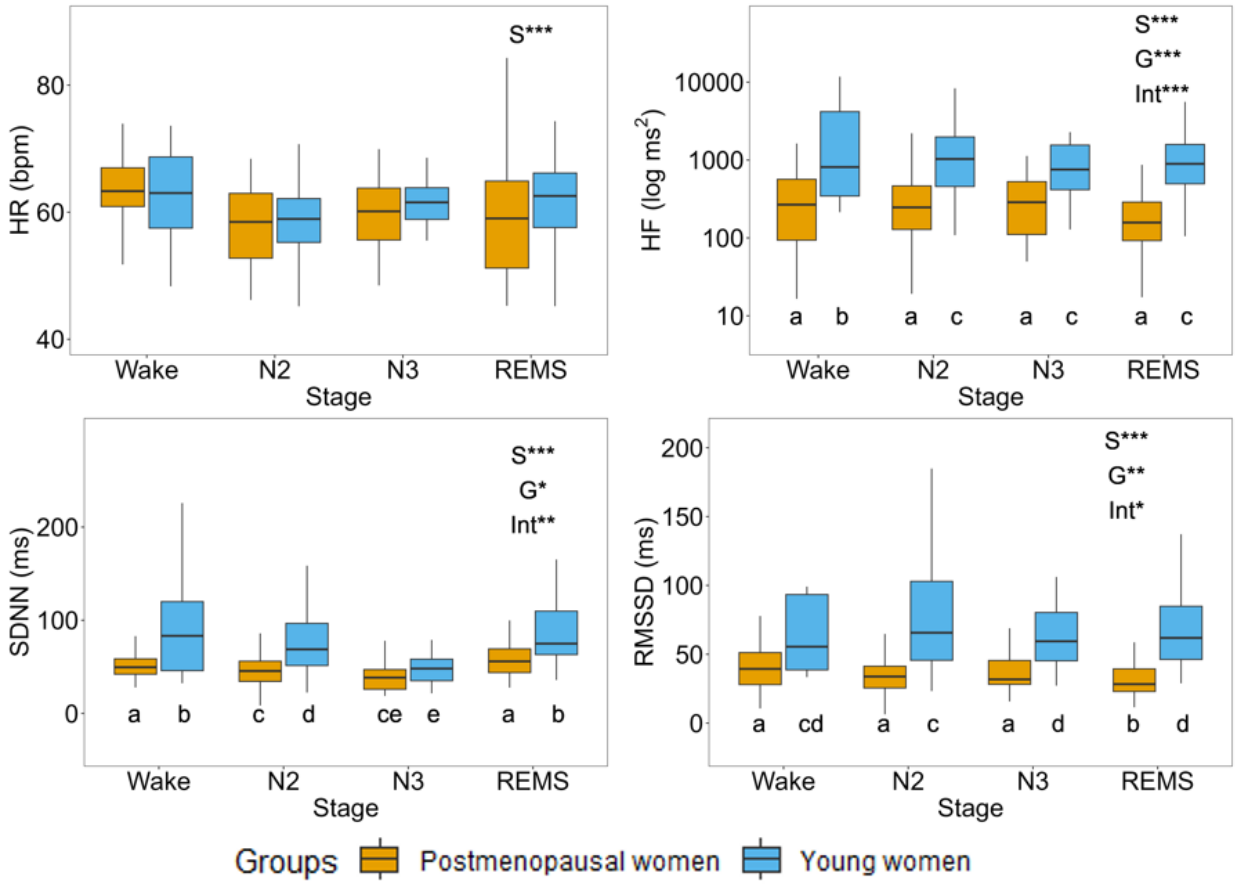

Figure 4. 2. HRV differences by stages (wake epochs, stage N2 sleep, stage N3 sleep, REM sleep) in postmenopausal and young women during the 8-hour baseline sleep period.

S = significant effect of stage. G = significant effect of group. Int = significant interaction between factors stage and time. *P<0.05, **p<0.01, ***p<0.001. Columns with the same letter present a statistically similar mean. Conversely, columns with different letters present a statistically significant difference (p<0.05). Data are presented as median ± IQR. Statistics are provided in Table 4.2.

HRV parameters during the baseline sleep period were also analyzed by sleep stages and were depicted in Figure 4.2. The results of the linear mixed-effects model are described in Table 4.2. HR, SDNN, RMSSD, and HF showed a significant sleep stage effect. A significant group effect was observed in SDNN, RMSSD, and HF, in which postmenopausal women presented a lower SDNN, RMSSD, and HF than younger women. A significant group by sleep stage interaction was observed in SDNN, RMSSD, and HF. In SDNN, Tukey's *post hoc* test yielded significantly lower SDNN in postmenopausal than young women during wake epochs, N2 and REM sleep ($p \leq 0.022$). In RMSSD and HF, Tukey's *post hoc* test showed that postmenopausal women had lower RMSSD and HF than young women throughout their wake epochs and all sleep stages. In RMSSD and HF, the effect of sleep stage was not significant for postmenopausal women, apart from REM sleep during RMSSD ($p \leq 0.036$).

4.5.2 USW procedure

The circadian variation of HRV parameters during NREM sleep and wake epochs is depicted in Figure 4.3. The p-values assessing circadian rhythmicity and circadian parameters are described in Tables 4.3 and 4.4, respectively.

Young women presented a significant circadian variation of HR during both NREM sleep and wake epochs whereas postmenopausal women did not. During NREM sleep and wake epochs, young women presented a peak of HR during the habitual daytime and a trough during the habitual nocturnal sleep period. Group differences were not observed in HR. Additionally, these group differences in HR were not observed even when naps with a preceding walking period were excluded (Supplementary Figure 4.1).

Young women presented a significant circadian variation of SDNN during NREM sleep and wake epochs. Postmenopausal women presented a significant circadian variation of SDNN in wake epochs but not NREM sleep. During NREM sleep, young women presented a peak of SDNN during the day. Conversely, during wake epochs, both postmenopausal and young women presented a peak of SDNN during the habitual sleep period at night. Group differences in mesor were observed during wakefulness with a lower SDNN in postmenopausal women. No differences in mesor were observed for

SDNN during NREM sleep. Group differences were not observed in either amplitude or phase.

Young women presented a significant circadian variation of RMSSD during wake epochs but not in NREM sleep. Conversely, postmenopausal women presented a significant circadian variation of RMSSD in NREM sleep but not in wake epochs. During wake epochs, young women presented a peak of RMSSD during the habitual sleep period. During NREM sleep, postmenopausal women presented a peak of RMSSD during the day. Group differences in mesor were observed during wakefulness and NREM sleep, such that postmenopausal women had lower RMSSD than young women.

Neither postmenopausal nor young women presented significant circadian variations during NREM sleep or wake epochs. Group differences in HF mesor were observed during NREM sleep and wake epochs, where postmenopausal women showed a lower HF than young women in both stages.

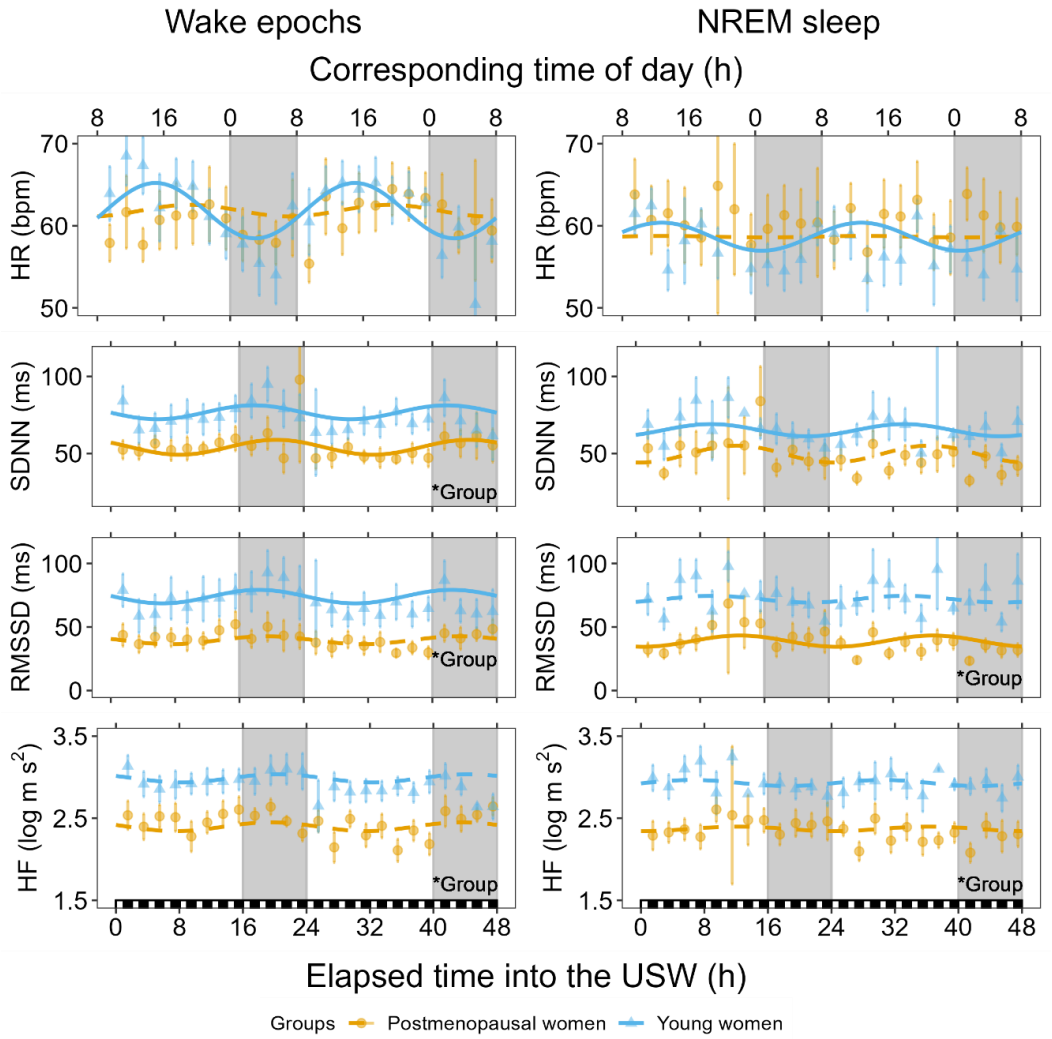

Figure 4. 3. Circadian variation of HR and HRV parameters throughout the USW procedure.

Data were aligned on the time elapsed into the USW procedure (USW, bottom X axis). Solid lines represent significant cosinor regressions, whereas dashed lines depict non-significant regressions. Black (~0 lux) and white (~10 lux) small squares above the bottom X-axis represent the nap and wake periods across the first 48 h of the USW procedure. The top X-axis depicts the corresponding time of day for a participant with a hypothetical bedtime of 00:00 h to 08:00 h. As reported in the results section, habitual sleep times were not the same for both groups. Large grey rectangles represent the projected time of the habitual nocturnal sleep period, corresponding to the time between habitual bedtime and wake time. *Group = group differences in mesor. Values are presented as mean ± SEM.

4.6 Discussion

The objective of this study was to better understand HRV changes during sleep and their circadian variation in postmenopausal women, as these may contribute to their increased risk of CVD. Compared to young naturally ovulating women, postmenopausal women consistently presented lower HRV and parasympathetic activity during the nocturnal sleep period and the USW procedure. Furthermore, postmenopausal women presented a dampened circadian variation of HR, SDNN, and RMSSD during the USW procedure.

4.6.1 Heart rate (HR)

No significant HR differences were observed between the two groups during the baseline sleep period, whether analyzed by thirds of time in bed or by sleep stages. However, both groups experienced a decline in HR overtime across the baseline sleep period. This decreased HR during nocturnal sleep has been previously described to be influenced by sleep stages or the circadian variation of HRV (Eddie et al., 2022). Indeed, the reduced HR during NREM sleep has been described as a consequence of the increased parasympathetic activity, whereas increased sympathetic activity may contribute to the higher HR during wake epochs and REM sleep (de Zambotti et al., 2018). Importantly, sympathetic activity cannot be reliably determined from HRV (Laborde et al., 2017), so we cannot draw solid conclusions about its role in the current study. The results of our study indicate that the expected increase in HR during wake epochs and REM sleep at the end of the night is not observed in both young and postmenopausal women. Rather, our findings suggest a declining HR across the sleep period in both groups. Given the HR troughs during the night, as depicted in Figure 4.3, it is likely that the declining HR is influenced by its circadian variation rather than sleep stage shifting. The lack of group differences in HR during the baseline sleep period is worth mentioning since age differences have been widely described in the literature, wherein HR is increased in aged individuals compared to younger ones (Accardo et al., 2021; Gonzales et al., 2023). Based on the reported literature, the small sample size may have contributed to this lack of difference, as the group-by-time interaction was significant, but *post hoc* tests did not reveal further significant results.

Circadian rhythmicity (p-values)			
Parameters		Postmenopausal women	Young women
HR			
	NREM	0.83	**0.047**
	Wake	0.06	**<0.001**
SDNN			
	NREM	0.06	**0.021**
	Wake	**0.038**	**0.023**
RMSSD			
	NREM	**0.045**	0.19
	Wake	0.10	**0.043**
HF			
	NREM	0.57	0.13
	Wake	0.25	0.28

Table 4. 3. P-values of circadian rhythmicity assessment of HRV parameters during the USW procedure.
Bold values denote statistical significance ($p<0.05$).

Circadian parameters		Postmenopausal women Mean ± SEM	Young women Mean ± SEM	*p-values*
HR NREM	Mesor (bpm)	59.96 ± 2.98	57.80 ± 3.85	0.55
	Amplitude (bpm)	-	1.71 ± 0.39	-
	Acrophase (elapsed time, h)	-	4.74 ± 0.84	-
	Acrophase (clock time, h)	-	12.91± 0.85	-
HR Wake	Mesor (bpm)	61.87 ± 3.22	61.82 ± 4.16	0.98
	Amplitude (bpm)	-	3.37 ± 0.37	-
	Acrophase (elapsed time, h)	-	7.02 ± 6.26	-
	Acrophase (clock time, h)	-	14.86 ± 0.42	-
SDNN NREM	Mesor (ms)	49.65 ± 6.66	65.12 ± 8.67	0.08
	Amplitude (ms)	-	3.96 ± 2.23	-
	Acrophase (elapsed time, h)	-	9.36 ± 1.97	-
	Acrophase (clock time, h)	-	17.11 ± 2.37	-
SDNN Wake	Mesor (ms)	54.05 ± 6.93	76.77 ± 8.98	**0.012**
	Amplitude (ms)	4.84 ± 2.31	4.51 ± 1.80	0.91
	Acrophase (elapsed time, h)	20.56 ± 1.86	17.79 ± 2.01	0.31
	Acrophase (clock time, h)	2.71 ± 2.23	0.73 ± 2.36	0.54
RMSSD NREM	Mesor (ms)	38.89 ± 9.32	71.85 ± 12.07	**0.009**
	Amplitude (ms)	4.40 ± 1.85	-	-
	Acrophase (elapsed time, h)	12.43 ± 1.97	-	-
	Acrophase (clock time, h)	20.17 ± 1.16	-	-
RMSSD Wake	Mesor (ms)	39.60 ± 10.62	73.87 ± 13.73	**0.015**
	Amplitude (ms)	-	5.33 ± 2.25	-
	Acrophase (elapsed time, h)	-	18.41 ± 1.76	-
	Acrophase (clock time, h)	-	2.17 ± 2.26	-
HF NREM	Mesor (log ms^2)	2.37 ± 0.11	2.93 ± 0.14	**<0.001**
	Amplitude (log ms^2)	-	-	-
	Acrophase (elapsed time, h)	-	-	-
	Acrophase (clock time, h)	-	-	-
HF Wake	Mesor (log ms^2)	2.40 ± 0.13	2.99 ± 0.17	**0.001**
	Amplitude (log ms^2)	-	-	-
	Acrophase (elapsed time, h)	-	-	-
	Acrophase (clock time, h)	-	-	-

Table 4. 4. Circadian parameters of HRV parameters throughout the USW procedure. Amplitude and acrophase were not calculated when the circadian variation was not significant. Acrophase is based on the elapsed time since the habitual wake time (elapsed time into the USW) and on time of day (clock time). P-values were based on a two-tailed t-test. Bold values denote statistical significance ($p<0.05$).

In the USW procedure, young women presented a significant circadian variation of HR during wake epochs and NREM sleep, whereas postmenopausal women did not. In the current study, we observed that HR peaked during wake epochs about 7 h after starting the USW procedure (corresponding to a clock time of ~15:00 h). The circadian variation of HR has been previously described in different protocols such as constant routine (Anders et al., 2010; Glos et al., 2014; Krauchi & Wirz-Justice, 1994; Shea et al., 2011), USW (Boudreau et al., 2013), forced desynchrony (Hu et al., 2004), and 24-hour ambulatory EKG recordings (Li et al., 2011; Otsuka et al., 2021; Vandeput et al., 2012). In line with our results, the literature consistently shows that HR peaks during wakefulness and occurs during the habitual daytime (Anders et al., 2010; Glos et al., 2014; Krauchi & Wirz-Justice, 1994; Shea et al., 2011). Particularly, our results are consistent with those of Anders et al. (2010), who studied nine healthy young women in their luteal phase during a constant routine and showed that HR peaks in the afternoon. The circadian variation of HR during NREM sleep has been less described in the literature. Our research group previously studied the circadian variation of RR intervals among 13 healthy young participants (11 men, 2 women) undergoing a USW procedure. In this study, the trough of RR intervals (thus the peak in HR) during stages N1, N2, and N3 occurred between 14:00 and 16:00 h (Boudreau et al., 2013)., This afternoon peak is consistent with our results but was slightly later than that observed in young women in the present study (~13:00 h). This earlier peak time in our young women could be influenced by sex differences, as women tend to have earlier circadian rhythms and sleep schedules than men (Boivin et al., 2016). However, sex differences in the circadian variation of HRV in controlled laboratory conditions have not been described and need further investigation.

In contrast to the younger women, postmenopausal women had an absent circadian variation of HR during both wake epochs and NREM sleep. This outcome supports the hypothesis of a weakened circadian signal in aged individuals (Dijk & Duffy, 2020) and is consistent with previous observations of a dampened circadian amplitude of melatonin, sleep, and alertness in postmenopausal women (Perez-Medina-Carballo et al., 2023). This dampened circadian variation of HR is consistent with the results of a longitudinal study using 24-hour Holter monitoring in elderly subjects aged ≥70 years (Tasaki et al., 2006). Moreover, a recent study in young, middle-aged, and elderly

individuals using 24-hour Holter monitoring showed reduced HR variation in the elderly group (Accardo et al., 2021). However, the previously mentioned research was performed in ambulatory conditions, in which environmental factors such as activity and sleep may influence the circadian variation of HR.

4.6.2 Heart rate variability (SDNN)

In the current study, we consistently found that postmenopausal women have a lower SDNN than young women. This was observed during baseline sleep and the naps of the USW procedure. SDNN represents the variability of heart rate and is known as a marker of cardiovascular health (Hillebrand et al., 2013). A meta-analysis of various epidemiological studies has shown that a low SDNN is associated with a 32-45% increased risk of a first cardiovascular event in populations without cardiovascular disease. In their linear model between SDNN and CVD, they showed that a decrease of 1% in SDNN was associated with an approximate 1% increase in the risk of developing CVD (Hillebrand et al., 2013).

During the baseline sleep period, the SDNN was substantially lower in postmenopausal women compared to younger women, with increasing values as time in bed increased in young women. The increasing SDNN values over the course of the night have been previously described (Eddie et al., 2022) and could be due to increased sympathetic activity during phasic REM sleep and the increasing presence of microarousals in the second half of the sleep period (de Zambotti et al., 2018). During the transitions from NREM sleep to a state of arousal, the variability of HR increases, leading to an increase in SDNN. SDNN during wake epochs peaks during the night in both groups, indicating the presence of this variability created by wakefulness. Additionally, SDNN increases over time during both NREM and REM sleep, with higher variability occurring during REM sleep (Eddie et al., 2022). These variations across sleep stages are consistent in both groups of the current study, with lower SDNN values in postmenopausal compared to young women. SDNN variability has been established as a protective factor against CVD due to its impact on heart reactivity and variability (Hillebrand et al., 2013). However, in young women, a rise in SDNN across the sleep period may merely reflect

the variability generated by sleep stage shifting rather than a CVD risk, as the parasympathetic activity in young women remained stable throughout the night.

During the USW procedure, a circadian variation of SDNN was observed in young women during both wake epochs and NREM sleep, whereas postmenopausal women displayed a significant circadian variation only during wake epochs. Interestingly, the circadian variation differed in pattern between wake epochs and NREM sleep. For wake epochs, the SDNN rhythm peaked during the habitual night and the trough occurred during the habitual day in both groups. These findings are consistent with studies that have been conducted using 24-hour Holter recordings (Accardo et al., 2021; Li et al., 2011) and constant routine protocols where participants remained awake at all times (Glos et al., 2014; Vandewalle et al., 2007; Viola et al., 2008). On the other hand, for NREM sleep, the SDNN rhythm peaked during the habitual day, while the minimum occurred during the habitual night in young women. To our knowledge, the circadian variation of SDNN has not been previously assessed during NREM sleep. This increased SDNN during the day, observed in NREM sleep, might reflect the higher number of arousals occurring during daytime compared to nighttime naps in young women (Perez-Medina-Carballo et al., 2023). In contrast, in postmenopausal women, the SDNN rhythm during NREM sleep was undetected, which is consistent with the undetected rhythm of HR during NREM sleep. As described by age-related changes in HRV, postmenopausal women presented lower SDNN than younger women throughout the USW procedure (Gonzales et al., 2023). This consistent observation of reduced SDNN persisted in both wake epochs and NREM sleep, which could be speculated to represent a potential association with adverse cardiovascular outcomes across all circadian phases in postmenopausal women.

4.6.3 Parasympathetic activity (RMSSD and HF)

Postmenopausal women showed lower parasympathetic activity than young women, measured by RMSSD and HF. This occurred during the baseline sleep period and the naps of the USW procedure. RMSSD and HF are two HRV metrics that indicate parasympathetic activity of the heart, specifically within a mechanism known as respiratory sinus arrhythmia. This mechanism involves a decrease in HR during expiration

due to the parasympathetic activation of a branch of the vagus nerve, and an increase in HR during inspiration due to a withdrawal of parasympathetic activity (Ottaviani et al., 2020). Epidemiological studies have shown that a high parasympathetic activity measured by RMSSD or HF represents a protective factor for CVD, which decreases with aging (Tegegne et al., 2018; Thayer et al., 2010).

During the baseline sleep period, parasympathetic activity was lower in postmenopausal than in young women when the analysis was divided into thirds of the period time in bed and in stages N2, N3, REM sleep, and wake epochs. Our results are consistent with the literature wherein older populations showed a lower RMSSD and HF than younger populations (Tegegne et al., 2018). It was also described that parasympathetic activity increases during NREM sleep stages in young individuals (Trinder et al., 2001), which is observed in young women but not in postmenopausal women. In contrast, the parasympathetic activity of postmenopausal women remained constantly low throughout the sleep period, possibly representing a higher CVD risk during their sleep (Hillebrand et al., 2013; Thayer et al., 2010).

During naps of the USW procedure, postmenopausal women presented a significant circadian variation of RMSSD during NREM sleep and not during wake epochs. In contrast, young women presented a significant circadian variation of RMSSD during wake epochs and not during NREM sleep. A circadian RMSSD variation has been previously described in the literature in a constant routine (Glos et al., 2014) and during ambulatory 24-hour EKG recordings (Accardo et al., 2021; Bonnemeier et al., 2003; Li et al., 2011). The circadian variation of RMSSD during wake epochs is consistent with that reported in a group of young men studied in a constant routine, in which RMSSD peaked at about 4:00 h (Glos et al., 2014). The RMSSD peak in our group of young women occurred about 18 h after the USW procedure started, roughly equivalent to a clock time of 2:00 h, which is earlier than in prior studies. Sex differences in RMSSD in constant routine studies have not been described; however, the earlier circadian rhythms described in women in the present study may contribute to this difference (Boivin et al., 2016). On the other hand, the RMSSD rhythm was dampened during wake epochs in postmenopausal women. The nocturnal peak of RMSSD has been previously described

to be reduced with aging in both men and women, using 24-hour ambulatory recordings (Accardo et al., 2021; Bonnemeier et al., 2003). Based on the literature, the reduced nocturnal peak of RMSSD occurring with aging might explain the undetected rhythmicity in postmenopausal women. This dampened rhythm of RMSSD during wakefulness in postmenopausal women, along with the dampened rhythms in HR and SDNN, are consistent with the weakened circadian output with aging (Dijk & Duffy, 2020).

It is worth noting that postmenopausal women presented a significant circadian variation of RMSSD during NREM sleep, with a peak at about 20:00 h. RMSSD during NREM sleep has not been specifically described in the literature. The circadian variation of stage N1 sleep in postmenopausal women reaches its lowest point in the evening, while the peak of RMSSD occurs at the same time, suggesting that stage N1 variation may be a contributing factor to RMSSD values (Perez-Medina-Carballo et al., 2023). Moreover, studies in men and women using 24-hour Holter recordings report the RMSSD peak during the night in middle-aged individuals (Li et al., 2011), in which RMSSD might be recorded during the nocturnal sleep comprising NREM sleep. However, ambulatory Holter studies are insufficient to understand the RMSSD circadian variation during NREM sleep, and this requires further investigation to better understand its relevance.

4.6.4. Potential physiological mechanisms

The observed dampened or absent rhythms in HR, SDNN, and RMSSD in postmenopausal women compared to young women supports the hypothesis that the output of the circadian system weakens with aging (Dijk & Duffy, 2020). The suprachiasmatic nucleus (SCN) of the hypothalamus centrally controls the circadian timing system (Patke et al., 2020). Previous studies have shown that decreasing the firing rate and neuron population of the SCN with aging can reduce its output signal, thereby dampening circadian rhythms (Kessler et al., 2011; Miller et al., 1989). On the other hand, the cardiovascular system is centrally regulated by several nuclei in the brain stem, including the nucleus of the solitary tract (NTS), nucleus ambiguous (NA), and the rostral ventrolateral medulla (RVLM) (Spary et al., 2009). Reflexes such as respiratory sinus arrhythmia are integrated into the NTS and NA. Additionally, the SCN projects to the NTS, which in turn projects to various brain nuclei that ultimately regulate the cardiovascular

system. The NTS also projects back to the SCN to complete the loop (Scheer et al., 2003). The circadian timing system regulates the circadian variation of the cardiovascular system, specifically HR, and possibly parasympathetic activity (Spary et al., 2009).

According to the literature, the dampened circadian rhythmicity in aged women may be due to aging mechanisms. Dampened circadian rhythms have been described in various outputs of the SCN in aged populations, such as CBT, melatonin, cortisol, and the circadian regulation of sleep and alertness (Buysse et al., 2005; Dijk et al., 1999; Munch et al., 2005; Perez-Medina-Carballo et al., 2023, Van Cauter et al., 1996). It is thus reasonable to hypothesize that the connection between the SCN and the NTS (Spary et al., 2009) might be affected, dampening the HR, SDNN, and RMSSD rhythms.

Age and sex differences in CVDs have been widely studied, as young women experience fewer cardiovascular events compared to young men (Thayer et al., 2010). More importantly, the risk of CVD drastically increases in women after menopause, suggesting a strong effect of sex hormones. Cardiovascular death rates decrease after midlife in men, while they continue to rise steadily throughout women's lifespan (Merz & Cheng, 2016). Indeed, estrogen benefits the cardiovascular system through its effect on the central nervous system and the periphery (Aryan et al., 2020; Spary et al., 2009). Estrogen receptors have been found in the NTS, NA, and RVLM, and direct estrogen injections in these areas have been shown to induce parasympathetic activity and sensitivity to baroreceptor reflexes in animal models (Saleh et al., 2000). Moreover, estrogen administration promotes vasodilation and decreases atherosclerosis and heart fibrosis (Aryan et al., 2020). On the other hand, progesterone has also been proposed to have a synergistic effect with estradiol on CVD, but its effect has been understudied (Gersh et al., 2024). Since estrogen administration affects brain nuclei regulating HR and parasympathetic activity, it is reasonable to hypothesize that reduced estrogen production at menopause might be one of the involved mechanisms dampening the rhythms and reducing the values of HR, HRV, and parasympathetic activity in postmenopausal women. Nevertheless, disentangling the effect of declining estrogen and progesterone and that of other aging events remains complex and further research is needed.

4.6.5. Clinical implications

The dampened or absent of rhythms and decreased HR, SDNN, and RMSSD values in postmenopausal women may have important clinical implications. Multiple studies have suggested that a decrease in HRV and parasympathetic activity can increase the risk of developing CVD. This correlation has been observed in healthy individuals and those with pre-existing CVD (Hillebrand et al., 2013; Patel et al., 2017; Stein et al., 2008; Tegegne et al., 2018). Our study found that during the USW procedure, postmenopausal women had 24-30% lower SDNN and approximately 46% lower RMSSD than young women. It is important to note that despite having low values of SDNN and RMSSD, our group of postmenopausal women did not display any symptoms of CVD based on our screening procedures. According to the literature, however, such low values may suggest a higher risk of developing CVD.

It has been observed that the risk of cardiovascular disease and stroke show a cyclical pattern across the day, with a higher risk between 6:00 am and noon. This risk is linked to the morning rise in HR and blood pressure (Manfredini et al., 2005; Thosar et al., 2018). Two HRV measures coincides with a higher CVD risk during the day for postmenopausal women. Specifically, the trough of SDNN rhythm during wake epochs occurred during the day and the trough of RMSSD during NREM sleep occurred in the early morning, possibly representing a higher risk of CVD during the daytime of postmenopausal women. It is worth noting that postmenopausal women experience a significant decrease in HRV and parasympathetic activity throughout the day and night. Even their highest values are lower than the lowest values observed in young women. Furthermore, this reduced HRV and parasympathetic activity is consistently observed in both NREM sleep and wake epochs. As a result, even stage N3 sleep does not exhibit its beneficial effects through parasympathetic activity on the HRV of postmenopausal women.

Insomnia symptoms are also associated with decreased HRV and parasympathetic activity (Cosgrave et al., 2021; Zhao & Jiang, 2023). This link is especially relevant in the postmenopausal population as they have a high risk of developing sleep disturbances. Insomnia is characterized by a hyperarousal state

involving the autonomic nervous system. For example, de Zambotti et al. found that perimenopausal women with insomnia have lower parasympathetic activity compared to perimenopausal controls (de Zambotti et al., 2017). Although our postmenopausal women did not have insomnia and reported good sleep quality, they had a higher number of arousals than younger women (Perez-Medina-Carballo et al., 2023). This is consistent with the observed trough of the RMSSD rhythm in postmenopausal women, which occurred during the late night. As such, the decreased parasympathetic activity at night may illustrate a risk for developing sleep disturbances in this group of healthy-sleeping postmenopausal women.

4.6.6. Strengths and limitations

The small sample size of the current study is a significant limitation that may have prevented us from observing group differences, particularly in HR. Consequently, we could not observe group differences in HR when the baseline sleep period was divided into thirds of time in bed or sleep stages. Moreover, the circadian pattern of HR appears to be similar between groups, but the rhythm in postmenopausal women did not reach significance. Our small sample size also increases the possibility of type 2 errors. Despite this, our results are consistent with a reduced parasympathetic modulation of the heart in postmenopausal women in line with an increased risk for CVD. However, the observational design of our study does not allow us to draw a causal relationship between HRV measures and CVD.

Our HRV measurements have some limitations. For instance, sympathetic activity cannot be accurately measured using HRV measures, and as such, a conclusion cannot be made about its role. Notably, parasympathetic activity measured by HRV only evaluates the regulation of the respiratory sinus arrhythmia and does not necessarily reflect the overall parasympathetic activity.

Additionally, the HRV data was analyzed into 5-minute intervals, which resulted in limited resolution and ignored various sleep epochs. This was applicable to both the baseline sleep period and USW analysis.

One participant taking hormone replacement therapy was included in the postmenopausal women group. However, her results were within 2 SD of the group data and were deemed appropriate for inclusion in the analyses.

We must interpret our results cautiously as postmenopausal women in this study underwent rigorous screening and thus represent only a small, healthy proportion of this population. This may limit the generalizability of our findings to the broader postmenopausal population.

Despite these limitations, our circadian measures are noteworthy. The specialized methodology employed in our study allowed us to record sleep patterns across day and night for at least 48 hours, which enabled us to acquire HRV measures during sleep. Additionally, the meticulous screening procedures excluded various conditions that could influence the studied parameters.

4.7 Conclusion

The present study sheds light on the circadian variation of HRV measures during the sleep of postmenopausal compared to young naturally ovulating women. Although cardiovascular risk was not directly evaluated in this study, postmenopausal women consistently presented lower HRV (SDNN) and parasympathetic activity (RMSSD and HF) during their nocturnal sleep period and across circadian phases which are associated with the risk of developing CVD. Furthermore, the dampened circadian rhythm observed in their HR, SDNN, and RMSSD suggests a weakened circadian output signal, possibly due to declining estrogen levels and age-related changes. Our findings highlight the importance of investigating ovarian hormones in postmenopausal women to better understand their cardiovascular risk and circadian variation.

4.8 Acknowledgments

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4.9 Data and Material Availability

The data underlying this article cannot be shared publicly because participants did not agree that their data be placed in a publicly accessible database. Therefore, for ethical and confidentiality reasons, the authors cannot provide public access to them. Nevertheless, materials, data, and protocols will be made available for investigation of scientific integrity if necessary. Readers are free to contact the principal investigator if they wish to discuss collaborations to build on these published data.

4.10 Disclosure Statement

Financial disclosure: D.B.B. provides conferences and legal expert advice on sleep-related topics. Non-financial disclosure: None.

4.11 References

Accardo, A., Merlo, M., Silveri, G., Del Popolo, L., Dalla Libera, L., Restivo, L., Cinquetti, M., Cannata, A., & Sinagra, G. (2021). Influence of ageing on circadian rhythm of heart rate variability in healthy subjects. J Cardiovasc Med (Hagerstown), 22(5), 405-413. https://doi.org/10.2459/JCM.0000000000001048

Anders, D., Vollenweider, S., Cann, J., Hofstetter, M., Flammer, J., Orgul, S., & Krauchi, K. (2010). Heart-rate variability in women during 40-hour prolonged wakefulness. Chronobiol Int, 27(8), 1609-1628. https://doi.org/10.3109/07420528.2010.504317

Aryan, L., Younessi, D., Zargari, M., Banerjee, S., Agopian, J., Rahman, S., Borna, R., Ruffenach, G., Umar, S., & Eghbali, M. (2020). The Role of Estrogen Receptors in Cardiovascular Disease. Int J Mol Sci, 21(12). https://doi.org/10.3390/ijms21124314

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Usinglme4. J. Stat. Softw., 67(1), 1-48. https://doi.org/10.18637/jss.v067.i01

Berry, R. B., Quan, S. F., Abreu, A. R., Bibbs, M. L., DelRosso, L., Harding, S. M., Mao, M.-M., Plante, D. T., Pressman, M. R., Troester, M. M., & Vaughn, B. V. (2020). The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.6.

Boivin, D. B., Shechter, A., Boudreau, P., Begum, E. A., & Ng Ying-Kin, N. M. (2016). Diurnal and circadian variation of sleep and alertness in men vs. naturally cycling

women. Proc Natl Acad Sci U S A, 113(39), 10980-10985. https://doi.org/10.1073/pnas.1524484113

Bonnemeier, H., Richardt, G., Potratz, J., Wiegand, U. K., Brandes, A., Kluge, N., & Katus, H. A. (2003). Circadian profile of cardiac autonomic nervous modulation in healthy subjects: differing effects of aging and gender on heart rate variability. J Cardiovasc Electrophysiol, 14(8), 791-799. https://doi.org/10.1046/j.1540-8167.2003.03078.x

Boudreau, P., Yeh, W. H., Dumont, G. A., & Boivin, D. B. (2013). Circadian variation of heart rate variability across sleep stages. Sleep, 36(12), 1919-1928. https://doi.org/10.5665/sleep.3230

Brockbank, C. L., Chatterjee, F., Bruce, S. A., & Woledge, R. C. (2000). Heart rate and its variability change after the menopause. Exp Physiol, 85(3), 327-330. https://www.ncbi.nlm.nih.gov/pubmed/10825420

Buysse, D. J., Monk, T. H., Carrier, J., & Begley, A. (2005). Circadian patterns of sleep, sleepiness, and performance in older and younger adults. Sleep, 28(11), 1365-1376. https://doi.org/10.1093/sleep/28.11.1365

Cosgrave, J., Phillips, J., Haines, R., Foster, R. G., Steinsaltz, D., & Wulff, K. (2021). Revisiting nocturnal heart rate and heart rate variability in insomnia: A polysomnography-based comparison of young self-reported good and poor sleepers. J Sleep Res, 30(4), e13278. https://doi.org/10.1111/jsr.13278

de Zambotti, M., Trinder, J., Colrain, I. M., & Baker, F. C. (2017). Menstrual cycle-related variation in autonomic nervous system functioning in women in the early menopausal transition with and without insomnia disorder. Psychoneuroendocrinology, 75, 44-51. https://doi.org/10.1016/j.psyneuen.2016.10.009

de Zambotti, M., Trinder, J., Silvani, A., Colrain, I. M., & Baker, F. C. (2018). Dynamic coupling between the central and autonomic nervous systems during sleep: A review. Neurosci Biobehav Rev, 90, 84-103. https://doi.org/10.1016/j.neubiorev.2018.03.027

Dijk, D. J., & Duffy, J. F. (2020). Novel Approaches for Assessing Circadian Rhythmicity in Humans: A Review. J Biol Rhythms, 35(5), 421-438. https://doi.org/10.1177/0748730420940483

Dijk, D. J., Duffy, J. F., Riel, E., Shanahan, T. L., & Czeisler, C. A. (1999). Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J Physiol, 516 (Pt 2)(2), 611-627. https://doi.org/10.1111/j.1469-7793.1999.0611v.x

Eddie, D., Bentley, K. H., Bernard, R., Mischoulon, D., & Winkelman, J. W. (2022). Aggregating heart rate variability indices across sleep stage epochs ignores significant variance through the night. Sleep Med, 90, 262-266. https://doi.org/10.1016/j.sleep.2021.11.020

El Khoudary, S. R. (2017). Gaps, limitations and new insights on endogenous estrogen and follicle stimulating hormone as related to risk of cardiovascular disease in women traversing the menopause: A narrative review. Maturitas, 104, 44-53. https://doi.org/10.1016/j.maturitas.2017.08.003

El Khoudary, S. R., Aggarwal, B., Beckie, T. M., Hodis, H. N., Johnson, A. E., Langer, R. D., Limacher, M. C., Manson, J. E., Stefanick, M. L., Allison, M. A., American Heart Association Prevention Science Committee of the Council on, E., Prevention, Council on, C., & Stroke, N. (2020). Menopause Transition and Cardiovascular Disease Risk: Implications for Timing of Early Prevention: A Scientific Statement From the American Heart Association. Circulation, 142(25), e506-e532. https://doi.org/10.1161/CIR.0000000000000912

Gersh, F., O'Keefe, J. H., Elagizi, A., Lavie, C. J., & Laukkanen, J. A. (2024). Estrogen and cardiovascular disease. Prog Cardiovasc Dis. https://doi.org/10.1016/j.pcad.2024.01.015

Glos, M., Fietze, I., Blau, A., Baumann, G., & Penzel, T. (2014). Cardiac autonomic modulation and sleepiness: physiological consequences of sleep deprivation due to 40 h of prolonged wakefulness. Physiol Behav, 125, 45-53. https://doi.org/10.1016/j.physbeh.2013.11.011

Gonzales, J. U., Elavsky, S., Cipryan, L., Jandackova, V., Burda, M., & Jandacka, D. (2023). Influence of sleep duration and sex on age-related differences in heart rate variability: Findings from program 4 of the HAIE study. Sleep Med, 106, 69-77. https://doi.org/10.1016/j.sleep.2023.03.029

Harlow, S. D., Gass, M., Hall, J. E., Lobo, R., Maki, P., Rebar, R. W., Sherman, S., Sluss, P. M., de Villiers, T. J., & Group, S. C. (2012). Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. J Clin Endocrinol Metab, 97(4), 1159-1168. https://doi.org/10.1210/jc.2011-3362

Hillebrand, S., Gast, K. B., de Mutsert, R., Swenne, C. A., Jukema, J. W., Middeldorp, S., Rosendaal, F. R., & Dekkers, O. M. (2013). Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. Europace, 15(5), 742-749. https://doi.org/10.1093/europace/eus341

Hu, K., Ivanov, P., Hilton, M. F., Chen, Z., Ayers, R. T., Stanley, H. E., & Shea, S. A. (2004). Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. Proc Natl Acad Sci U S A, 101(52), 18223-18227. https://doi.org/10.1073/pnas.0408243101

Iber, C. (2007). The AASM manual for the scoring of sleep and associated events: Rules. Terminology and Technical Specification.

Jain, N., Lehrer, H. M., Chin, B. N., Tracy, E. L., Evans, M. A., Krafty, R. T., Buysse, D. J., & Hall, M. H. (2023). Heart rate and heart rate variability following sleep deprivation in retired night shift workers and retired day workers. Psychophysiology, 60(12), e14374. https://doi.org/10.1111/psyp.14374

Kessler, B. A., Stanley, E. M., Frederick-Duus, D., & Fadel, J. (2011). Age-related loss of orexin/hypocretin neurons. Neuroscience, 178, 82-88. https://doi.org/10.1016/j.neuroscience.2011.01.031

Knowles, J. E. F., Carl. (2020). merTools: Tools for Analyzing Mixed Effect Regression Models. In (Version R package version 0.5.2) https://CRAN.R-project.org/package=merTools

Krauchi, K., & Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. Am J Physiol, 267(3 Pt 2), R819-829. https://doi.org/10.1152/ajpregu.1994.267.3.R819

Laborde, S., Mosley, E., & Thayer, J. F. (2017). Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research - Recommendations for Experiment

Planning, Data Analysis, and Data Reporting. Front Psychol, 8, 213. https://doi.org/10.3389/fpsyg.2017.00213

Li, X., Shaffer, M. L., Rodriguez-Colon, S., He, F., Wolbrette, D. L., Alagona, P., Jr., Wu, C., & Liao, D. (2011). The circadian pattern of cardiac autonomic modulation in a middle-aged population. Clin Auton Res, 21(3), 143-150. https://doi.org/10.1007/s10286-010-0112-4

Liu, C. C., Kuo, T. B., & Yang, C. C. (2003). Effects of estrogen on gender-related autonomic differences in humans. Am J Physiol Heart Circ Physiol, 285(5), H2188-2193. https://doi.org/10.1152/ajpheart.00256.2003

Manfredini, R., Boari, B., Smolensky, M. H., Salmi, R., la Cecilia, O., Maria Malagoni, A., Haus, E., & Manfredini, F. (2005). Circadian variation in stroke onset: identical temporal pattern in ischemic and hemorrhagic events. Chronobiol Int, 22(3), 417-453. https://doi.org/10.1081/CBI-200062927

Merz, A. A., & Cheng, S. (2016). Sex differences in cardiovascular ageing. Heart, 102(11), 825-831. https://doi.org/10.1136/heartjnl-2015-308769

Miller, M. M., Gould, B. E., & Nelson, J. F. (1989). Aging and long-term ovariectomy alter the cytoarchitecture of the hypothalamic-preoptic area of the C57BL/6J mouse. Neurobiol Aging, 10(6), 683-690. https://doi.org/10.1016/0197-4580(89)90005-5

Moodithaya, S. S., & Avadhany, S. T. (2009). Comparison of cardiac autonomic activity between pre and post menopausal women using heart rate variability. Indian J Physiol Pharmacol, 53(3), 227-234. https://www.ncbi.nlm.nih.gov/pubmed/20329369

Munch, M., Knoblauch, V., Blatter, K., Schroder, C., Schnitzler, C., Krauchi, K., Wirz-Justice, A., & Cajochen, C. (2005). Age-related attenuation of the evening circadian arousal signal in humans. Neurobiol Aging, 26(9), 1307-1319. https://doi.org/10.1016/j.neurobiolaging.2005.03.004

Otsuka, K., Cornelissen, G., Furukawa, S., Kubo, Y., Shibata, K., Mizuno, K., Ohshima, H., & Mukai, C. (2021). Astronauts well-being and possibly anti-aging improved during long-duration spaceflight. Sci Rep, 11(1), 14907. https://doi.org/10.1038/s41598-021-94478-w

Ottaviani, M. M., Wright, L., Dawood, T., & Macefield, V. G. (2020). In vivo recordings from the human vagus nerve using ultrasound-guided microneurography. J Physiol, 598(17), 3569-3576. https://doi.org/10.1113/JP280077

Parsons, R., Parsons, R., Garner, N., Oster, H., & Rawashdeh, O. (2020). CircaCompare: a method to estimate and statistically support differences in mesor, amplitude and phase, between circadian rhythms. Bioinformatics, 36(4), 1208-1212. https://doi.org/10.1093/bioinformatics/btz730

Patel, V. N., Pierce, B. R., Bodapati, R. K., Brown, D. L., Ives, D. G., & Stein, P. K. (2017). Association of Holter-Derived Heart Rate Variability Parameters With the Development of Congestive Heart Failure in the Cardiovascular Health Study. JACC Heart Fail, 5(6), 423-431. https://doi.org/10.1016/j.jchf.2016.12.015

Patke, A., Young, M. W., & Axelrod, S. (2020). Molecular mechanisms and physiological importance of circadian rhythms. Nat Rev Mol Cell Biol, 21(2), 67-84. https://doi.org/10.1038/s41580-019-0179-2

Perez-Medina-Carballo, R., Kosmadopoulos, A., Boudreau, P., Robert, M., Walker, C. D., & Boivin, D. B. (2023). The circadian variation of sleep and alertness of postmenopausal women. Sleep, 46(2). https://doi.org/10.1093/sleep/zsac272

Pichot, V., Roche, F., Celle, S., Barthelemy, J. C., & Chouchou, F. (2016). HRVanalysis: A Free Software for Analyzing Cardiac Autonomic Activity. Front Physiol, 7, 557. https://doi.org/10.3389/fphys.2016.00557

R Core Team. (2020). R: A Language and Environment for Statistical Computing. In R Foundation for Statistical Computing. https://www.R-project.org/

Ribeiro, T. F., Azevedo, G. D., Crescencio, J. C., Maraes, V. R., Papa, V., Catai, A. M., Verzola, R. M., Oliveira, L., Silva de Sa, M. F., Gallo Junior, L., & Silva, E. (2001). Heart rate variability under resting conditions in postmenopausal and young women. Braz J Med Biol Res, 34(7), 871-877. https://doi.org/10.1590/s0100-879x2001000700006

Saleh, M. C., Connell, B. J., & Saleh, T. M. (2000). Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. Brain Res, 879(1-2), 105-114. https://doi.org/10.1016/s0006-8993(00)02757-8

Scheer, F. A., Kalsbeek, A., & Buijs, R. M. (2003). Cardiovascular control by the suprachiasmatic nucleus: neural and neuroendocrine mechanisms in human and rat. Biol Chem, 384(5), 697-709. https://doi.org/10.1515/BC.2003.078

Shaver, J. L., & Woods, N. F. (2015). Sleep and menopause: a narrative review. Menopause, 22(8), 899-915. https://doi.org/10.1097/GME.0000000000000499

Shea, S. A., Hilton, M. F., Hu, K., & Scheer, F. A. (2011). Existence of an endogenous circadian blood pressure rhythm in humans that peaks in the evening. Circ Res, 108(8), 980-984. https://doi.org/10.1161/CIRCRESAHA.110.233668

Shechter, A., Varin, F., & Boivin, D. B. (2010). Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. Sleep, 33(5), 647-656. https://doi.org/10.1093/sleep/33.5.647

Spary, E. J., Maqbool, A., & Batten, T. F. (2009). Oestrogen receptors in the central nervous system and evidence for their role in the control of cardiovascular function. J Chem Neuroanat, 38(3), 185-196. https://doi.org/10.1016/j.jchemneu.2009.05.008

Stein, P. K., Barzilay, J. I., Chaves, P. H., Mistretta, S. Q., Domitrovich, P. P., Gottdiener, J. S., Rich, M. W., & Kleiger, R. E. (2008). Novel measures of heart rate variability predict cardiovascular mortality in older adults independent of traditional cardiovascular risk factors: the Cardiovascular Health Study (CHS). J Cardiovasc Electrophysiol, 19(11), 1169-1174. https://doi.org/10.1111/j.1540-8167.2008.01232.x

Tasaki, H., Serita, T., Ueyama, C., Kitano, K., Seto, S., & Yano, K. (2006). Long-Term follow-up of the circadian rhythm of heart rate and heart rate variability in healthy elderly patients. Circ J, 70(7), 889-895. https://doi.org/10.1253/circj.70.889

Tegegne, B. S., Man, T., van Roon, A. M., Riese, H., & Snieder, H. (2018). Determinants of heart rate variability in the general population: The Lifelines Cohort Study. Heart Rhythm, 15(10), 1552-1558. https://doi.org/10.1016/j.hrthm.2018.05.006

Thayer, J. F., Yamamoto, S. S., & Brosschot, J. F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol, 141(2), 122-131. https://doi.org/10.1016/j.ijcard.2009.09.543

Thosar, S. S., Butler, M. P., & Shea, S. A. (2018). Role of the circadian system in cardiovascular disease. J Clin Invest, 128(6), 2157-2167. https://doi.org/10.1172/JCI80590

Trinder, J., Kleiman, J., Carrington, M., Smith, S., Breen, S., Tan, N., & Kim, Y. (2001). Autonomic activity during human sleep as a function of time and sleep stage. J Sleep Res, 10(4), 253-264. https://doi.org/10.1046/j.1365-2869.2001.00263.x

Van Cauter, E., Leproult, R., & Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab, 81(7), 2468-2473. https://doi.org/10.1210/jcem.81.7.8675562

Vandeput, S., Verheyden, B., Aubert, A. E., & Van Huffel, S. (2012). Nonlinear heart rate dynamics: circadian profile and influence of age and gender. Med Eng Phys, 34(1), 108-117. https://doi.org/10.1016/j.medengphy.2011.07.004

Vandewalle, G., Middleton, B., Rajaratnam, S. M., Stone, B. M., Thorleifsdottir, B., Arendt, J., & Dijk, D. J. (2007). Robust circadian rhythm in heart rate and its variability: influence of exogenous melatonin and photoperiod. J Sleep Res, 16(2), 148-155. https://doi.org/10.1111/j.1365-2869.2007.00581.x

Viola, A. U., James, L. M., Archer, S. N., & Dijk, D. J. (2008). PER3 polymorphism and cardiac autonomic control: effects of sleep debt and circadian phase. Am J Physiol Heart Circ Physiol, 295(5), H2156-2163. https://doi.org/10.1152/ajpheart.00662.2008

von Holzen, J. J., Capaldo, G., Wilhelm, M., & Stute, P. (2016). Impact of endo- and exogenous estrogens on heart rate variability in women: a review. Climacteric, 19(3), 222-228. https://doi.org/10.3109/13697137.2016.1145206

Woods, N. F., & Mitchell, E. S. (2010). Sleep symptoms during the menopausal transition and early postmenopause: observations from the Seattle Midlife Women's Health Study. Sleep, 33(4), 539-549. https://doi.org/10.1093/sleep/33.4.539

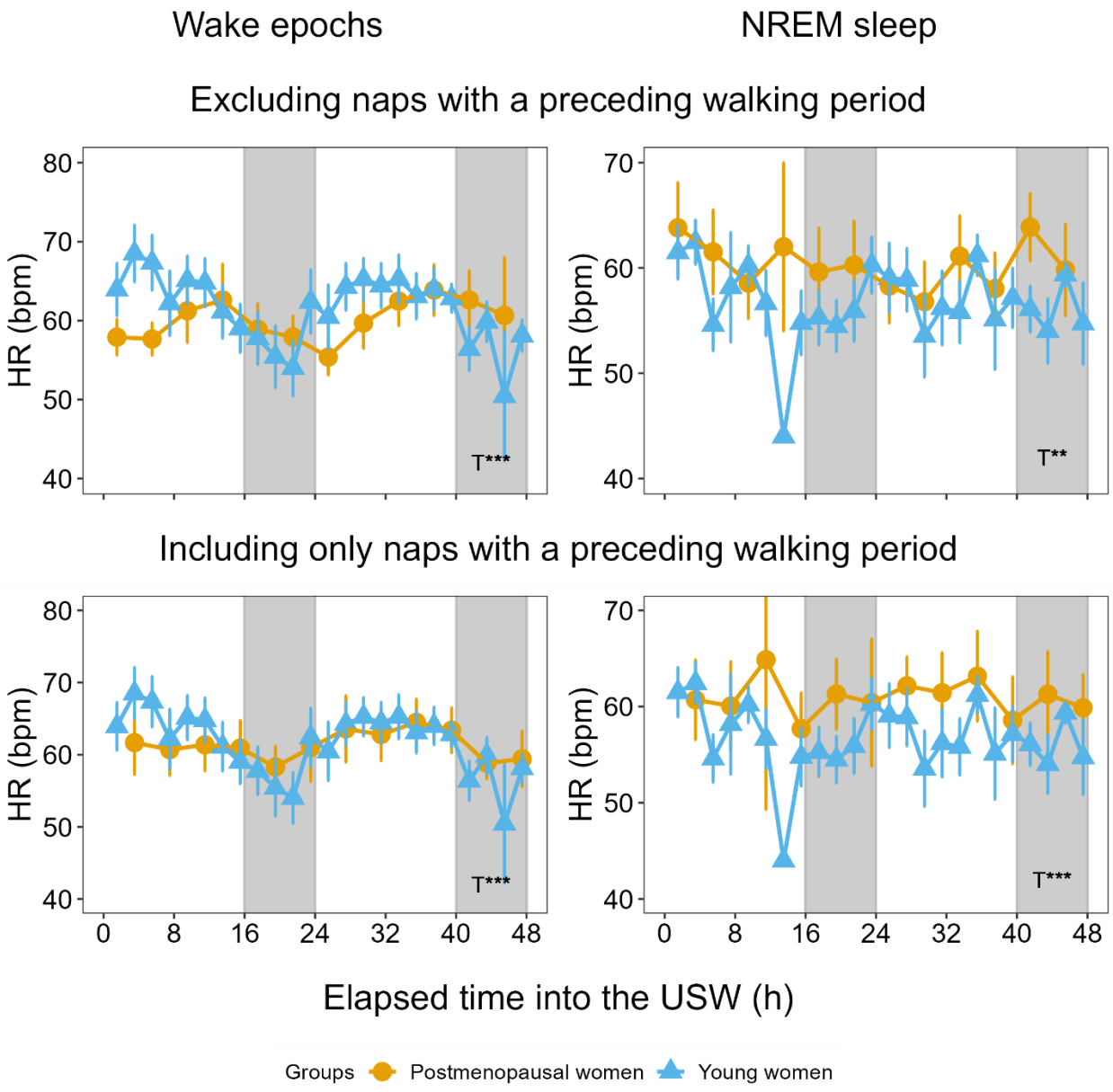
Wulsin, L. R., Horn, P. S., Perry, J. L., Massaro, J. M., & D'Agostino, R. B. (2015). Autonomic Imbalance as a Predictor of Metabolic Risks, Cardiovascular Disease, Diabetes, and Mortality. J Clin Endocrinol Metab, 100(6), 2443-2448. https://doi.org/10.1210/jc.2015-1748

Yeh, C. H., Kuo, T. B. J., Li, J. Y., Kuo, K. L., Chern, C. M., Yang, C. C. H., & Huang, H. Y. (2022). Effects of age and sex on vasomotor activity and baroreflex sensitivity during the sleep-wake cycle. Sci Rep, 12(1), 22424. https://doi.org/10.1038/s41598-022-26440-3

Zhao, W., & Jiang, B. (2023). Heart rate variability in patients with insomnia disorder: a systematic review and meta-analysis. Sleep Breath, 27(4), 1309-1313. https://doi.org/10.1007/s11325-022-02720-0

Zolfaghari, S., Yao, C., Thompson, C., Gosselin, N., Desautels, A., Dang-Vu, T. T., Postuma, R. B., & Carrier, J. (2020). Effects of menopause on sleep quality and sleep disorders: Canadian Longitudinal Study on Aging. Menopause, 27(3), 295-304. https://doi.org/10.1097/GME.0000000000001462

4.12 Supplementary material



Supplementary Figure 4. 1. Variation of HR excluding naps with a preceding walking period in postmenopausal women.

Data were aligned to elapsed time into the USW procedure. Large grey rectangles represent the projected time of the habitual nocturnal sleep period, corresponding to the time between habitual bedtime and wake time. T = significant main effect of time. **P<0.01, ***P<0.001. Values are presented as mean ± SEM.

Chapter 5. Discussion

5.1 General discussion

The presented evidence in Chapters 2, 3, and 4 indicates a substantial influence of menopause on the various outputs of the circadian timing system. These postmenopausal differences might result from hormonal changes at menopause, other aging effects, or a combination of both. Our studies further suggest that these circadian differences in healthy postmenopausal women may contribute to their increased susceptibility to sleep disturbances and heightened risk of cardiovascular disease.

5.2 Summary of main findings

The study in Chapter 2 showed a detailed analysis of the changes in the circadian rhythm of CBT and melatonin as well as the circadian variation of sleep and alertness in postmenopausal women, thereby confirming our hypotheses 1 and 2. The findings of this study revealed a dampened melatonin rhythm and a disorganization of the circadian process of the sleep-wake cycle, which was reflected in the reduced amplitude of TST, SOL, SWS, and alertness in postmenopausal women. Notably, the reduced amplitude of the rhythm of TST in postmenopausal women was characterized by more time spent asleep during the day than in young women, with no evident group changes at night. In addition, postmenopausal women were found to be more alert during the night as compared to young women.

The study in Chapter 3 subsequently analyzed sleep EEG activity to further expand on the circadian and sleep changes occurring at menopause, which confirmed our hypothesis 3. Using EEG power spectra, a more refined insight into the sleep changes of postmenopausal women was obtained. This study observed dampened or undetectable rhythms of delta, theta, and sigma power in postmenopausal women compared to young women. Furthermore, during the nighttime naps of the USW procedure, postmenopausal women had more power within beta and less within delta and sigma frequencies.

Chapters 2 and 3 presented complementary results regarding the baseline sleep period. Based on visually-scored sleep parameters, postmenopausal women had more arousals, awakenings, and stage N1 sleep. Remarkably, other postmenopausal

differences in the sleep architecture were not observed in TST, SE, SWS, and REM sleep. Nevertheless, the study on the sleep EEG activity in Chapter 3 showed that postmenopausal women had less power within delta and sigma and more power within the alpha frequencies. These findings suggest that subtle changes in visually-scored sleep may occur in healthy-sleeping postmenopausal women. However, from the EEG activity perspective, significant postmenopausal changes in EEG power spectra can be observed, accompanied by alterations in delta, sigma, and alpha frequencies. Together, these changes represent a greater fragility of postmenopausal sleep.

Chapter 4 of the study confirmed our hypothesis 4 and focused on the circadian control of HR, HRV, and parasympathetic activity across the sleep episodes of the USW procedure. Particularly, young women displayed a circadian variation in HR, HRV, and parasympathetic activity measured by RMSSD, which was not observed in postmenopausal women. Additionally, postmenopausal women consistently displayed lower HRV and parasympathetic activity during sleep at both the baseline sleep period and the USW procedure.

Taken together, Chapters 2, 3, and 4 presented evidence of a dampened circadian variation of melatonin, sleep, alertness, EEG activity, HRV, and parasympathetic activity in postmenopausal women. Overall, these results present the contribution of the circadian timing system on sleep changes occurring at menopause and suggest a weakened circadian signal from the SCN regulating its various outputs. It is important to note that this study compared postmenopausal women aged about 55 years with young women aged about 25 years, and the observed results might reflect menopause or aging due to this age gap. Based on relevant literature, we attempt to explore the impact of ovarian hormone changes at menopause and other age-related changes.

5.3 Contribution of circadian rhythm changes to sleep disturbances

The findings presented in Chapters 2, 3, and 4 of this thesis have confirmed our hypotheses regarding the impact of menopause on the various outputs of the circadian timing system, such as CBT, melatonin, sleep and alertness, and HRV measures.

The dampened circadian amplitude of the SCN outputs reflect a decreased robustness of the circadian signal, and it is in accordance with the weakened circadian

signal described in aged populations (Buysse et al., 2005; Dijk & Duffy, 1999, 2020; Kim et al., 2014; Munch et al., 2005; Yoon et al., 2003). For instance, aged mice exhibit alterations in the SCN output, characterized by a decreased circadian amplitude of wheel-running activity (Nakamura et al., 2011) and *in vivo* neural activity of the SCN and SPZ (Nakamura et al., 2011; Nygard et al., 2005). Additionally, reduced firing rates of SCN neurons have been observed in cultured neurons of aged mice (Aujard et al., 2001), aged rats demonstrate fewer arginine vasopressin-expressing neurons in the SCN (Roozendaal et al., 1987), and aged hamsters show a reduction in the gene expression of BMAL1 and Clock (Kolker et al., 2003). The reduced circadian amplitude of rat activity levels is consistent with these previous observations (Kondratova & Kondratov, 2012; Yin et al., 2019). In humans, circadian amplitude reduction, particularly in melatonin (Kim et al., 2014; Munch et al., 2005; van Coevorden et al., 1991; Walters et al., 2005; Yoon et al., 2003) and CBT rhythms (Carrier et al., 1996; Czeisler et al., 1992; Dijk & Duffy, 1999; Duffy et al., 1998; Kim et al., 2014), has been consistently observed in aged individuals across various laboratory-controlled conditions. A reduction in diurnal amplitude was also observed in human activity levels (Huang et al., 2002). Light intervention studies further support the assumption that SCN changes are reflected in CBT and melatonin rhythms, as circadian amplitude dampening in CBT correlates with that of melatonin after light exposure (Dijk et al., 2012).

In our group of postmenopausal women, dampened circadian amplitudes were observed in several output rhythms of the circadian timing system. Firstly, the affected circadian rhythmicity of the sleep organization is reflected in the dampened rhythms of TST, SOL, and alertness levels, leading to increased daytime sleepiness and nighttime wakefulness. This is consistent with the sleep disturbance experienced by postmenopausal women, as evidenced by the higher number of arousals and N1 sleep stage. Secondly, the dampened variation of SWS and SWA during NREM sleep results in objectively reduced sleep pressure, quality, and depth, particularly at night. Thirdly, the dampened variation of SFA during NREM sleep translates to lower sleep protection at night. Finally, the absence of rhythms in HR, HRV, and parasympathetic activity indicates an autonomic dysregulation of the cardiovascular system during the sleep of postmenopausal women. These cardiovascular changes during sleep have been

associated with CVD and hyperarousability leading to an increased risk of insomnia (de Zambotti, Trinder, Colrain, et al., 2017; de Zambotti, Trinder, Javitz, et al., 2017; Silvani et al., 2015).

Postmenopausal women are at a high risk of developing sleep-onset insomnia (Zolfaghari et al., 2020), which may be linked to circadian rhythm changes. For instance, sleep-onset insomnia is associated with a 2-3-hour delay in circadian rhythms, which brings the wake maintenance zone closer to the individual's bedtime, thereby postponing the timing of sleep propensity that is necessary for initiating sleep (Lack et al., 2017). However, we did not observe any significant postmenopausal differences in the phase of CBT, melatonin, or alertness that might suggest a risk of developing sleep-onset insomnia. Nevertheless, we noted that postmenopausal women showed delayed rhythms of TST, SOL, and N1 sleep, indicating a circadian misalignment. For instance, the peak of TST occurred closer to the habitual rise time of postmenopausal women, whereas the peak in young women occurred approximately two hours before their rise time. However, there were no group differences in SOL during the baseline sleep period or the biological night of the USW procedure that might suggest an association with sleep-onset insomnia. Our study also revealed that postmenopausal women exhibit advanced rhythms of alpha power, which could be potentially linked to earlier sleep times than young women or early morning awakenings. This observation is consistent with previous reports that have suggested earlier rhythms to be related to aging (Kim et al., 2014; Van Cauter et al., 1996; Youngstedt et al., 2019). However, again, our study did not find any significant differences in CBT, melatonin, and alertness between postmenopausal and young women, which does not support a substantial shift in the circadian timing system of postmenopausal women. Our findings indeed suggest that there is a shift in the circadian organization of sleep reflected in the rhythms of TST, SOL, N1 sleep, and alpha power. Nevertheless, the clinical relevance of these observations requires further investigation and remains to be elucidated.

It is worth noting that our postmenopausal women are healthy sleepers, as indicated by a PSG screening conducted at the laboratory and a clinical evaluation. Indeed, their SE during the baseline sleep period and self-reported sleep quality during

the USW procedure were similar to that of the young women. The findings of this study highlight the fact that postmenopausal women experience changes in sleep patterns, even when they subjectively assess their sleep quality as good, and suggest an elevated risk of developing sleep disorders in this particular population.

5.4 Proposed mechanisms by which menopause influences circadian rhythms and sleep

Circadian rhythm changes in postmenopausal women may be influenced by reduced levels of ovarian hormones such as estrogen and progesterone, in addition to age-related changes. These sex hormones are known to impact multiple neural circuits and neurotransmitters in the brain. Sex hormones have been implicated in different mechanisms of neural plasticity, such as neural outgrowth, synaptogenesis, dendritic branching, and myelination. Estrogen receptors (ERα, ERβ, GPR30) and progesterone receptors (A and B) have been widely identified in various brain regions and may interact with glutamatergic and GABAergic pathways (Barth et al., 2015; Zhang et al., 2021).

Ovarian hormones can affect circadian rhythms through the SCN. Estrogen receptors have been found in the SCN, with a higher expression in the shell than the core; however, their overall expression is not abundant (Alvord et al., 2022; Karatsoreos & Silver, 2007; Yan & Silver, 2016). Studies on animal models suggest that the administration of estrogen affects the levels and timing of daily activity. Ovariectomy of mice reduces daily activity and makes it more fragmented, which is re-established with estrogen administration (Blattner & Mahoney, 2014; Brockman et al., 2011). Additionally, in animal models, estrogen has been observed to shorten the free-running period length and advance the onset of daily activity (Albers, 1981; Morin et al., 1977). On the other hand, the administration of progesterone has been found to delay the daily activity cycle (Axelson et al., 1981). Due to the reduced number of estrogen receptors in the SCN, studies suggest that the effect of ovarian hormones on circadian rhythms is given via extra-SCN sites projecting to the SCN, such as the intergeniculate leaflet and the dorsal RN (Mong et al., 2011).

The research conducted in this thesis does not provide a direct measure of the function of the SCN. Therefore, in the following chapters, we will explore the literature

regarding the impact of aging and ovarian hormones on the various outputs of the SCN, namely CBT, melatonin, sleep and alertness, and the cardiovascular system.

5.4.1 CBT and ovarian hormones

In Chapter 2, our investigation revealed that postmenopausal women exhibited a lower mesor of CBT than their younger counterparts throughout the USW procedure, with no apparent group differences in circadian amplitude or phase. The observed results in CBT can be attributed to the interplay of ovarian hormone effects and age-related changes.

Focusing first on ovarian hormones, estrogen and progesterone exert both peripheral and central effects on the vascular system and the hypothalamus, respectively. Within the central mechanisms, various nuclei, including the MPO, MnPO, ARC, DMH, and VMH, express estrogen receptors (ERα and ERβ). For instance, estrogen administration increases the firing rate of warm-sensitive neurons in the POA, promoting vasodilation in the peripheral vascular system and heat dissipation in mice (Silva & Boulant, 1986; Zhang et al., 2020). In contrast, the administration of progesterone has been less studied, but has shown to decrease the firing rate of warm-sensitive neurons in the POA of rabbits (Nakayama et al., 1975). Moreover, ovarian hormones interact with the peripheral vascular system. More specifically, estrogen administration stimulates the vasodilator pathway mediated by nitric oxide (NO) (Aryan et al., 2020). Human studies reveal that estrogen promotes heat dissipation through peripheral vasodilation, while progesterone triggers heat retention through peripheral vasoconstriction (Charkoudian et al., 2017; Stachenfeld et al., 2000). This aligns with increased CBT levels observed during the luteal phase, characterized by higher progesterone levels, compared to the follicular phase in naturally menstruating women (Shechter et al., 2011). The interaction between estrogen and progesterone levels may be responsible for CBT values, as shown by a study by Grant et al. (2020), where an increased progesterone/estradiol ratio during the luteal phase was associated with increased CBT.

With aging, there is a reduction in basal metabolic rate. This decrease in metabolism translates to fewer chemical reactions responsible for heat production, such as diminished glucose metabolism in skeletal muscle or decreased lipolysis in the liver.

The consequence of this reduced heat production is reflected in decreasing CBT values (Blatteis, 2012; Neff et al., 2016; Van Someren, 2007). Alternatively, increased blood flow in the fingertips of older individuals has also been proposed as a potential mechanism (Frank et al., 2000). Rich arteriovenous anastomosis in fingertips can increase heat dissipation in older individuals, decreasing overall body temperature (Frank et al., 2000).

As discussed in “Chapter 2.8.2, Discussion — Circadian rhythms”, our results suggest that the magnitude of the CBT reduction in postmenopausal women is similar to that observed in aging studies conducted in both men and women under controlled laboratory conditions (Buysse et al., 2005; Duffy et al., 1998; Yoon et al., 2003). Notably, the progesterone/estradiol ratio was substantially lower in postmenopausal women (~6) compared to young women (estimated at ~30). Based on these findings, we can hypothesize potential mechanisms through which CBT may be reduced in postmenopausal women.

Firstly, it is hypothesized that the output of the SCN toward the POA remains unaffected, reflected on the comparable circadian amplitude of CBT between our groups. Instead, age-related reductions in metabolic activity directly contribute to the decrease in CBT. The low progesterone/estradiol ratio further contributes to this reduction by upregulating heat dissipation mechanisms mediated by the MPO, MnPO and ARC.

Secondly, a low progesterone/estradiol ratio may reduce CBT by lowering the thermostat threshold via the DMH. This alternative possibility is coherent with a study showing that DMH lesions in animal models resulted in decreased CBT without changes in amplitude or phase (Chou et al., 2003). Estrogen may decrease CBT through its effect on the DMH (Zhang et al., 2020), and thus a low progesterone/estradiol ratio might contribute to the decreased CBT observed in postmenopausal women. This is an assumption based on the overall antagonizing effect of progesterone on estrogen in terms of body temperature. However, the absence of research identifying progesterone receptors in the DMH complicates understanding the specific impact of progesterone in this brain nucleus.

These hypotheses offer insights into the complex interplay between hormonal changes and other age-related factors influencing CBT in postmenopausal women, as

well as highlighting the need for further research to differentiate the effect of hormones and aging.

5.4.2 Melatonin and ovarian hormones

In Chapter 2, postmenopausal women displayed a reduced melatonin rhythm amplitude and mesor compared to young women. These results can be influenced by the declining ovarian hormone levels and other age-related effects. The effect of ovarian hormones on melatonin is a complex topic, as a bidirectional relationship between sex steroids and melatonin has been reported, though the exact mechanism by which these molecules interact remains unclear (Cipolla-Neto et al., 2022). This chapter examines the mechanisms that may lead to a decrease in melatonin levels and dampening of its circadian amplitude in postmenopausal women. To comprehend these mechanisms, research related to melatonin from animal studies and human studies are described, with an additional focus on menstrual phase variations and hormonal administration.

Studies have shown that estrogen and progesterone receptors are expressed in the pineal gland of nocturnal rodents (Cipolla-Neto et al., 2022; Luboshitzky et al., 1997; Vacas et al., 1979). For instance, melatonin secretion decreases during proestrus, characterized by elevated estrogen levels (Ozaki et al., 1978), and estradiol implants in female rats decrease melatonin secretion (Okatani et al., 1999). Progesterone, on the other hand, has a similar effect to estradiol and decreases melatonin synthesis in the female rat pineal gland (San Martin & Touitou, 2000).

Menstrual phase variations and the use of oral contraceptives further contribute to the complexity of melatonin levels in women. A within-subject analysis of eight young naturally ovulating women in their mid-follicular and mid-luteal phase using a USW protocol found no significant differences in salivary melatonin levels (Shechter et al., 2010). Similarly, Burgess and Fogg (2008) found no significant differences in salivary melatonin levels between menstrual phases in young ovulating women. However, Burgess and Fogg (2008) observed higher melatonin levels in women on oral contraceptives than in naturally ovulating women. Another study in young premenopausal women found increased melatonin secretion with oral contraceptives (Wright Jr & Badia, 1999). However, neither of these studies specified their oral contraceptives formulation

(Burgess & Fogg, 2008; Wright Jr & Badia, 1999), as it may be necessary to clarify the role of each formulation on melatonin secretion.

Melatonin secretion is also affected during the menopausal transition, which coincides with high hormonal fluctuations. Okatani et al. (2000) conducted a study which showed that melatonin levels increase in women aged between 45 and 50 compared to women aged between 30 and 39, with a subsequent and progressive decrease for several years after the FMP. Studies exploring the impact of estrogen and progesterone administration on melatonin levels in postmenopausal women present opposing results compared to those presented in young women. In postmenopausal women, estrogen administration may decrease melatonin levels (Okatani et al., 2000), but no effect on melatonin levels were observed when estrogen and progesterone treatment were combined (Toffol et al., 2014). The administration of progesterone alone has been less studied but has shown to decrease melatonin levels in postmenopausal women (Caufriez et al., 2011). The role of melatonin on the reproductive system might contribute to the discrepancies in melatonin levels after hormonal administration between premenopausal and postmenopausal women. Indeed, melatonin has been shown to promote GnRH synthesis and release, as well as modulate estrogen and progesterone production in the ovaries (Chuffa et al., 2011; Roy & Belsham, 2002).

With aging, various mechanisms can impact the synthesis and secretion of melatonin. Firstly, adrenergic neurons originating from the superior cervical ganglion promote melatonin synthesis within the pineal gland. However, a decreased density of β adrenergic receptors in pinealocytes leads to a reduction in the synthesis of serotonin N-acetyltransferase, thereby decreasing melatonin synthesis (Dax & Sugden, 1988). Secondly, gene expression of serotonin N-acetyltransferase has also been found to be reduced in aging individuals, which can further contribute to decreased melatonin synthesis (Paltsev et al., 2016). Lastly, melatonin can neutralize increased reactive oxygen species generated in aging cells. The overproduction of reactive oxygen species and constant inhibition by melatonin may lead to decreased melatonin levels (Tan et al., 2018).

The literature suggests that hormonal fluctuations around menopause may increase melatonin values and reach similar levels to those observed in young women. Our postmenopausal women have been without a menstrual period for at least two years, and hormonal fluctuations should not have affected their melatonin levels. The fact that melatonin levels decrease further away from the FMP when the menopausal transition is finalized suggests that aging processes other than ovarian hormones play an important role in melatonin levels. Moreover, oophorectomy with subsequent reduction of ovarian hormone levels lead to increased melatonin levels (Okatani et al., 2000). Therefore, the decreased melatonin values and amplitude observed in this group of postmenopausal women should be primarily attributed to age-related changes in the pineal gland rather than to the decreased ovarian hormones characteristic of menopause. Nevertheless, the complete mechanism explaining the interaction between sex hormones and melatonin remains to be further investigated.

5.4.3 Sleep, alertness, and ovarian hormones

In Chapters 2 and 3, postmenopausal women exhibited a dampened circadian variation of the sleep architecture, alertness, and EEG activity compared to young women. To understand these results, we must explore the contribution of ovarian hormones and aging to sleep. Indeed, estrogen and progesterone regulate sleep and alertness through their central effect in specific brain regions.

Our group of postmenopausal women displayed a dampened TST and SOL rhythm characterized by shorter sleep latency and more sleep during the day, with no group differences in sleep observed at night. Moreover, postmenopausal women showed more alertness at night compared to younger women. A dampened circadian variation of sleep and alertness has been described with aging, characterized by decreased sleep propensity at night. Conversely, the dampened circadian variation of sleep in our postmenopausal women is given by increased sleep propensity during the day. Animal research has shown that estradiol may synchronize the sleep-wake cycle to the correct time of day by promoting wakefulness during the active phase of rats (Mong & Cusmano, 2016; Smith et al., 2020), and has been further shown in a menopausal rat model (Deurveilher et al., 2013). Indeed, inhibition of prostaglandin D2 by estradiol in the POA

is a well-documented mechanism promoting wakefulness (Hadjimarkou et al., 2008; Mong, Devidze, Frail, et al., 2003; Mong, Devidze, Goodwillie, et al., 2003; Ribeiro et al., 2009; Smith et al., 2020). Estradiol may also promote wakefulness through its action on wake-promoting areas such as the TMN, DRN, LHA, and LC (Deurveilher et al., 2008; Krajnak et al., 2003; Porkka-Heiskanen et al., 2004; Ribeiro et al., 2009; Silveyra et al., 2009). Since decreased estrogen accompanies menopause, it is reasonable to hypothesize that the declining estrogen levels may disrupt the sleep-wake cycle by promoting more sleep during the day and alertness at night in postmenopausal women. Our research outcomes are consistent with the research performed by Mong and Cusmano (2016), and confirm their results in human subjects, which had not been previously established.

Moreover, the low progesterone levels of postmenopausal women may further support the dampened variation of sleep in postmenopausal women. This is because progesterone may also promote wakefulness, but there is limited research on the effect of progesterone in regulating the sleep-wake cycle (Deurveilher et al., 2008; Dorsey et al., 2020). Progesterone receptors have been found in a few sleep-regulating areas, such as the DRN, LHA, TMN, POA, and thalamic reticular nucleus (TRN) (Barth et al., 2015; Belelli & Lambert, 2005; Deurveilher et al., 2008; Dorsey et al., 2020; Smith et al., 2020). Although progesterone receptors are present in these brain areas regulating sleep and alertness, the exact mechanisms by which progesterone may influence the sleep-wake cycle via these nuclei have not yet been described.

Notably, the reduced levels of progesterone of postmenopausal women may contribute to the dampened circadian variation of sigma power as described in Chapter 3.6.1. Within the thalamocortical network, progesterone potentially enhances the production of sleep spindles, as supported by research studies conducted on both animals and humans (Fernandez & Luthi, 2020). The TRN generates sleep spindles by connecting with thalamocortical neurons through a GABAergic projection. These neurons also connect to the TRN and send signals to the cortex through glutamatergic projections, while the corticothalamic projections complete the network (Fernandez & Luthi, 2020). Phasic and tonic inhibition of the TRN neurons promotes sleep spindle production,

modulated by the neurosteroid pregnenolone, a precursor of progesterone, promoting spindle generation (Belelli & Lambert, 2005; Brown et al., 2015; Christian, 2020). The role of progesterone in the thalamocortical network is supported by human research. For instance, Plante and Goldstein (2013) compared the effect of medroxyprogesterone acetate on matched controls not taking any medication and observed higher power in spindle frequency activity in women on medroxyprogesterone acetate compared to controls.

The dampened circadian variation of SWS, SWA, and sigma power in our group of postmenopausal women might be influenced by aging processes other than hormonal changes. Brain atrophy, for instance, has been widely described as affecting sleep in aged individuals. Slow oscillations, such as sleep spindles and SWA, are generated in the thalamus and the basal forebrain and then distributed to the cortex. Consequently, increased sulcal atrophy has been associated with decreased SWS, SWA, and sleep spindles (Guazzelli et al., 1986; Jones, 2020; Liu et al., 2017; Niethard et al., 2018). Other aging studies have also found a reduction in neural density in the POA (Miller et al., 1989) and decreased levels of orexin and MCH neurons in the LHA (Kessler et al., 2011). The projection from the SCN to the POA and LH suggests that age-related changes may also influence the circadian process of sleep regulation (Saper, Lu, et al., 2005). The extent to which age-related changes in the cortex, POA, and LHA contribute to the effect of declining estrogen and progesterone levels on various sleep parameters of postmenopausal women remains unclear. As such, further investigation is necessary to better comprehend the relationship between hormonal changes, aging, and sleep after menopause.

5.4.4 The cardiovascular system and ovarian hormones

In the analysis of our results, we have observed a decrease in values and a dampened rhythm of HRV and parasympathetic activity during the sleep of postmenopausal women, compared to young women. These findings suggest a potential risk for developing cardiovascular disease in a healthy postmenopausal population. These HRV results might be given by the effect of ovarian hormones of other age-related changes.

The existing literature has extensively investigated age and sex differences in CVD, as young women have fewer cardiovascular events compared to young men. As individuals age, there is a decrease in HRV and parasympathetic activity (Hillebrand et al., 2013; Wulsin et al., 2015). This decrease has been observed in both healthy aging populations, as well as those affected by CVD (Jandackova et al., 2016; Tegegne et al., 2018). Notably, the risk of CVD significantly increases in women after menopause, indicating a strong influence of sex hormones (El Khoudary et al., 2019). Indeed, estrogen has been shown to strongly influence the cardiovascular system through its effect on the central nervous system and the peripheral vascular system (Aryan et al., 2020).

Estrogen plays a fundamental role in the vascular system by inducing vasodilation and reducing the risk of heart problems such as atherosclerosis and heart fibrosis (Haas et al., 2007; Shaw et al., 2001). This mechanism is activated through estrogen receptors in arteries (ERα, ERβ, and GPR30), leading to vasodilation via endothelial nitric oxide synthase (Fredette et al., 2018; Widder et al., 2003) and cardioprotective effects by preventing cardiomyocyte apoptosis (Deschamps & Murphy, 2009; Liu et al., 2011). In the central nervous system, estrogen receptors have been identified in various brain regions responsible for regulating the cardiovascular system, including the PVN, LHA, RVLM, NAmb, and NTS (Spary et al., 2009). Direct estradiol injections in the central nervous system of rats indicate the estrogen modulation of the ANS. For instance, studies conducted in ovariectomized female rats showed a decrease in renal sympathetic nerve activity with estradiol microinjections in the NTS, RVLM, PB, and intrathecal space (Saleh et al., 2000a, 2000b). They also observed an increased baroreflex function with estradiol microinjections in the NTS, NAamb, PB, and intrathecal space. On the other hand, progesterone receptors have been characterized in brain regions regulating the cardiovascular system, including the NTS, VLM, ARC and PVN (Greco et al., 2001; Haywood et al., 1999). However, the specific role of these progesterone receptors in the cardiovascular system and their interaction with estrogenic effects in various brain nuclei have yet to be fully elucidated.

The overall decreased values of HRV and parasympathetic activity in postmenopausal women might be given by cardiovascular changes and not by the

circadian timing system. As stated by Chen et al. (2022), estrogen activity can protect against cardiovascular disease by improving cardiac function and contractility, attenuating myocyte apoptosis, decreasing heart fibrosis, and promoting vasodilation in the peripheral vascular system. Aging has also been associated with impaired cardiac acetylcholine release, decreased cardiac muscarinic receptor activity, and density (Jandackova et al., 2016).

A connection between the SCN and pathways regulating the autonomic function, such as the PVN and the DMH, has been described (Buijs et al., 2014; Ueyama et al., 1999). Our findings support this connection between the SCN and the cardiovascular regulatory nuclei, as confirmed by the circadian variation in HRV parameters in young women. Additionally, the literature presented in this chapter suggests that estradiol administration affects central nuclei such as the RVLM, Namb, NTS, and PB promoting parasympathetic activity. Therefore, the lack of estrogen in central regulatory nuclei of the cardiovascular system due to decreased levels of ovarian hormones in postmenopausal women may contribute to the dampened rhythm of HRV measures. Moreover, as the NTS and PB nuclei have been proposed to connect the ANS and the sleep-wake cycle (Silvani et al., 2015), the decline estrogenic activity in these nuclei may contribute to the sleep disruption associated with autonomic changes in postmenopausal women. However, the specific contribution of RVLM, Namb, NTS, and PVN to circadian rhythmicity and sleep regulation remains unclear. Overall, this collective evidence shows the role of ovarian hormones as central modulators of autonomic tone. It is important to acknowledge that studies on menopause are scarce, and the full extent of ovarian hormones' contribution to the circadian rhythms of cardiovascular variables remains to be largely investigated.

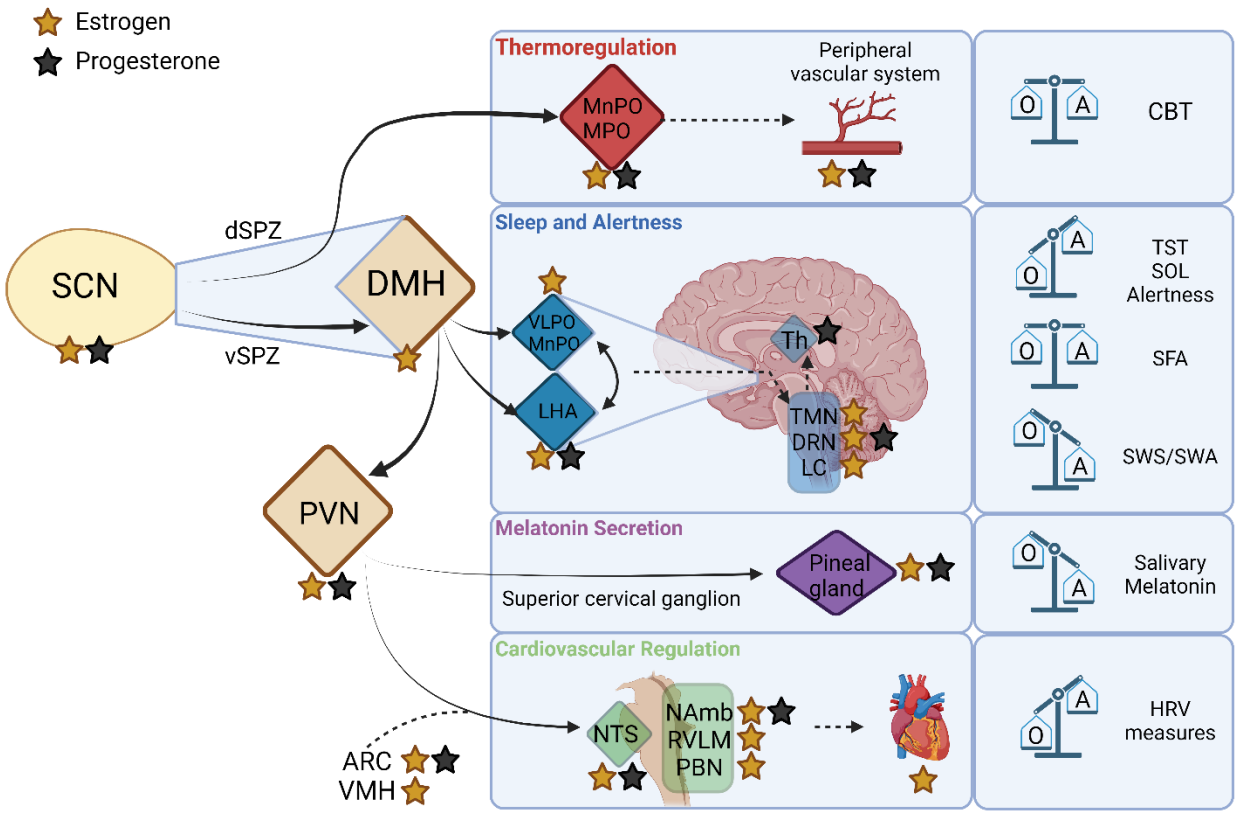


Figure 5. 1. Sites of estrogen and progesterone effects on the various SCN outputs. This model illustrates the relationship between the thermoregulatory centers, melatonin secretion, sleep and alertness, and cardiovascular system modulation, as well as the body regions where ovarian hormones may affect these systems. Balance scales on the right side illustrate the hypothesized contribution of ovarian hormones (O) vs aging changes (A) for the dampened circadian variations in postmenopausal women presented in this thesis. Created with BioRender.com

5.5 A proposed model on the influence of menopause on the circadian timing system outputs

The previous chapters have described the effects of estrogen and progesterone on various brain areas that regulate physiological functions such as CBT, melatonin

secretion, sleep and alertness, and HRV, and their possible contribution to the results observed in the present thesis. Figure 5.1 presents a model illustrating the multiple sites of estrogen and progesterone effects and their relationship with the various SCN outputs. The sites of action range from central effects in the hypothalamus to peripheral effects in the vascular system. Notably, some nuclei can affect multiple systems, such as the SCN, DMH and PVN, and thus the effect of ovarian hormones in these nuclei may affect the circadian rhythms of our postmenopausal women. Firstly, the SCN can be affected by estrogen and progesterone, altering the timing and duration of activity patterns in rodents. Secondly, estrogen receptors in the DMH play a role in regulating body temperature and the cardiovascular system (Uchida et al., 2010; Ueyama et al., 2006; Zhang et al., 2020). Finally, the PVN expresses estradiol and progesterone receptors (Gingerich & Krukoff, 2006; Xue et al., 2013), which can be involved in cardiovascular regulation and melatonin secretion. Although the PVN is a relay from the SCN to the pineal gland, the local effects of ovarian hormones and their impact on melatonin secretion has not yet been explored. However, research is currently investigating the effect of estrogen on the PVN for various physiological functions, such as reproduction, immune response, metabolism, and autonomic functions of the kidneys and the gastrointestinal tract (Grassi et al., 2022).

Figure 5.1 also illustrates the hypothesized contribution of aging and ovarian hormones to the various dampened circadian rhythms of postmenopausal women in the present thesis, based on the literature summarized in Chapters 5.4.1 – 5.4.4. According to the available literature, age-related changes could significantly impact the circadian variation of SWS, SWA, and melatonin secretion of our group of postmenopausal women, whereas the effects of ovarian hormones on these outcomes might be reduced. Conversely, the effect of ovarian hormones may strongly influence the circadian variation of TST, SOL, alertness, and HRV of postmenopausal women, while the effect of aging may have a lowered impact on these measures. We acknowledge the complexity of separating the effects of sex hormones on aging based on the results of our experiment, but we highlight the lack of research on ovarian hormones and emphasize that common age-related changes do not necessarily account for all our findings.

Although the decline in levels of estrogen and progesterone during menopause might affect the aforementioned brain nuclei, it is important to perform further research to comprehend the whole network functioning. For example, the lack of estrogen affects the regulation of the cardiovascular system and may lead to the observed dampened rhythms in postmenopausal women. However, the individual contribution of estrogen at each step of the network, i.e., the SCN projecting to the PVN, which projects to the NTS, and the cumulative effects of aging, remains to be further studied.

5.6 Limitations

The current thesis has some limitations that need to be addressed. One significant limitation is the small sample size, which may have limited the statistical power and the possibility of identifying statistical significance in some results. In our initial recruitment efforts, we aimed to align the number of postmenopausal women with that of young women for our study. However, recruiting postmenopausal women was challenging. Since we wanted to study the effect of menopause and not the effect of numerous common disorders occurring at menopause, only a small proportion of women were selected. First, participants experiencing insomnia were ineligible for this study if they self-reported experiencing difficulties falling asleep, staying asleep, waking up early in the morning, or impaired daytime functioning at least three times per week for a minimum of three months. Participants were not excluded based on their self-reported chronotype using the Horne & Östberg morningness-eveningness questionnaire. However, we excluded participants whose bedtimes fell before 9 p.m. or after 2 a.m. and whose rise times were earlier than 5 a.m. Common reasons for exclusion included depression, anxiety, psychological and gynecological conditions, use of medications that potentially affected sleep and circadian rhythms, sleep apnea and restless legs syndrome, obesity, and cancer, among others. A total of 1312 women were initially assessed for eligibility, of which 29 underwent our screening procedures at our research facility. Ultimately, only ten postmenopausal women met the eligibility criteria, and two withdrew from the study, resulting in a final sample of 8 postmenopausal women.

Differences in methodology were observed between postmenopausal and young women due to variations in data collection timing. The protocol for postmenopausal

women was shorter for feasibility and safety reasons, given their increased thrombosis risk due to reduced mobility. Salivary melatonin collection methods differed, necessitating a correction calculation detailed in Chapter 2.6.7. Postmenopausal women were permitted to utilize the ensuite bathroom and move around the room, whereas young women remained in bed. While these differences had the potential to impact certain measures (CBT, alertness, HRV), as discussed in Chapters 2.7.3 and 4.5.2, their overall influence was non-significant.

The AHI cutoff differed between postmenopausal (≥15) and young women (≥5), which potentially impacted our sleep results. Nevertheless, the mean AHI value for postmenopausal women was 4.02, falling within the normal AHI range. More specifically, the actual AHI values were <5 in 5 postmenopausal women, reflecting a healthy AHI. Three postmenopausal women exhibited an AHI >5 and <10, categorized as mild sleep apnea. It is important to consider that sleep apnea may potentially influence SWS and REM sleep (Schwarz et al., 2017). However, SWS and REM sleep results were comparable between postmenopausal and young women during the baseline sleep period. Therefore, it is improbable that the small differences in AHI between postmenopausal and young women influenced our results.

Moreover, we decided to interpolate the EEG data from 250 Hz to 512 Hz to obtain frequency bins of 0.25 Hz following the Fourier Transform. The sum of specific 0.25 Hz bins enabled us to obtain the power of conventional fixed frequency bands (e.g., 4-8 Hz for delta power). While it was feasible to down-sample the 512 Hz data to 250 Hz, the resulting conversion would have produced frequency bins of 0.244 Hz instead of the desired 0.25 Hz. This would lead to a delta frequency range from 3.904 Hz to 8.052 Hz, and thus, interpolation of data from 250 Hz to 512 Hz was deemed more convenient. Interpolating the sampling frequency from 250 to 512 Hz could have led to some imprecisions in the highest frequency bands and represents a study limitation. Nevertheless, the highest frequency we reported is within the beta range (16-24.5 Hz) and below the lowest sampling frequency of 250 Hz. Thus, we do not believe this limitation to have a major impact on our results.

Another possible limitation in EEG analysis could be the introduction of high-frequency artifacts. Possible high-frequency artifacts were reviewed by the research team. For instance, we observed a narrow peak in young women that appeared to drive group differences within beta power (Figure 3.2, panel C, p.117). Since data from this figure depict relative power (in %) between night and day EEG activity, the peak is given by a relative difference of ~50% in a high frequency with very low power (<0.1 μv2). Thus, this peak does not represent an artifact.

We recognize that every additional statistical test using a p-value to determine statistical significance inflates the risk of Type I error. We understand the importance of correcting Type I errors, as it has been done in prior manuscripts published in our research group (Kervezee et al., 2019; Kervezee et al., 2018) . However, controversy still exists about the pros and cons of correcting for multiple tests, especially in an exploratory study design such as ours (Streiner, 2015). Correcting for multiple testing in the current study would disproportionately increase the risk of Type 2 errors while reducing that of Type I. In accordance with Althouse (2016), adjustments for multiple tests were not performed, and subsequent studies will be necessary to explore our results further.

The strict inclusion criteria focused only on healthy participants, which may have limited the generalization of the findings to the postmenopausal population, especially those with associated diseases. Furthermore, since most women included in our study were Caucasian, we did not analyze the effect of ethnicity on sleep or circadian rhythm changes at menopause.

The observational design of our study prevents any causal inference. It remains unknown to what extent the SCN controls the circadian amplitude changes observed in this thesis and their functional consequences. Moreover, we did not investigate how the various parameters studied influenced each other. For instance, we did not test how low melatonin or ovarian hormone levels could lead to changes in alertness, sleep architecture, EEG power spectrum, or HRV.

It remains challenging to separate the effect of sex hormones from other age-related changes that occur during menopause, and an age-matched group of men was not available for testing. As highlighted in Chapter 5.5, this is a crucial area for future

research. Unfortunately, we could not establish correlations between hormones, age, and sleep parameters. We attempted to see whether some correlations could be found within the postmenopausal group since blood samples were not collected in young women. The scope of this analysis was limited as the analysis was based on a single blood sample per postmenopausal woman during the USW procedure. No significant effects of age, date since the final menstrual period, or sex hormones on sleep parameters in postmenopausal women were observed. Altogether, we find it risky to draw any conclusions from these limited analyses. Additionally, as all our postmenopausal women were at least two years post their final menstrual period, when hormonal levels had already stabilized, the factor of time since the final menstrual period does not yield substantial information.

It is important to acknowledge that there are different types of chronobiology protocols, including the USW procedure. During this protocol, nap episodes may have masked the circadian variation of some measures, such as CBT, alertness, and HRV. Furthermore, the nature and length of the experimental phase in the laboratory may have influenced self-reported measures. Our experimental design did not allow us to directly assess the homeostatic process of sleep, as it would have required evaluating a recovery sleep period after sleep deprivation. Nevertheless, this unique protocol was necessary to obtain endogenous circadian rhythms and allowed us to record sleep at different circadian phases.

5.7 Perspectives and Future Steps

Results presented in Chapters 2, 3, and 4 of the current thesis showed postmenopausal changes in nocturnal sleep and the circadian variation of various outputs of the circadian timing system. As a follow-up study to further investigate the circadian basis of sleep disturbances, various SCN outputs should be assessed in postmenopausal women with insomnia. Conducting this study in highly controlled laboratory conditions, such as the USW or forced desynchrony, would be ideal. A comparison of the circadian rhythms in healthy-sleeping postmenopausal women and individuals with menopausal insomnia would provide valuable insights into the circadian disruptions observed in the current thesis. It is important to account for the type of insomnia during the study, as

sleep-onset and early-morning-awakening insomnia have been found to be significantly associated with specific circadian rhythm changes (Lack et al., 2017). Overall, there is a lack of research on circadian rhythms at menopause, and more investigations are necessary. Furthermore, the prevalence of circadian disruption in postmenopausal women remains to be investigated.

A remarkable result in Chapter 2 is the reduced melatonin secretion in postmenopausal women compared to young women. Clinical studies support the potential benefits of exogenous melatonin in older adults with insomnia due to its sleep-promoting effect (Ferracioli-Oda et al., 2018). One possible approach involves administering exogenous melatonin to menopausal women suffering from insomnia. This should be done while measuring melatonin rhythms before and after treatment. This would help us determine whether melatonin administration improves sleep by raising melatonin levels or by shifting the relationship between endogenous rhythms and the sleep-wake cycle. As stated in Roenneberg et al. (2022), circadian rhythm changes are often linked to sleep disturbances, but the causal relationship is not always clear. A randomized clinical trial using exogenous melatonin could help establish whether changes in circadian rhythms cause insomnia. Additionally, light therapy may be useful in establishing causation between circadian rhythm changes and insomnia. While light therapy has been explored as a treatment for insomnia, the results in objective sleep measures have been minimal (Chambe et al., 2023). Its primary benefit may be shifting circadian rhythms for some types of insomnia, such as sleep-onset insomnia, rather than directly improving sleep measures.

Methodological variations have resulted in significant variability in the results of EEG power spectra in the literature. Consequently, further studies are required to gain a more comprehensive understanding of microstructural changes in the EEG of postmenopausal women. For example, we decided to analyze the central lead (C3) of the EEG recording. However, topographical differences in EEG power have been described. These differences include increased SWA and LSFA in frontal leads compared to central and occipital leads, as well as increased HSFA in central leads compared to frontal and occipital leads (Cox et al., 2017; Knoblauch et al., 2003; Zhang et al., 2022). Additionally,

EEG power spectra exhibit variations across different sleep stages. There is increased EEG power in SWA during stage N3 compared to other sleep stages, increased HSFA in stage N2 compared to other sleep stages, and decreased sigma activity and sleep spindles during REM sleep compared to NREM sleep (Adamantidis et al., 2019; Cox et al., 2017). EEG spectral analyses could be additionally performed in adapted frequency bands to account for interindividual differences, such as correcting for peak alpha activity and identifying individual spindle frequency activity (Fernandez & Luthi, 2020; Hooper, 2005).

The investigation of ovarian hormones and their contribution to circadian rhythm changes requires further elucidation. As mentioned in Chapter 5.4, separating the effects of hormonal changes after menopause from age-related changes is complex. As a follow-up study to further examine the effect of hormones in circadian and sleep disruptions of postmenopausal women, randomized clinical trials with HRT are necessary. Ideally, estrogen treatment alone should be compared to progesterone treatment alone. This can be accomplished in postmenopausal women with a hysterectomy, where estrogen alone can be administered. In addition, the results should be compared to age-matched men. There is still much to explore regarding the effects of estrogen and progesterone on the brain. This is of critical importance, as estrogen receptors are implicated not only in sleep but in CVD (Chen et al., 2022).

A comprehensive understanding of women's sleep is important for improving their treatments. Dib et al. (2021) highlighted the lack of research on the sleep of women. In the past five years of their study, approximately 25% of non-human studies on sleep included females, while less than 7% of studies on circadian phase shifts involved female rodents. These statistics are concerning and emphasize the significance of sleep and circadian research for women's health.

5.8 Conclusion

The research presented in this thesis indicates that menopause has a notable influence on circadian rhythms, sleep and alertness, and the cardiovascular system. Our findings indicate that postmenopausal women exhibit a dampened circadian variation

across various outputs of the circadian timing system, which can result in increased daytime sleep propensity, nocturnal wake propensity, sleep disruption and fragility, and disrupted autonomic regulation during sleep. Based on the literature, circadian rhythm differences presented in this thesis may be linked to menopause-specific hormonal changes, age-related changes, or a combination of both factors. However, further investigations remain necessary to disentangle the effect of ovarian sex hormones at menopause and other aging changes. Finally, our study highlighted the lack of research regarding circadian rhythms during menopause and emphasized the importance of our findings in understanding the increased risk of sleep disorders and CVD in postmenopausal women. The research presented in this thesis is expected to lay the foundations for further studies aimed at improving treatment options for sleep disturbances in postmenopausal women.

Non-manuscript references

Adamantidis, A. R., Gutierrez Herrera, C., & Gent, T. C. (2019). Oscillating circuitries in the sleeping brain. *Nat Rev Neurosci*, *20*(12), 746-762. https://doi.org/10.1038/s41583-019-0223-4

Ahmady, F., Niknami, M., & Khalesi, Z. B. (2022). Quality of sleep in women with menopause and its related factors. *Sleep Sci*, *15*(Spec 1), 209-214. https://doi.org/10.5935/1984-0063.20220021

Ahokas, E. K., Hanstock, H. G., Lofberg, I., Nyman, M., Wenning, P., Kyrolainen, H., Mikkonen, R. S., & Ihalainen, J. K. (2023). Nocturnal Heart Rate Variability in Women Discordant for Hormonal Contraceptive Use. *Med Sci Sports Exerc*, *55*(7), 1342-1349. https://doi.org/10.1249/MSS.0000000000003158

Albers, H. E. (1981). Gonadal hormones organize and modulate the circadian system of the rat. *Am J Physiol*, *241*(1), R62-66. https://doi.org/10.1152/ajpregu.1981.241.1.R62

Althouse, A. D. (2016). Adjust for Multiple Comparisons? It's Not That Simple. *Ann Thorac Surg*, *101*(5), 1644-1645. https://doi.org/10.1016/j.athoracsur.2015.11.024

Alvord, V. M., Kantra, E. J., & Pendergast, J. S. (2022). Estrogens and the circadian system. *Semin Cell Dev Biol*, *126*, 56-65. https://doi.org/10.1016/j.semcdb.2021.04.010

Anaclet, C., & Fuller, P. M. (2017). Brainstem regulation of slow-wave-sleep. *Curr Opin Neurobiol*, *44*, 139-143. https://doi.org/10.1016/j.conb.2017.04.004

Anagnostis, P., & Stevenson, J. C. (2024). Cardiovascular health and the menopause, metabolic health. *Best Pract Res Clin Endocrinol Metab*, *38*(1), 101781. https://doi.org/10.1016/j.beem.2023.101781

Aryan, L., Younessi, D., Zargari, M., Banerjee, S., Agopian, J., Rahman, S., Borna, R., Ruffenach, G., Umar, S., & Eghbali, M. (2020). The Role of Estrogen Receptors in Cardiovascular Disease. *Int J Mol Sci*, *21*(12). https://doi.org/10.3390/ijms21124314

Attarian, H., Hachul, H., Guttuso, T., & Phillips, B. (2015). Treatment of chronic insomnia disorder in menopause: evaluation of literature. *Menopause*, *22*(6), 674-684. https://doi.org/10.1097/GME.0000000000000348

Aujard, F., Herzog, E. D., & Block, G. D. (2001). Circadian rhythms in firing rate of individual suprachiasmatic nucleus neurons from adult and middle-aged mice. *Neuroscience*, *106*(2), 255-261. https://doi.org/10.1016/s0306-4522(01)00285-8

Avis, N. E., Crawford, S. L., Greendale, G., Bromberger, J. T., Everson-Rose, S. A., Gold, E. B., Hess, R., Joffe, H., Kravitz, H. M., Tepper, P. G., Thurston, R. C., & Study of Women's Health Across the, N. (2015). Duration of menopausal vasomotor symptoms over the menopause transition. *JAMA Intern Med*, *175*(4), 531-539. https://doi.org/10.1001/jamainternmed.2014.8063

Axelson, J. F., Gerall, A. A., & Albers, H. E. (1981). Effect of progesterone on the estrous activity cycle of the rat. *Physiol Behav*, *26*(4), 631-635. https://doi.org/10.1016/0031-9384(81)90137-2

Baker, F. C. (2023a). It's Not Just About the Hot Flashes: Menopausal Hormone Changes and Disrupted Sleep. *J Clin Endocrinol Metab*, *108*(2), e25-e26. https://doi.org/10.1210/clinem/dgac628

Baker, F. C. (2023b). Optimizing sleep across the menopausal transition. *Climacteric*, *26*(3), 198-205. https://doi.org/10.1080/13697137.2023.2173569

Barth, C., Villringer, A., & Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front Neurosci*, *9*, 37. https://doi.org/10.3389/fnins.2015.00037

Belelli, D., & Lambert, J. J. (2005). Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci*, *6*(7), 565-575. https://doi.org/10.1038/nrn1703

Berry, R. B., Quan, S. F., Abreu, A. R., Bibbs, M. L., DelRosso, L., Harding, S. M., Mao, M.-M., Plante, D. T., Pressman, M. R., Troester, M. M., & Vaughn, B. V. (2020). *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.6*.

Blatteis, C. M. (2012). Age-dependent changes in temperature regulation - a mini review. *Gerontology*, *58*(4), 289-295. https://doi.org/10.1159/000333148

Blattner, M. S., & Mahoney, M. M. (2014). Estrogen receptor 1 modulates circadian rhythms in adult female mice. *Chronobiol Int*, *31*(5), 637-644. https://doi.org/10.3109/07420528.2014.885528

Boivin, D. B., Shechter, A., Boudreau, P., Begum, E. A., & Ng Ying-Kin, N. M. (2016). Diurnal and circadian variation of sleep and alertness in men vs. naturally cycling women. *Proc Natl Acad Sci U S A*, *113*(39), 10980-10985. https://doi.org/10.1073/pnas.1524484113

Borbely, A. (2022). The two-process model of sleep regulation: Beginnings and outlook. *J Sleep Res*, *31*(4), e13598. https://doi.org/10.1111/jsr.13598

Borbely, A. A. (1982). A two process model of sleep regulation. *Hum Neurobiol*, *1*(3), 195-204. https://www.ncbi.nlm.nih.gov/pubmed/7185792

Borbely, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: a reappraisal. *J Sleep Res*, *25*(2), 131-143. https://doi.org/10.1111/jsr.12371

Borjigin, J., Zhang, L. S., & Calinescu, A. A. (2012). Circadian regulation of pineal gland rhythmicity. *Mol Cell Endocrinol*, *349*(1), 13-19. https://doi.org/10.1016/j.mce.2011.07.009

Boudreau, P., Yeh, W. H., Dumont, G. A., & Boivin, D. B. (2013). Circadian variation of heart rate variability across sleep stages. *Sleep*, *36*(12), 1919-1928. https://doi.org/10.5665/sleep.3230

Brockman, R., Bunick, D., & Mahoney, M. M. (2011). Estradiol deficiency during development modulates the expression of circadian and daily rhythms in male and female aromatase knockout mice. *Horm Behav*, *60*(4), 439-447. https://doi.org/10.1016/j.yhbeh.2011.07.011

Brown, A. R., Herd, M. B., Belelli, D., & Lambert, J. J. (2015). Developmentally regulated neurosteroid synthesis enhances GABAergic neurotransmission in mouse thalamocortical neurones. *J Physiol*, *593*(1), 267-284. https://doi.org/10.1113/jphysiol.2014.280263

Buijs, F. N., Cazarez, F., Basualdo, M. C., Scheer, F. A., Perusquia, M., Centurion, D., & Buijs, R. M. (2014). The suprachiasmatic nucleus is part of a neural feedback circuit adapting blood pressure response. *Neuroscience*, *266*, 197-207. https://doi.org/10.1016/j.neuroscience.2014.02.018

Bullock, B., Murray, G., Anderson, J. L., Cooper-O'Neill, T., Gooley, J. J., Cain, S. W., & Lockley, S. W. (2017). Constraint is associated with earlier circadian phase and morningness: Confirmation of relationships between personality and circadian phase using a constant routine protocol. *Pers Individ Dif*, *104*, 69-74. https://doi.org/10.1016/j.paid.2016.07.036

Burgess, H. J., & Fogg, L. F. (2008). Individual differences in the amount and timing of salivary melatonin secretion. *PloS one*, *3*(8), e3055. https://doi.org/10.1371/journal.pone.0003055

Burgess, H. J., Wyatt, J. K., Park, M., & Fogg, L. F. (2015). Home Circadian Phase Assessments with Measures of Compliance Yield Accurate Dim Light Melatonin Onsets. *Sleep*, *38*(6), 889-897. https://doi.org/10.5665/sleep.4734

Buysse, D. J., Monk, T. H., Carrier, J., & Begley, A. (2005). Circadian patterns of sleep, sleepiness, and performance in older and younger adults. *Sleep*, *28*(11), 1365-1376. https://doi.org/10.1093/sleep/28.11.1365

Cain, S. W., Dennison, C. F., Zeitzer, J. M., Guzik, A. M., Khalsa, S. B., Santhi, N., Schoen, M. W., Czeisler, C. A., & Duffy, J. F. (2010). Sex differences in phase angle of entrainment and melatonin amplitude in humans. *J Biol Rhythms*, *25*(4), 288-296. https://doi.org/10.1177/0748730410374943

Carrier, J., Land, S., Buysse, D. J., Kupfer, D. J., & Monk, T. H. (2001). The effects of age and gender on sleep EEG power spectral density in the middle years of life (ages 20-60 years old). *Psychophysiology*, *38*(2), 232-242. https://doi.org/10.1111/1469-8986.3820232

Carrier, J., Monk, T. H., Buysse, D. J., & Kupfer, D. J. (1996). Amplitude reduction of the circadian temperature and sleep rhythms in the elderly. *Chronobiol Int*, *13*(5), 373-386. https://doi.org/10.3109/07420529609012661

Carrier, J., Paquet, J., Morettini, J., & Touchette, E. (2002). Phase advance of sleep and temperature circadian rhythms in the middle years of life in humans. *Neurosci Lett*, *320*(1-2), 1-4. https://doi.org/10.1016/s0304-3940(02)00038-1

Carrier, J., Semba, K., Deurveilher, S., Drogos, L., Cyr-Cronier, J., Lord, C., & Sekerovick, Z. (2017). Sex differences in age-related changes in the sleep-wake cycle. *Front Neuroendocrinol*, *47*, 66-85. https://doi.org/10.1016/j.yfrne.2017.07.004

Caufriez, A., Leproult, R., L'Hermite-Baleriaux, M., Kerkhofs, M., & Copinschi, G. (2011). Progesterone prevents sleep disturbances and modulates GH, TSH, and melatonin secretion in postmenopausal women. *J Clin Endocrinol Metab*, *96*(4), E614-623. https://doi.org/10.1210/jc.2010-2558

Chambe, J., Reynaud, E., Maruani, J., Fraih, E., Geoffroy, P. A., & Bourgin, P. (2023). Light therapy in insomnia disorder: A systematic review and meta-analysis. *J Sleep Res*, *32*(6), e13895. https://doi.org/10.1111/jsr.13895

Charkoudian, N., Hart, E. C. J., Barnes, J. N., & Joyner, M. J. (2017). Autonomic control of body temperature and blood pressure: influences of female sex hormones. *Clin Auton Res*, *27*(3), 149-155. https://doi.org/10.1007/s10286-017-0420-z

Chen, P., Li, B., & Ou-Yang, L. (2022). Role of estrogen receptors in health and disease. *Front Endocrinol (Lausanne)*, *13*, 839005. https://doi.org/10.3389/fendo.2022.839005

Chokroverty, S., & Bhat, S. (2021). Functional neuroanatomy of the peripheral autonomic nervous system. *Autonomic Nervous System and Sleep: Order and Disorder*, 19-28.

Chou, T. C., Scammell, T. E., Gooley, J. J., Gaus, S. E., Saper, C. B., & Lu, J. (2003). Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. *J Neurosci*, *23*(33), 10691-10702. https://doi.org/10.1523/JNEUROSCI.23-33-10691.2003

Christian, C. A. (2020). Nucleus-specific modulation of phasic and tonic inhibition by endogenous neurosteroidogenesis in the murine thalamus. *Synapse*, *74*(5), e22144. https://doi.org/10.1002/syn.22144

Chuffa, L. G., Seiva, F. R., Favaro, W. J., Teixeira, G. R., Amorim, J. P., Mendes, L. O., Fioruci, B. A., Pinheiro, P. F., Fernandes, A. A., Franci, J. A., Delella, F. K., Martinez, M., & Martinez, F. E. (2011). Melatonin reduces LH, 17 beta-estradiol and induces differential regulation of sex steroid receptors in reproductive tissues during rat ovulation. *Reprod Biol Endocrinol*, *9*, 108. https://doi.org/10.1186/1477-7827-9-108

Cipolla-Neto, J., Amaral, F. G., Soares Jr, J. M., Gallo, C. C., Furtado, A., Cavaco, J. E., Gonçalves, I., Santos, C. R. A., & Quintela, T. (2022). The crosstalk between melatonin and sex steroid hormones. *Neuroendocrinology*, *112*(2), 115-129. https://doi.org/10.1159/000516148

Cox, R., Schapiro, A. C., Manoach, D. S., & Stickgold, R. (2017). Individual Differences in Frequency and Topography of Slow and Fast Sleep Spindles. *Front Hum Neurosci*, *11*, 433. https://doi.org/10.3389/fnhum.2017.00433

Crandall, C. J., Mehta, J. M., & Manson, J. E. (2023). Management of Menopausal Symptoms: A Review. *JAMA*, *329*(5), 405-420. https://doi.org/10.1001/jama.2022.24140

Czeisler, C. A., Dumont, M., Duffy, J. F., Steinberg, J. D., Richardson, G. S., Brown, E. N., Sanchez, R., Rios, C. D., & Ronda, J. M. (1992). Association of sleep-wake habits in older people with changes in output of circadian pacemaker. *Lancet*, *340*(8825), 933-936. https://doi.org/10.1016/0140-6736(92)92817-y

Dax, E. M., & Sugden, D. (1988). Age-associated changes in pineal adrenergic receptors and melatonin synthesizing enzymes in the Wistar rat. *J Neurochem*, *50*(2), 468-472. https://doi.org/10.1111/j.1471-4159.1988.tb02934.x

de Zambotti, M., Colrain, I. M., & Baker, F. C. (2015). Interaction between reproductive hormones and physiological sleep in women. *J Clin Endocrinol Metab*, *100*(4), 1426-1433. https://doi.org/10.1210/jc.2014-3892

de Zambotti, M., Trinder, J., Colrain, I. M., & Baker, F. C. (2017). Menstrual cycle-related variation in autonomic nervous system functioning in women in the early menopausal transition with and without insomnia disorder. *Psychoneuroendocrinology*, *75*, 44-51. https://doi.org/10.1016/j.psyneuen.2016.10.009

de Zambotti, M., Trinder, J., Javitz, H., Colrain, I. M., & Baker, F. C. (2017). Altered nocturnal blood pressure profiles in women with insomnia disorder in the menopausal transition. *Menopause*, *24*(3), 278-287. https://doi.org/10.1097/GME.0000000000000754

de Zambotti, M., Trinder, J., Silvani, A., Colrain, I. M., & Baker, F. C. (2018). Dynamic coupling between the central and autonomic nervous systems during sleep: A review. *Neurosci Biobehav Rev*, *90*, 84-103. https://doi.org/10.1016/j.neubiorev.2018.03.027

Deboer, T. (2018). Sleep homeostasis and the circadian clock: Do the circadian pacemaker and the sleep homeostat influence each other's functioning? *Neurobiol Sleep Circadian Rhythms*, *5*, 68-77. https://doi.org/10.1016/j.nbscr.2018.02.003

Deboer, T. (2020). Circadian regulation of sleep in mammals. *Current Opinion in Physiology*, *15*, 89-95.

Deschamps, A. M., & Murphy, E. (2009). Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. *Am J Physiol Heart Circ Physiol*, *297*(5), H1806-1813. https://doi.org/10.1152/ajpheart.00283.2009

Deurveilher, S., Cumyn, E. M., Peers, T., Rusak, B., & Semba, K. (2008). Estradiol replacement enhances sleep deprivation-induced c-Fos immunoreactivity in forebrain arousal regions of ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol*, *295*(4), R1328-1340. https://doi.org/10.1152/ajpregu.90576.2008

Deurveilher, S., Seary, M. E., & Semba, K. (2013). Ovarian hormones promote recovery from sleep deprivation by increasing sleep intensity in middle-aged ovariectomized rats. *Hormones and behavior*, *63*(4), 566-576.

Dib, R., Gervais, N. J., & Mongrain, V. (2021). A review of the current state of knowledge on sex differences in sleep and circadian phenotypes in rodents. *Neurobiol Sleep Circadian Rhythms*, *11*, 100068. https://doi.org/10.1016/j.nbscr.2021.100068

Dijk, D. J., Beersma, D. G., & Bloem, G. M. (1989). Sex differences in the sleep EEG of young adults: visual scoring and spectral analysis. *Sleep*, *12*(6), 500-507. https://doi.org/10.1093/sleep/12.6.500

Dijk, D. J., Beersma, D. G., & van den Hoofdakker, R. H. (1989). All night spectral analysis of EEG sleep in young adult and middle-aged male subjects. *Neurobiol Aging*, *10*(6), 677-682. https://doi.org/10.1016/0197-4580(89)90004-3

Dijk, D. J., & Czeisler, C. A. (1995). Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. *J Neurosci*, *15*(5 Pt 1), 3526-3538. https://doi.org/10.1523/JNEUROSCI.15-05-03526.1995

Dijk, D. J., & Duffy, J. F. (1999). Circadian regulation of human sleep and age-related changes in its timing, consolidation and EEG characteristics. *Ann Med*, *31*(2), 130-140. https://doi.org/10.3109/07853899908998789

Dijk, D. J., & Duffy, J. F. (2020). Novel Approaches for Assessing Circadian Rhythmicity in Humans: A Review. *J Biol Rhythms*, *35*(5), 421-438. https://doi.org/10.1177/0748730420940483

Dijk, D. J., Duffy, J. F., Riel, E., Shanahan, T. L., & Czeisler, C. A. (1999). Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. *J Physiol*, *516 (Pt 2)*(2), 611-627. https://doi.org/10.1111/j.1469-7793.1999.0611v.x

Dijk, D. J., Duffy, J. F., Silva, E. J., Shanahan, T. L., Boivin, D. B., & Czeisler, C. A. (2012). Amplitude reduction and phase shifts of melatonin, cortisol and other circadian rhythms after a gradual advance of sleep and light exposure in humans. *PloS one*, *7*(2), e30037. https://doi.org/10.1371/journal.pone.0030037

Dorsey, A., de Lecea, L., & Jennings, K. J. (2020). Neurobiological and Hormonal Mechanisms Regulating Women's Sleep. *Front Neurosci*, *14*, 625397. https://doi.org/10.3389/fnins.2020.625397

Duffy, J., Dijk, D., Hall, E., & Czeisler, C. (1999). Relationship of endogenous circadian melatonin and temperature rhythms to self-reported preference for morning or evening activity in young and older people. *Journal of investigative medicine: the official publication of the American Federation for Clinical Research*, *47*(3), 141-150.

Duffy, J. F., Cain, S. W., Chang, A. M., Phillips, A. J., Munch, M. Y., Gronfier, C., Wyatt, J. K., Dijk, D. J., Wright, K. P., Jr., & Czeisler, C. A. (2011). Sex difference in the near-24-hour intrinsic period of the human circadian timing system. *Proc Natl Acad Sci U S A*, *108 Suppl 3*, 15602-15608. https://doi.org/10.1073/pnas.1010666108

Duffy, J. F., Dijk, D. J., Klerman, E. B., & Czeisler, C. A. (1998). Later endogenous circadian temperature nadir relative to an earlier wake time in older people. *Am J Physiol*, *275*(5 Pt 2), R1478-1487. https://doi.org/10.1152/ajpregu.1998.275.5.r1478

Duffy, J. F., Zeitzer, J. M., Rimmer, D. W., Klerman, E. B., Dijk, D.-J., & Czeisler, C. A. (2002). Peak of circadian melatonin rhythm occurs later within the sleep of older subjects. *Am J Physiol Endocrinol Metab*, *282*(2), E297-E303. https://doi.org/10.1152/ajpendo.00268.2001

Duffy, J. F., Zitting, K. M., & Chinoy, E. D. (2015). Aging and Circadian Rhythms. *Sleep Med Clin*, *10*(4), 423-434. https://doi.org/10.1016/j.jsmc.2015.08.002

El Khoudary, S. R. (2017). Gaps, limitations and new insights on endogenous estrogen and follicle stimulating hormone as related to risk of cardiovascular disease in

women traversing the menopause: A narrative review. *Maturitas*, *104*, 44-53. https://doi.org/10.1016/j.maturitas.2017.08.003

El Khoudary, S. R., Aggarwal, B., Beckie, T. M., Hodis, H. N., Johnson, A. E., Langer, R. D., Limacher, M. C., Manson, J. E., Stefanick, M. L., Allison, M. A., American Heart Association Prevention Science Committee of the Council on, E., Prevention, Council on, C., & Stroke, N. (2020). Menopause Transition and Cardiovascular Disease Risk: Implications for Timing of Early Prevention: A Scientific Statement From the American Heart Association. *Circulation*, *142*(25), e506-e532. https://doi.org/10.1161/CIR.0000000000000912

El Khoudary, S. R., Greendale, G., Crawford, S. L., Avis, N. E., Brooks, M. M., Thurston, R. C., Karvonen-Gutierrez, C., Waetjen, L. E., & Matthews, K. (2019). The menopause transition and women's health at midlife: a progress report from the Study of Women's Health Across the Nation (SWAN). *Menopause*, *26*(10), 1213-1227. https://doi.org/10.1097/GME.0000000000001424

Fernandes, E. O., Moraes, R. S., Ferlin, E. L., Wender, M. C., & Ribeiro, J. P. (2005). Hormone replacement therapy does not affect the 24-hour heart rate variability in postmenopausal women: results of a randomized, placebo-controlled trial with two regimens. *Pacing Clin Electrophysiol*, *28 Suppl 1*, S172-177. https://doi.org/10.1111/j.1540-8159.2005.00041.x

Fernandez, L. M. J., & Luthi, A. (2020). Sleep Spindles: Mechanisms and Functions. *Physiol Rev*, *100*(2), 805-868. https://doi.org/10.1152/physrev.00042.2018

Ferracioli-Oda, E., Qawasmi, A., & Bloch, M. H. (2018). Meta-Analysis: Melatonin for the Treatment of Primary Sleep Disorders. *Focus (Am Psychiatr Publ)*, *16*(1), 113-118. https://doi.org/10.1176/appi.focus.16101

Frank, S. M., Raja, S. N., Bulcao, C., & Goldstein, D. S. (2000). Age-related thermoregulatory differences during core cooling in humans. *Am J Physiol Regul Integr Comp Physiol*, *279*(1), R349-354. https://doi.org/10.1152/ajpregu.2000.279.1.R349

Fredette, N. C., Meyer, M. R., & Prossnitz, E. R. (2018). Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. *J Steroid Biochem Mol Biol*, *176*, 65-72. https://doi.org/10.1016/j.jsbmb.2017.05.006

Freedman, R. R. (2014). Menopausal hot flashes: mechanisms, endocrinology, treatment. *J Steroid Biochem Mol Biol*, *142*, 115-120. https://doi.org/10.1016/j.jsbmb.2013.08.010

Freedman, R. R., & Roehrs, T. A. (2004). Lack of sleep disturbance from menopausal hot flashes. *Fertil Steril*, *82*(1), 138-144. https://doi.org/10.1016/j.fertnstert.2003.12.029

Freeman, E. W., Sammel, M. D., & Sanders, R. J. (2014). Risk of long-term hot flashes after natural menopause: evidence from the Penn Ovarian Aging Study cohort. *Menopause*, *21*(9), 924-932. https://doi.org/10.1097/GME.0000000000000196

Gabbay, I. E., & Lavie, P. (2012). Age- and gender-related characteristics of obstructive sleep apnea. *Sleep Breath*, *16*(2), 453-460. https://doi.org/10.1007/s11325-011-0523-z

Geiger, P. J., Eisenlohr-Moul, T., Gordon, J. L., Rubinow, D. R., & Girdler, S. S. (2019). Effects of perimenopausal transdermal estradiol on self-reported sleep, independent of its effect on vasomotor symptom bother and depressive

symptoms. *Menopause*, *26*(11), 1318-1323. https://doi.org/10.1097/GME.0000000000001398

Gingerich, S., & Krukoff, T. L. (2006). Estrogen in the paraventricular nucleus attenuates L-glutamate-induced increases in mean arterial pressure through estrogen receptor beta and NO. *Hypertension*, *48*(6), 1130-1136. https://doi.org/10.1161/01.HYP.0000248754.67128.ff

Grant, L. K., Gooley, J. J., St Hilaire, M. A., Rajaratnam, S. M. W., Brainard, G. C., Czeisler, C. A., Lockley, S. W., & Rahman, S. A. (2020). Menstrual phase-dependent differences in neurobehavioral performance: the role of temperature and the progesterone/estradiol ratio. *Sleep*, *43*(2), zsz227. https://doi.org/10.1093/sleep/zsz227

Grassi, D., Marraudino, M., Garcia-Segura, L. M., & Panzica, G. C. (2022). The hypothalamic paraventricular nucleus as a central hub for the estrogenic modulation of neuroendocrine function and behavior. *Front Neuroendocrinol*, *65*, 100974. https://doi.org/10.1016/j.yfrne.2021.100974

Gravholt, C. H., Viuff, M. H., Brun, S., Stochholm, K., & Andersen, N. H. (2019). Turner syndrome: mechanisms and management. *Nat Rev Endocrinol*, *15*(10), 601-614. https://doi.org/10.1038/s41574-019-0224-4

Greco, B., Allegretto, E. A., Tetel, M. J., & Blaustein, J. D. (2001). Coexpression of ER beta with ER alpha and progestin receptor proteins in the female rat forebrain: effects of estradiol treatment. *Endocrinology*, *142*(12), 5172-5181. https://doi.org/10.1210/endo.142.12.8560

Guazzelli, M., Feinberg, I., Aminoff, M., Fein, G., Floyd, T. C., & Maggini, C. (1986). Sleep spindles in normal elderly: comparison with young adult patterns and relation to nocturnal awakening, cognitive function and brain atrophy. *Electroencephalogr Clin Neurophysiol*, *63*(6), 526-539. https://doi.org/10.1016/0013-4694(86)90140-9

Guillaumin, M. C. C., McKillop, L. E., Cui, N., Fisher, S. P., Foster, R. G., de Vos, M., Peirson, S. N., Achermann, P., & Vyazovskiy, V. V. (2018). Cortical region-specific sleep homeostasis in mice: effects of time of day and waking experience. *Sleep*, *41*(7). https://doi.org/10.1093/sleep/zsy079

Gunn, P. J., Middleton, B., Davies, S. K., Revell, V. L., & Skene, D. J. (2016). Sex differences in the circadian profiles of melatonin and cortisol in plasma and urine matrices under constant routine conditions. *Chronobiol Int*, *33*(1), 39-50. https://doi.org/10.3109/07420528.2015.1112396

Haas, E., Meyer, M. R., Schurr, U., Bhattacharya, I., Minotti, R., Nguyen, H. H., Heigl, A., Lachat, M., Genoni, M., & Barton, M. (2007). Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. *Hypertension*, *49*(6), 1358-1363. https://doi.org/10.1161/HYPERTENSIONAHA.107.089995

Hachul, H., Bittencourt, L. R., Soares, J. M., Jr., Tufik, S., & Baracat, E. C. (2009). Sleep in post-menopausal women: differences between early and late post-menopause. *Eur J Obstet Gynecol Reprod Biol*, *145*(1), 81-84. https://doi.org/10.1016/j.ejogrb.2009.03.019

Hachul, H., Frange, C., Bezerra, A. G., Hirotsu, C., Pires, G. N., Andersen, M. L., Bittencourt, L., & Tufik, S. (2015). The effect of menopause on objective sleep parameters: data from an epidemiologic study in Sao Paulo, Brazil. *Maturitas*, *80*(2), 170-178. https://doi.org/10.1016/j.maturitas.2014.11.002

Hadjimarkou, M. M., Benham, R., Schwarz, J. M., Holder, M. K., & Mong, J. A. (2008). Estradiol suppresses rapid eye movement sleep and activation of sleep-active neurons in the ventrolateral preoptic area. *Eur J Neurosci*, *27*(7), 1780-1792. https://doi.org/10.1111/j.1460-9568.2008.06142.x

Hall, J. E. (2015). Endocrinology of the Menopause. *Endocrinol Metab Clin North Am*, *44*(3), 485-496. https://doi.org/10.1016/j.ecl.2015.05.010

Hall, J. E. (2019). Neuroendocrine control of the menstrual cycle. In *Yen and Jaffe's reproductive endocrinology* (pp. 149-166. e145). Elsevier.

Harlow, S. D., Gass, M., Hall, J. E., Lobo, R., Maki, P., Rebar, R. W., Sherman, S., Sluss, P. M., de Villiers, T. J., & Group, S. C. (2012). Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. *J Clin Endocrinol Metab*, *97*(4), 1159-1168. https://doi.org/10.1210/jc.2011-3362

Hautamaki, H., Mikkola, T. S., Sovijarvi, A. R., Piirila, P., & Haapalahti, P. (2013). Menopausal hot flushes do not associate with changes in heart rate variability in controlled testing: a randomized trial on hormone therapy. *Acta Obstet Gynecol Scand*, *92*(8), 902-908. https://doi.org/10.1111/aogs.12164

Haywood, S. A., Simonian, S. X., van der Beek, E. M., Bicknell, R. J., & Herbison, A. E. (1999). Fluctuating estrogen and progesterone receptor expression in brainstem norepinephrine neurons through the rat estrous cycle. *Endocrinology*, *140*(7), 3255-3263. https://doi.org/10.1210/endo.140.7.6869

Herrmann, C. S., Struber, D., Helfrich, R. F., & Engel, A. K. (2016). EEG oscillations: From correlation to causality. *Int J Psychophysiol*, *103*, 12-21. https://doi.org/10.1016/j.ijpsycho.2015.02.003

Hillebrand, S., Gast, K. B., de Mutsert, R., Swenne, C. A., Jukema, J. W., Middeldorp, S., Rosendaal, F. R., & Dekkers, O. M. (2013). Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace*, *15*(5), 742-749. https://doi.org/10.1093/europace/eus341

Hooper, G. S. (2005). Comparison of the distributions of classical and adaptively aligned EEG power spectra. *Int J Psychophysiol*, *55*(2), 179-189. https://doi.org/10.1016/j.ijpsycho.2004.07.008

Huang, Y. L., Liu, R. Y., Wang, Q. S., Van Someren, E. J., Xu, H., & Zhou, J. N. (2002). Age-associated difference in circadian sleep-wake and rest-activity rhythms. *Physiol Behav*, *76*(4-5), 597-603. https://doi.org/10.1016/s0031-9384(02)00733-3

Jandackova, V. K., Scholes, S., Britton, A., & Steptoe, A. (2016). Are Changes in Heart Rate Variability in Middle-Aged and Older People Normative or Caused by Pathological Conditions? Findings From a Large Population-Based Longitudinal Cohort Study. *J Am Heart Assoc*, *5*(2). https://doi.org/10.1161/JAHA.115.002365

Jehan, S., Masters-Isarilov, A., Salifu, I., Zizi, F., Jean-Louis, G., Pandi-Perumal, S. R., Gupta, R., Brzezinski, A., & McFarlane, S. I. (2015). Sleep Disorders in

Postmenopausal Women. *J Sleep Disord Ther*, *4*(5). https://www.ncbi.nlm.nih.gov/pubmed/26512337

Jones, B. E. (2017). Principal cell types of sleep-wake regulatory circuits. *Curr Opin Neurobiol*, *44*, 101-109. https://doi.org/10.1016/j.conb.2017.03.018

Jones, B. E. (2020). Arousal and sleep circuits. *Neuropsychopharmacology*, *45*(1), 6-20. https://doi.org/10.1038/s41386-019-0444-2

Kalleinen, N., Aittokallio, J., Lampio, L., Kaisti, M., Polo-Kantola, P., Polo, O., Heinonen, O. J., & Saaresranta, T. (2020). Sleep during menopausal transition: a 10-year follow-up. *Sleep*. https://doi.org/10.1093/sleep/zsaa283

Kalleinen, N., Polo-Kantola, P., Himanen, S.-L., Alhola, P., Joutsen, A., Urrila, A. S., & Polo, O. (2008). Sleep and the menopause–do postmenopausal women experience worse sleep than premenopausal women? *Menopause Int*, *14*(3), 97-104. https://doi.org/10.1258/mi.2008.008013

Kalsbeek, A., Palm, I. F., La Fleur, S. E., Scheer, F. A., Perreau-Lenz, S., Ruiter, M., Kreier, F., Cailotto, C., & Buijs, R. M. (2006). SCN outputs and the hypothalamic balance of life. *J Biol Rhythms*, *21*(6), 458-469. https://doi.org/10.1177/0748730406293854

Karatsoreos, I. N., & Silver, R. (2007). Minireview: The neuroendocrinology of the suprachiasmatic nucleus as a conductor of body time in mammals. *Endocrinology*, *148*(12), 5640-5647. https://doi.org/10.1210/en.2007-1083

Kervezee, L., Cermakian, N., & Boivin, D. B. (2019). Individual metabolomic signatures of circadian misalignment during simulated night shifts in humans. *PLoS Biol*, *17*(6), e3000303. https://doi.org/10.1371/journal.pbio.3000303

Kervezee, L., Cuesta, M., Cermakian, N., & Boivin, D. B. (2018). Simulated night shift work induces circadian misalignment of the human peripheral blood mononuclear cell transcriptome. *Proc Natl Acad Sci U S A*, *115*(21), 5540-5545. https://doi.org/10.1073/pnas.1720719115

Kessler, B. A., Stanley, E. M., Frederick-Duus, D., & Fadel, J. (2011). Age-related loss of orexin/hypocretin neurons. *Neuroscience*, *178*, 82-88. https://doi.org/10.1016/j.neuroscience.2011.01.031

Kim, J. H., Elkhadem, A. R., & Duffy, J. F. (2022). Circadian Rhythm Sleep-Wake Disorders in Older Adults. *Sleep Med Clin*, *17*(2), 241-252. https://doi.org/10.1016/j.jsmc.2022.02.003

Kim, S. J., Benloucif, S., Reid, K. J., Weintraub, S., Kennedy, N., Wolfe, L. F., & Zee, P. C. (2014). Phase-shifting response to light in older adults. *J Physiol*, *592*(1), 189-202. https://doi.org/10.1113/jphysiol.2013.262899

Kingsberg, S. A., Schulze-Rath, R., Mulligan, C., Moeller, C., Caetano, C., & Bitzer, J. (2023). Global view of vasomotor symptoms and sleep disturbance in menopause: a systematic review. *Climacteric*, *26*(6), 537-549. https://doi.org/10.1080/13697137.2023.2256658

Klerman, E. B., Brager, A., Carskadon, M. A., Depner, C. M., Foster, R., Goel, N., Harrington, M., Holloway, P. M., Knauert, M. P., LeBourgeois, M. K., Lipton, J., Merrow, M., Montagnese, S., Ning, M., Ray, D., Scheer, F., Shea, S. A., Skene, D. J., Spies, C., . . . Burgess, H. J. (2022). Keeping an eye on circadian time in clinical research and medicine. *Clin Transl Med*, *12*(12), e1131. https://doi.org/10.1002/ctm2.1131

Klerman, E. B., Davis, J. B., Duffy, J. F., Dijk, D.-J., & Kronauer, R. E. (2004). Older people awaken more frequently but fall back asleep at the same rate as younger people. *Sleep*, *27*(4), 793-798. https://doi.org/10.1093/sleep/27.4.793

Klerman, E. B., Lee, Y., Czeisler, C. A., & Kronauer, R. E. (1999). Linear demasking techniques are unreliable for estimating the circadian phase of ambulatory temperature data. *J Biol Rhythms*, *14*(4), 260-274. https://doi.org/10.1177/074873099129000678

Knoblauch, V., Martens, W., Wirz-Justice, A., Krauchi, K., & Cajochen, C. (2003). Regional differences in the circadian modulation of human sleep spindle characteristics. *Eur J Neurosci*, *18*(1), 155-163. https://doi.org/10.1046/j.1460-9568.2003.02729.x

Kolker, D. E., Fukuyama, H., Huang, D. S., Takahashi, J. S., Horton, T. H., & Turek, F. W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. *J Biol Rhythms*, *18*(2), 159-169. https://doi.org/10.1177/0748730403251802

Kondratova, A. A., & Kondratov, R. V. (2012). The circadian clock and pathology of the ageing brain. *Nat Rev Neurosci*, *13*(5), 325-335. https://doi.org/10.1038/nrn3208

Krajnak, K., Rosewell, K. L., Duncan, M. J., & Wise, P. M. (2003). Aging, estradiol and time of day differentially affect serotonin transporter binding in the central nervous system of female rats. *Brain Res*, *990*(1-2), 87-94. https://doi.org/10.1016/s0006-8993(03)03441-3

Krauchi, K., & Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am J Physiol*, *267*(3 Pt 2), R819-829. https://doi.org/10.1152/ajpregu.1994.267.3.R819

Kravitz, H. M., Janssen, I., Bromberger, J. T., Matthews, K. A., Hall, M. H., Ruppert, K., & Joffe, H. (2017). Sleep Trajectories Before and After the Final Menstrual Period in The Study of Women's Health Across the Nation (SWAN). *Curr Sleep Med Rep*, *3*(3), 235-250. https://doi.org/10.1007/s40675-017-0084-1

Kripke, D. F., Elliott, J. A., Youngstedt, S. D., & Rex, K. M. (2007). Circadian phase response curves to light in older and young women and men. *J Circadian Rhythms*, *5*, 4. https://doi.org/10.1186/1740-3391-5-4

Kripke, D. F., Youngstedt, S. D., Elliott, J. A., Tuunainen, A., Rex, K. M., Hauger, R. L., & Marler, M. R. (2005). Circadian phase in adults of contrasting ages. *Chronobiol Int*, *22*(4), 695-709. https://doi.org/10.1080/07420520500180439

Laborde, S., Mosley, E., & Thayer, J. F. (2017). Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research - Recommendations for Experiment Planning, Data Analysis, and Data Reporting. *Front Psychol*, *8*, 213. https://doi.org/10.3389/fpsyg.2017.00213

Lack, L. C., Lovato, N., & Micic, G. (2017). Circadian rhythms and insomnia. *Sleep and Biological Rhythms*, *15*, 3-10.

Lampio, L., Polo-Kantola, P., Himanen, S. L., Kurki, S., Huupponen, E., Engblom, J., Heinonen, O. J., Polo, O., & Saaresranta, T. (2017). Sleep During Menopausal Transition: A 6-Year Follow-Up. *Sleep*, *40*(7), zsx090. https://doi.org/10.1093/sleep/zsx090

Lazar, A. S., Santhi, N., Hasan, S., Lo, J. C., Johnston, J. D., Von Schantz, M., Archer, S. N., & Dijk, D. J. (2013). Circadian period and the timing of melatonin onset in

men and women: predictors of sleep during the weekend and in the laboratory. *J Sleep Res*, *22*(2), 155-159. https://doi.org/10.1111/jsr.12001

Lim, A. S., Myers, A. J., Yu, L., Buchman, A. S., Duffy, J. F., De Jager, P. L., & Bennett, D. A. (2013). Sex difference in daily rhythms of clock gene expression in the aged human cerebral cortex. *J Biol Rhythms*, *28*(2), 117-129. https://doi.org/10.1177/0748730413478552

Liu, C. C., Kuo, T. B., & Yang, C. C. (2003). Effects of estrogen on gender-related autonomic differences in humans. *Am J Physiol Heart Circ Physiol*, *285*(5), H2188-2193. https://doi.org/10.1152/ajpheart.00256.2003

Liu, H., Pedram, A., & Kim, J. K. (2011). Oestrogen prevents cardiomyocyte apoptosis by suppressing p38alpha-mediated activation of p53 and by down-regulating p53 inhibition on p38beta. *Cardiovasc Res*, *89*(1), 119-128. https://doi.org/10.1093/cvr/cvq265

Liu, H., Yang, Y., Xia, Y., Zhu, W., Leak, R. K., Wei, Z., Wang, J., & Hu, X. (2017). Aging of cerebral white matter. *Ageing Res Rev*, *34*, 64-76. https://doi.org/10.1016/j.arr.2016.11.006

Luboshitzky, R., Dharan, M., Goldman, D., Herer, P., Hiss, Y., & Lavie, P. (1997). Seasonal variation of gonadotropins and gonadal steroids receptors in the human pineal gland. *Brain Res Bull*, *44*(6), 665-670. https://doi.org/10.1016/s0361-9230(97)00106-8

Magri, F., Gabellieri, E., Busconi, L., Guazzoni, V., Cravello, L., Valdes, V., Sorrentino, A. R., Chytiris, S., & Ferrari, E. (2006). Cardiovascular, anthropometric and neurocognitive features of healthy postmenopausal women: effects of hormone replacement therapy. *Life Sci*, *78*(22), 2625-2632. https://doi.org/10.1016/j.lfs.2005.10.036

Mehra, V. M., Costanian, C., McCague, H., Riddell, M. C., & Tamim, H. (2023). The association between diabetes type, age of onset, and age at natural menopause: a retrospective cohort study using the Canadian Longitudinal Study on Aging. *Menopause*, *30*(1), 37-44. https://doi.org/10.1097/GME.0000000000002085

Miller, M. M., Gould, B. E., & Nelson, J. F. (1989). Aging and long-term ovariectomy alter the cytoarchitecture of the hypothalamic-preoptic area of the C57BL/6J mouse. *Neurobiol Aging*, *10*(6), 683-690. https://doi.org/10.1016/0197-4580(89)90005-5

Mitterling, T., Högl, B., Schönwald, S. V., Hackner, H., Gabelia, D., Biermayr, M., & Frauscher, B. (2015). Sleep and respiration in 100 healthy caucasian sleepers—a polysomnographic study according to American Academy of Sleep Medicine standards. *Sleep*, *38*(6), 867-875. https://doi.org/10.5665/sleep.4730

Mong, J. A., Baker, F. C., Mahoney, M. M., Paul, K. N., Schwartz, M. D., Semba, K., & Silver, R. (2011). Sleep, rhythms, and the endocrine brain: influence of sex and gonadal hormones. *J Neurosci*, *31*(45), 16107-16116. https://doi.org/10.1523/JNEUROSCI.4175-11.2011

Mong, J. A., & Cusmano, D. M. (2016). Sex differences in sleep: impact of biological sex and sex steroids. *Philos Trans R Soc Lond B Biol Sci*, *371*(1688), 20150110. https://doi.org/10.1098/rstb.2015.0110

Mong, J. A., Devidze, N., Frail, D. E., O'Connor, L. T., Samuel, M., Choleris, E., Ogawa, S., & Pfaff, D. W. (2003). Estradiol differentially regulates lipocalin-type prostaglandin D synthase transcript levels in the rodent brain: Evidence from

high-density oligonucleotide arrays and in situ hybridization. *Proc Natl Acad Sci U S A*, *100*(1), 318-323. https://doi.org/10.1073/pnas.262663799

Mong, J. A., Devidze, N., Goodwillie, A., & Pfaff, D. W. (2003). Reduction of lipocalin-type prostaglandin D synthase in the preoptic area of female mice mimics estradiol effects on arousal and sex behavior. *Proc Natl Acad Sci U S A*, *100*(25), 15206-15211. https://doi.org/10.1073/pnas.2436540100

Morin, L. P., Fitzgerald, K. M., & Zucker, I. (1977). Estradiol shortens the period of hamster circadian rhythms. *Science*, *196*(4287), 305-307. https://doi.org/10.1126/science.557840

Motlani, V., Motlani, G., Pamnani, S., Sahu, A., & Acharya, N. (2023). Endocrine Changes in Postmenopausal Women: A Comprehensive View. *Cureus*, *15*(12), e51287. https://doi.org/10.7759/cureus.51287

Munch, M., Knoblauch, V., Blatter, K., Schroder, C., Schnitzler, C., Krauchi, K., Wirz-Justice, A., & Cajochen, C. (2004). The frontal predominance in human EEG delta activity after sleep loss decreases with age. *Eur J Neurosci*, *20*(5), 1402-1410. https://doi.org/10.1111/j.1460-9568.2004.03580.x

Munch, M., Knoblauch, V., Blatter, K., Schroder, C., Schnitzler, C., Krauchi, K., Wirz-Justice, A., & Cajochen, C. (2005). Age-related attenuation of the evening circadian arousal signal in humans. *Neurobiol Aging*, *26*(9), 1307-1319. https://doi.org/10.1016/j.neurobiolaging.2005.03.004

Nakamura, K., & Morrison, S. F. (2008). A thermosensory pathway that controls body temperature. *Nat Neurosci*, *11*(1), 62-71. https://doi.org/10.1038/nn2027

Nakamura, K., & Morrison, S. F. (2010). A thermosensory pathway mediating heat-defense responses. *Proc Natl Acad Sci U S A*, *107*(19), 8848-8853. https://doi.org/10.1073/pnas.0913358107

Nakamura, T. J., Nakamura, W., Yamazaki, S., Kudo, T., Cutler, T., Colwell, C. S., & Block, G. D. (2011). Age-related decline in circadian output. *J Neurosci*, *31*(28), 10201-10205. https://doi.org/10.1523/JNEUROSCI.0451-11.2011

Nakayama, T., Suzuki, M., & Ishizuka, N. (1975). Action of progesterone on preoptic thermosensitive neurones. *Nature*, *258*(5530), 80. https://doi.org/10.1038/258080a0

Neff, L. M., Hoffmann, M. E., Zeiss, D. M., Lowry, K., Edwards, M., Rodriguez, S. M., Wachsberg, K. N., Kushner, R., & Landsberg, L. (2016). Core body temperature is lower in postmenopausal women than premenopausal women: potential implications for energy metabolism and midlife weight gain. *Cardiovasc Endocrinol*, *5*(4), 151-154. https://doi.org/10.1097/XCE.0000000000000078

Neves, V. F., Silva de Sa, M. F., Gallo, L., Jr., Catai, A. M., Martins, L. E., Crescencio, J. C., Perpetuo, N. M., & Silva, E. (2007). Autonomic modulation of heart rate of young and postmenopausal women undergoing estrogen therapy. *Braz J Med Biol Res*, *40*(4), 491-499. https://doi.org/10.1590/s0100-879x2007000400007

Ng, K. Y., Leong, M. K., Liang, H., & Paxinos, G. (2017). Melatonin receptors: distribution in mammalian brain and their respective putative functions. *Brain Struct Funct*, *222*(7), 2921-2939. https://doi.org/10.1007/s00429-017-1439-6

Niethard, N., Ngo, H. V., Ehrlich, I., & Born, J. (2018). Cortical circuit activity underlying sleep slow oscillations and spindles. *Proc Natl Acad Sci U S A*, *115*(39), E9220-E9229. https://doi.org/10.1073/pnas.1805517115

Nisenbaum, M. G., de Melo, N. R., Giribela, C. R., de Morais, T. L., Guerra, G. M., de Angelis, K., Mostarda, C., Baracat, E. C., & Consolim-Colombo, F. M. (2014). Effects of a contraceptive containing drospirenone and ethinyl estradiol on blood pressure and autonomic tone: a prospective controlled clinical trial. *Eur J Obstet Gynecol Reprod Biol*, *175*, 62-66. https://doi.org/10.1016/j.ejogrb.2014.01.006

Nygard, M., Hill, R. H., Wikstrom, M. A., & Kristensson, K. (2005). Age-related changes in electrophysiological properties of the mouse suprachiasmatic nucleus in vitro. *Brain Res Bull*, *65*(2), 149-154. https://doi.org/10.1016/j.brainresbull.2004.12.006

Ohayon, M. M. (2006). Severe hot flashes are associated with chronic insomnia. *Arch Intern Med*, *166*(12), 1262-1268. https://doi.org/10.1001/archinte.166.12.1262

Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep*, *27*(7), 1255-1273. https://doi.org/10.1093/sleep/27.7.1255

Okatani, Y., Morioka, N., & Hayashi, K. (1999). Changes in nocturnal pineal melatonin synthesis during the perimenopausal period: relation to estrogen levels in female rats. *J Pineal Res*, *27*(2), 65-72. https://doi.org/10.1111/j.1600-079x.1999.tb00598.x

Okatani, Y., Morioka, N., & Wakatsuki, A. (2000). Changes in nocturnal melatonin secretion in perimenopausal women: correlation with endogenous estrogen concentrations. *J Pineal Res*, *28*(2), 111-118. https://doi.org/10.1034/j.1600-079x.2001.280207.x

Ozaki, Y., Wurtman, R. J., Alonso, R., & Lynch, H. J. (1978). Melatonin secretion decreases during the proestrous stage of the rat estrous cycle. *Proc Natl Acad Sci U S A*, *75*(1), 531-534. https://doi.org/10.1073/pnas.75.1.531

Paltsev, M. A., Polyakova, V. O., Kvetnoy, I. M., Anderson, G., Kvetnaia, T. V., Linkova, N. S., Paltseva, E. M., Rubino, R., De Cosmo, S., De Cata, A., & Mazzoccoli, G. (2016). Morphofunctional and signaling molecules overlap of the pineal gland and thymus: role and significance in aging. *Oncotarget*, *7*(11), 11972-11983. https://doi.org/10.18632/oncotarget.7863

Pan, Z., Wen, S., Qiao, X., Yang, M., Shen, X., & Xu, L. (2022). Different regimens of menopausal hormone therapy for improving sleep quality: a systematic review and meta-analysis. *Menopause*, *29*(5), 627-635. https://doi.org/10.1097/GME.0000000000001945

Patke, A., Young, M. W., & Axelrod, S. (2020). Molecular mechanisms and physiological importance of circadian rhythms. *Nat Rev Mol Cell Biol*, *21*(2), 67-84. https://doi.org/10.1038/s41580-019-0179-2

Pennestri, M.-H., Whittom, S., Adam, B., Petit, D., Carrier, J., & Montplaisir, J. (2006). PLMS and PLMW in healthy subjects as a function of age: prevalence and interval distribution. *Sleep*, *29*(9), 1183-1187. https://doi.org/10.1093/sleep/29.9.1183

Pevet, P., & Challet, E. (2011). Melatonin: both master clock output and internal time-giver in the circadian clocks network. *J Physiol Paris*, *105*(4-6), 170-182. https://doi.org/10.1016/j.jphysparis.2011.07.001

Plante, D. T., & Goldstein, M. R. (2013). Medroxyprogesterone acetate is associated with increased sleep spindles during non-rapid eye movement sleep in women

referred for polysomnography. *Psychoneuroendocrinology*, *38*(12), 3160-3166. https://doi.org/10.1016/j.psyneuen.2013.08.012

Poniatowski, B. C., Grimm, P., & Cohen, G. (2001). Chemotherapy-induced menopause: a literature review. *Cancer Invest*, *19*(6), 641-648. https://doi.org/10.1081/cnv-100104292

Porkka-Heiskanen, T., Kalinchuk, A., Alanko, L., Huhtaniemi, I., & Stenberg, D. (2004). Orexin A and B levels in the hypothalamus of female rats: the effects of the estrous cycle and age. *Eur J Endocrinol*, *150*(5), 737-742. https://doi.org/10.1530/eje.0.1500737

Randolph, J. F., Jr., Zheng, H., Sowers, M. R., Crandall, C., Crawford, S., Gold, E. B., & Vuga, M. (2011). Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. *J Clin Endocrinol Metab*, *96*(3), 746-754. https://doi.org/10.1210/jc.2010-1746

Refinetti, R. (2020). Circadian rhythmicity of body temperature and metabolism. *Temperature (Austin)*, *7*(4), 321-362. https://doi.org/10.1080/23328940.2020.1743605

Ribeiro, A. C., Pfaff, D. W., & Devidze, N. (2009). Estradiol modulates behavioral arousal and induces changes in gene expression profiles in brain regions involved in the control of vigilance. *Eur J Neurosci*, *29*(4), 795-801. https://doi.org/10.1111/j.1460-9568.2009.06620.x

Roenneberg, T., Foster, R. G., & Klerman, E. B. (2022). The circadian system, sleep, and the health/disease balance: a conceptual review. *J Sleep Res*, *31*(4), e13621. https://doi.org/10.1111/jsr.13621

Roozendaal, B., van Gool, W. A., Swaab, D. F., Hoogendijk, J. E., & Mirmiran, M. (1987). Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. *Brain Res*, *409*(2), 259-264. https://doi.org/10.1016/0006-8993(87)90710-4

Rowley, J. A., & Badr, M. S. (2022). Normal sleep. In *Essentials of Sleep Medicine: A Practical Approach to Patients with Sleep Complaints* (pp. 3-19). Springer.

Roy, D., & Belsham, D. D. (2002). Melatonin receptor activation regulates GnRH gene expression and secretion in GT1-7 GnRH neurons. Signal transduction mechanisms. *J Biol Chem*, *277*(1), 251-258. https://doi.org/10.1074/jbc.M108890200

Salari, N., Hasheminezhad, R., Hosseinian-Far, A., Rasoulpoor, S., Assefi, M., Nankali, S., Nankali, A., & Mohammadi, M. (2023). Global prevalence of sleep disorders during menopause: a meta-analysis. *Sleep Breath*, *27*(5), 1883-1897. https://doi.org/10.1007/s11325-023-02793-5

Saleh, M. C., Connell, B. J., & Saleh, T. M. (2000a). Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. *Brain Res*, *879*(1-2), 105-114. https://doi.org/10.1016/s0006-8993(00)02757-8

Saleh, M. C., Connell, B. J., & Saleh, T. M. (2000b). Medullary and intrathecal injections of 17beta-estradiol in male rats. *Brain Res*, *867*(1-2), 200-209. https://doi.org/10.1016/s0006-8993(00)02313-1

San Martin, M., & Touitou, Y. (2000). Progesterone inhibits, on a circadian basis, the release of melatonin by rat pineal perifusion. *Steroids*, *65*(4), 206-209. https://doi.org/10.1016/s0039-128x(99)00105-1

Santhi, N., Lazar, A. S., McCabe, P. J., Lo, J. C., Groeger, J. A., & Dijk, D. J. (2016). Sex differences in the circadian regulation of sleep and waking cognition in humans. *Proc Natl Acad Sci U S A*, *113*(19), E2730-2739. https://doi.org/10.1073/pnas.1521637113

Santoro, N., Roeca, C., Peters, B. A., & Neal-Perry, G. (2021). The Menopause Transition: Signs, Symptoms, and Management Options. *J Clin Endocrinol Metab*, *106*(1), 1-15. https://doi.org/10.1210/clinem/dgaa764

Saper, C. B., & Fuller, P. M. (2017). Wake-sleep circuitry: an overview. *Curr Opin Neurobiol*, *44*, 186-192. https://doi.org/10.1016/j.conb.2017.03.021

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J., & Scammell, T. E. (2010). Sleep state switching. *Neuron*, *68*(6), 1023-1042. https://doi.org/10.1016/j.neuron.2010.11.032

Saper, C. B., Lu, J., Chou, T. C., & Gooley, J. (2005). The hypothalamic integrator for circadian rhythms. *Trends Neurosci*, *28*(3), 152-157. https://doi.org/10.1016/j.tins.2004.12.009

Saper, C. B., & Machado, N. L. S. (2020). Flipping the switch on the body's thermoregulatory system. *Nature*, *583*(7814), 34-35. https://doi.org/10.1038/d41586-020-01600-5

Saper, C. B., Scammell, T. E., & Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, *437*(7063), 1257-1263. https://doi.org/10.1038/nature04284

Scheer, F. A., Kalsbeek, A., & Buijs, R. M. (2003). Cardiovascular control by the suprachiasmatic nucleus: neural and neuroendocrine mechanisms in human and rat. *Biol Chem*, *384*(5), 697-709. https://doi.org/10.1515/BC.2003.078

Schwarz, J. F. A., Akerstedt, T., Lindberg, E., Gruber, G., Fischer, H., & Theorell-Haglow, J. (2017). Age affects sleep microstructure more than sleep macrostructure. *J Sleep Res*, *26*(3), 277-287. https://doi.org/10.1111/jsr.12478

Shaver, J. L., & Woods, N. F. (2015). Sleep and menopause: a narrative review. *Menopause*, *22*(8), 899-915. https://doi.org/10.1097/GME.0000000000000499

Shaw, L., Taggart, M., & Austin, C. (2001). Effects of the oestrous cycle and gender on acute vasodilatory responses of isolated pressurized rat mesenteric arteries to 17 beta-oestradiol. *Br J Pharmacol*, *132*(5), 1055-1062. https://doi.org/10.1038/sj.bjp.0703908

Shea, S. A., Hilton, M. F., Hu, K., & Scheer, F. A. (2011). Existence of an endogenous circadian blood pressure rhythm in humans that peaks in the evening. *Circ Res*, *108*(8), 980-984. https://doi.org/10.1161/CIRCRESAHA.110.233668

Shechter, A., Boudreau, P., Varin, F., & Boivin, D. B. (2011). Predominance of distal skin temperature changes at sleep onset across menstrual and circadian phases. *J Biol Rhythms*, *26*(3), 260-270. https://doi.org/10.1177/0748730411404677

Shechter, A., Varin, F., & Boivin, D. B. (2010). Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. *Sleep*, *33*(5), 647-656. https://doi.org/10.1093/sleep/33.5.647

Shieu, M. M., Braley, T. J., Becker, J., & Dunietz, G. L. (2023). The Interplay Among Natural Menopause, Insomnia, and Cognitive Health: A Population-Based Study. *Nat Sci Sleep*, *15*, 39-48. https://doi.org/10.2147/NSS.S398019

Shouman, K., & Benarroch, E. E. (2021). Central autonomic network. *Autonomic Nervous System and Sleep: Order and Disorder*, 9-18.

Silva, N. L., & Boulant, J. A. (1986). Effects of testosterone, estradiol, and temperature on neurons in preoptic tissue slices. *Am J Physiol*, *250*(4 Pt 2), R625-632. https://doi.org/10.1152/ajpregu.1986.250.4.R625

Silvani, A. (2019). Sleep disorders, nocturnal blood pressure, and cardiovascular risk: A translational perspective. *Auton Neurosci*, *218*, 31-42. https://doi.org/10.1016/j.autneu.2019.02.006

Silvani, A., Calandra-Buonaura, G., Benarroch, E. E., Dampney, R. A., & Cortelli, P. (2015). Bidirectional interactions between the baroreceptor reflex and arousal: an update. *Sleep Med*, *16*(2), 210-216. https://doi.org/10.1016/j.sleep.2014.10.011

Silveyra, P., Cataldi, N. I., Lux-Lantos, V., & Libertun, C. (2009). Gonadal steroids modulated hypocretin/orexin type-1 receptor expression in a brain region, sex and daytime specific manner. *Regul Pept*, *158*(1-3), 121-126. https://doi.org/10.1016/j.regpep.2009.08.002

Smith, P. C., Phillips, D. J., Viechweg, S. S., Schwartz, M. D., & Mong, J. A. (2020). Estradiol Action at the Median Preoptic Nucleus is Necessary and Sufficient for Sleep Suppression in Female rats. *bioRxiv*.

Song, Z., Jiang, R., Li, C., Jin, F., & Tao, M. (2022). Menopausal Symptoms and Sleep Quality in Women Aged 40-65 Years. *Biomed Res Int*, *2022*, 2560053. https://doi.org/10.1155/2022/2560053

Spary, E. J., Maqbool, A., & Batten, T. F. (2009). Oestrogen receptors in the central nervous system and evidence for their role in the control of cardiovascular function. *J Chem Neuroanat*, *38*(3), 185-196. https://doi.org/10.1016/j.jchemneu.2009.05.008

Stachenfeld, N. S., Silva, C., & Keefe, D. L. (2000). Estrogen modifies the temperature effects of progesterone. *J Appl Physiol (1985)*, *88*(5), 1643-1649. https://doi.org/10.1152/jappl.2000.88.5.1643

Streiner, D. L. (2015). Best (but oft-forgotten) practices: the multiple problems of multiplicity-whether and how to correct for many statistical tests. *Am J Clin Nutr*, *102*(4), 721-728. https://doi.org/10.3945/ajcn.115.113548

Szymusiak, R., Gvilia, I., & McGinty, D. (2007). Hypothalamic control of sleep. *Sleep Med*, *8*(4), 291-301. https://doi.org/10.1016/j.sleep.2007.03.013

Takahashi, J. S. (2017). Transcriptional architecture of the mammalian circadian clock. *Nat Rev Genet*, *18*(3), 164-179. https://doi.org/10.1038/nrg.2016.150

Tan, D. X., Xu, B., Zhou, X., & Reiter, R. J. (2018). Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and Rejuvenation of the Pineal Gland. *Molecules*, *23*(2), 301. https://doi.org/10.3390/molecules23020301

Tegegne, B. S., Man, T., van Roon, A. M., Riese, H., & Snieder, H. (2018). Determinants of heart rate variability in the general population: The Lifelines Cohort Study. *Heart Rhythm*, *15*(10), 1552-1558. https://doi.org/10.1016/j.hrthm.2018.05.006

Teixeira, A. L., Ramos, P. S., Vianna, L. C., & Ricardo, D. R. (2015). Heart rate variability across the menstrual cycle in young women taking oral contraceptives. *Psychophysiology*, *52*(11), 1451-1455. https://doi.org/10.1111/psyp.12510

Tepper, P. G., Randolph, J. F., Jr., McConnell, D. S., Crawford, S. L., El Khoudary, S. R., Joffe, H., Gold, E. B., Zheng, H., Bromberger, J. T., & Sutton-Tyrrell, K. (2012).

Trajectory clustering of estradiol and follicle-stimulating hormone during the menopausal transition among women in the Study of Women's Health across the Nation (SWAN). *J Clin Endocrinol Metab*, *97*(8), 2872-2880. https://doi.org/10.1210/jc.2012-1422

Tobaldini, E., Nobili, L., Strada, S., Casali, K. R., Braghiroli, A., & Montano, N. (2013). Heart rate variability in normal and pathological sleep. *Front Physiol*, *4*, 294. https://doi.org/10.3389/fphys.2013.00294

Toffol, E., Kalleinen, N., Haukka, J., Vakkuri, O., Partonen, T., & Polo-Kantola, P. (2014). The effect of hormone therapy on serum melatonin concentrations in premenopausal and postmenopausal women: a randomized, double-blind, placebo-controlled study. *Maturitas*, *77*(4), 361-369. https://doi.org/10.1016/j.maturitas.2014.01.015

Uchida, Y., Kano, M., Yasuhara, S., Kobayashi, A., Tokizawa, K., & Nagashima, K. (2010). Estrogen modulates central and peripheral responses to cold in female rats. *J Physiol Sci*, *60*(2), 151-160. https://doi.org/10.1007/s12576-009-0079-x

Ueyama, T., Krout, K. E., Nguyen, X. V., Karpitskiy, V., Kollert, A., Mettenleiter, T. C., & Loewy, A. D. (1999). Suprachiasmatic nucleus: a central autonomic clock. *Nat Neurosci*, *2*(12), 1051-1053. https://doi.org/10.1038/15973

Ueyama, T., Tanioku, T., Nuta, J., Kujira, K., Ito, T., Nakai, S., & Tsuruo, Y. (2006). Estrogen alters c-Fos response to immobilization stress in the brain of ovariectomized rats. *Brain Res*, *1084*(1), 67-79. https://doi.org/10.1016/j.brainres.2006.02.008

Vacas, M. I., Lowenstein, P. R., & Cardinali, D. P. (1979). Characterization of a cytosol progesterone receptor in bovine pineal gland. *Neuroendocrinology*, *29*(2), 84-89. https://doi.org/10.1159/000122909

Van Cauter, E., Leproult, R., & Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J Clin Endocrinol Metab*, *81*(7), 2468-2473. https://doi.org/10.1210/jcem.81.7.8675562

van Coevorden, A., Mockel, J., Laurent, E., Kerkhofs, M., L'Hermite-Baleriaux, M., Decoster, C., Neve, P., & Van Cauter, E. (1991). Neuroendocrine rhythms and sleep in aging men. *Am J Physiol*, *260*(4 Pt 1), E651-661. https://doi.org/10.1152/ajpendo.1991.260.4.E651

Van Someren, E. J. (2007). Thermoregulation and aging. *Am J Physiol Regul Integr Comp Physiol*, *292*(1), R99-102. https://doi.org/10.1152/ajpregu.00557.2006

Van Voorhis, B. J., Santoro, N., Harlow, S., Crawford, S. L., & Randolph, J. (2008). The relationship of bleeding patterns to daily reproductive hormones in women approaching menopause. *Obstet Gynecol*, *112*(1), 101-108. https://doi.org/10.1097/AOG.0b013e31817d452b

Velez, M. P., Alvarado, B. E., Rosendaal, N., da Camara, S. M., Belanger, E., Richardson, H., & Pirkle, C. M. (2019). Age at natural menopause and physical functioning in postmenopausal women: the Canadian Longitudinal Study on Aging. *Menopause*, *26*(9), 958-965. https://doi.org/10.1097/GME.0000000000001362

Virtanen, I., Polo-Kantola, P., & Kalleinen, N. (2023). Overnight Heart Rate Variability During Sleep Disturbance In Peri- And Postmenopausal Women. *Behav Sleep Med*, 1-11. https://doi.org/10.1080/15402002.2023.2255329

von Holzen, J. J., Capaldo, G., Wilhelm, M., & Stute, P. (2016). Impact of endo- and exogenous estrogens on heart rate variability in women: a review. *Climacteric*, *19*(3), 222-228. https://doi.org/10.3109/13697137.2016.1145206

Walters, J. F., Hampton, S. M., Ferns, G. A., & Skene, D. J. (2005). Effect of menopause on melatonin and alertness rhythms investigated in constant routine conditions. *Chronobiol Int*, *22*(5), 859-872. https://doi.org/10.1080/07420520500263193

Whitehurst, L. N., Naji, M., & Mednick, S. C. (2018). Comparing the cardiac autonomic activity profile of daytime naps and nighttime sleep. *Neurobiol Sleep Circadian Rhythms*, *5*, 52-57. https://doi.org/10.1016/j.nbscr.2018.03.001

Widder, J., Pelzer, T., von Poser-Klein, C., Hu, K., Jazbutyte, V., Fritzemeier, K. H., Hegele-Hartung, C., Neyses, L., & Bauersachs, J. (2003). Improvement of endothelial dysfunction by selective estrogen receptor-alpha stimulation in ovariectomized SHR. *Hypertension*, *42*(5), 991-996. https://doi.org/10.1161/01.HYP.0000098661.37637.89

Wilczak, A., Marciniak, K., Klapcinski, M., Rydlewska, A., Danel, D., & Jankowska, E. A. (2013). Relations between combined oral contraceptive therapy and indices of autonomic balance (baroreflex sensitivity and heart rate variability) in young healthy women. *Ginekol Pol*, *84*(11), 915-921. https://doi.org/10.17772/gp/1660

Woods, N. F., & Mitchell, E. S. (2010). Sleep symptoms during the menopausal transition and early postmenopause: observations from the Seattle Midlife Women's Health Study. *Sleep*, *33*(4), 539-549. https://doi.org/10.1093/sleep/33.4.539

Wright Jr, K. P., & Badia, P. (1999). Effects of menstrual cycle phase and oral contraceptives on alertness, cognitive performance, and circadian rhythms during sleep deprivation. *Behav Brain Res*, *103*(2), 185-194. https://doi.org/10.1016/s0166-4328(99)00042-x

Wulsin, L. R., Horn, P. S., Perry, J. L., Massaro, J. M., & D'Agostino, R. B. (2015). Autonomic Imbalance as a Predictor of Metabolic Risks, Cardiovascular Disease, Diabetes, and Mortality. *J Clin Endocrinol Metab*, *100*(6), 2443-2448. https://doi.org/10.1210/jc.2015-1748

Xu, M., Belanger, L., Ivers, H., Guay, B., Zhang, J., & Morin, C. M. (2011). Comparison of subjective and objective sleep quality in menopausal and non-menopausal women with insomnia. *Sleep Med*, *12*(1), 65-69. https://doi.org/10.1016/j.sleep.2010.09.003

Xu, Q., & Lang, C. P. (2014). Examining the relationship between subjective sleep disturbance and menopause: a systematic review and meta-analysis. *Menopause*, *21*(12), 1301-1318. https://doi.org/10.1097/GME.0000000000000240

Xue, B., Zhang, Z., Beltz, T. G., Johnson, R. F., Guo, F., Hay, M., & Johnson, A. K. (2013). Estrogen receptor-beta in the paraventricular nucleus and rostroventrolateral medulla plays an essential protective role in aldosterone/salt-induced hypertension in female rats. *Hypertension*, *61*(6), 1255-1262. https://doi.org/10.1161/HYPERTENSIONAHA.111.00903

Yan, L., & Silver, R. (2016). Neuroendocrine underpinnings of sex differences in circadian timing systems. *J Steroid Biochem Mol Biol*, *160*, 118-126. https://doi.org/10.1016/j.jsbmb.2015.10.007

Yang, S. G., Mlcek, M., & Kittnar, O. (2013). Estrogen can modulate menopausal women's heart rate variability. *Physiol Res*, *62*(Suppl 1), S165-171. https://doi.org/10.33549/physiolres.932612

Yao, Y., & Silver, R. (2022). Mutual Shaping of Circadian Body-Wide Synchronization by the Suprachiasmatic Nucleus and Circulating Steroids. *Front Behav Neurosci*, *16*, 877256. https://doi.org/10.3389/fnbeh.2022.877256

Yin, W., Borniger, J. C., Wang, X., Maguire, S. M., Munselle, M. L., Bezner, K. S., Tesfamariam, H. M., Garcia, A. N., Hofmann, H. A., Nelson, R. J., & Gore, A. C. (2019). Estradiol treatment improves biological rhythms in a preclinical rat model of menopause. *Neurobiol Aging*, *83*, 1-10. https://doi.org/10.1016/j.neurobiolaging.2019.08.029

Yoon, I. Y., Kripke, D. F., Elliott, J. A., Youngstedt, S. D., Rex, K. M., & Hauger, R. L. (2003). Age-related changes of circadian rhythms and sleep-wake cycles. *J Am Geriatr Soc*, *51*(8), 1085-1091. https://doi.org/10.1046/j.1532-5415.2003.51356.x

Young, T., Rabago, D., Zgierska, A., Austin, D., & Laurel, F. (2003). Objective and subjective sleep quality in premenopausal, perimenopausal, and postmenopausal women in the Wisconsin Sleep Cohort Study. *Sleep*, *26*(6), 667-672. https://doi.org/10.1093/sleep/26.6.667

Youngstedt, S. D., Elliott, J. A., & Kripke, D. F. (2019). Human circadian phase-response curves for exercise. *J Physiol*, *597*(8), 2253-2268. https://doi.org/10.1113/JP276943

Zeitzer, J. M., Daniels, J. E., Duffy, J. F., Klerman, E. B., Shanahan, T. L., Dijk, D. J., & Czeisler, C. A. (1999). Do plasma melatonin concentrations decline with age? *Am J Med*, *107*(5), 432-436. https://doi.org/10.1016/s0002-9343(99)00266-1

Zhang, X., Liu, X., Wang, Y., Liu, C., Zhang, N., Lu, J., & Lv, Y. (2022). Exploration of cortical inhibition and habituation in insomnia: Based on CNV and EEG. *Methods*, *204*, 73-83. https://doi.org/10.1016/j.ymeth.2022.01.012

Zhang, Z. (2019). Spectral and time-frequency analysis. *EEG Signal Processing and feature extraction*, 89-116.

Zhang, Z., DiVittorio, J. R., Joseph, A. M., & Correa, S. M. (2021). The Effects of Estrogens on Neural Circuits That Control Temperature. *Endocrinology*, *162*(8). https://doi.org/10.1210/endocr/bqab087

Zhang, Z., Reis, F., He, Y., Park, J. W., DiVittorio, J. R., Sivakumar, N., van Veen, J. E., Maesta-Pereira, S., Shum, M., Nichols, I., Massa, M. G., Anderson, S., Paul, K., Liesa, M., Ajijola, O. A., Xu, Y., Adhikari, A., & Correa, S. M. (2020). Estrogen-sensitive medial preoptic area neurons coordinate torpor in mice. *Nat Commun*, *11*(1), 6378. https://doi.org/10.1038/s41467-020-20050-1

Zhao, W., & Jiang, B. (2023). Heart rate variability in patients with insomnia disorder: a systematic review and meta-analysis. *Sleep Breath*, *27*(4), 1309-1313. https://doi.org/10.1007/s11325-022-02720-0

Zolfaghari, S., Yao, C., Thompson, C., Gosselin, N., Desautels, A., Dang-Vu, T. T., Postuma, R. B., & Carrier, J. (2020). Effects of menopause on sleep quality and sleep disorders: Canadian Longitudinal Study on Aging. *Menopause*, *27*(3), 295-304. https://doi.org/10.1097/GME.0000000000001462