Sleep Medicine 81 (2021) 52–61

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

Nighttime melatonin secretion and sleep architecture: different associations in perimenopausal and postmenopausal women☆

Elena Toffol [a, *], Nea Kalleinen [b, c], Sari-Leena Himanen [d, e], Timo Partonen [f], Jari Haukka [a, d], Päivi Polo-Kantola [c, g]

[a] Department of Public Health, Clinicum, Faculty of Medicine, University of Helsinki, Helsinki, Finland
[b] Heart Center, Turku University Hospital and University of Turku, Turku, Finland
[c] Sleep Research Center, Department of Pulmonary Diseases and Clinical Allergology, University of Turku, Turku, Finland
[d] Faculty of Medicine and Health Technology, Tampere University, Finland
[e] Department of Clinical Neurophysiology, Tampere University Hospital, Tampere, Finland
[f] Mental Health Unit, Department of Public Health Solutions, Finnish Institute for Health and Welfare, Helsinki, Finland
[g] Department of Obstetrics and Gynecology, Turku University Hospital and Turku University, Turku, Finland

ARTICLE INFO

Article history:
Received 31 August 2020
Received in revised form
1 February 2021
Accepted 2 February 2021
Available online 9 February 2021

Keywords:
Perimenopause
Postmenopause
Women
Sleep
Sleep stage
Melatonin

ABSTRACT

Background: Sleep quality typically decreases after menopause, but the underlying mechanisms are poorly understood. Concentrations of melatonin are lower and its secretion profiles different before and after menopause. However, whether and how melatonin and sleep architecture are associated in women of different reproductive states have not been examined to date.
Methods: Overnight serum melatonin samples were taken from 17 perimenopausal and 18 postmenopausal healthy women. Sleep quality was measured with all-night polysomnography recordings.
Results: Melatonin concentrations tended to be the lowest during NREM sleep, and were associated with higher odds of transitions from wake to NREM sleep. The curves of predicted overnight melatonin values from linear mixed models varied according to sleep phases (NREM, REM, Wake) in perimenopausal, but not in postmenopausal women. In perimenopause higher melatonin area under curve (AUC) correlated with higher slow-wave activity ($p = 0.043$), and higher minimum concentrations with shorter slow-wave sleep (SWS) latency ($p = 0.029$). In postmenopause higher mean and maximum melatonin concentrations and AUC correlated with lower SWS percentage ($p = 0.044$, $p = 0.029$, $p = 0.032$), and higher mean ($p = 0.032$), maximum ($p = 0.032$) and minimum ($p = 0.037$) concentrations with more awakenings from REM sleep. In the age- and BMI- adjusted regression models, the association between higher maximum ($p = 0.046$) melatonin concentration and lower SWS percentage remained.
Conclusions: The relationship between melatonin and sleep architecture differed in perimenopausal and postmenopausal women. After menopause, high melatonin concentrations were associated with worse sleep. Whether these different patterns are related to aging of the reproductive system, and to decrease in menopausal sleep quality, remains to be elucidated.

© 2021 Elsevier B.V. All rights reserved.

1. Introduction

The decrease in sleep quality during the menopausal transition, once the production of endogenous estrogens has stopped, is well established [1–3]. This evidence is further supported by the beneficial effects of replacing the lack of estrogens with menopausal hormone therapy (MHT), which is shown to abolish or at least alleviate sleep disturbances [4–6]. One of the most important underlying causes are the climacteric vasomotor symptoms, such as nocturnal hot flushes and sweating [5,7], symptoms which are significantly reduced with MHT. However, although a proportion of women with menopausal sleep disturbances do not report vasomotor symptoms, they similarly seem to benefit from MHT [4].

To date, sleep studies using polysomnography (PSG) and conventionally evaluating sleep time, sleep efficiency, sleep stage distributions and number of awakenings, have failed to explain the

☆ The study was registered as a European Research Project: Sleep disorders in menopausal and postmenopausal ageing women: prevalence, biological mechanisms, animal models, social aspects, treatment and prevention, https://cordis.europa.eu/project/id/QLK6-CT-2000-00499 (QLK6-CT-2000-00499).

* Corresponding author. Department of Public Health, Clinicum, University of Helsinki, Po Box 20 (Tukholmankatu 8 B), 00014, Helsinki, Finland.
E-mail address: elena.toffol@helsinki.fi (E. Toffol).

https://doi.org/10.1016/j.sleep.2021.02.011
1389-9457/© 2021 Elsevier B.V. All rights reserved.

mechanisms behind the increase of menopausal sleep disturbances [5,8]. Most of these studies have found worse self-reported sleep quality, but none or only minimal alterations in sleep architecture [3,5,9–14]. Rather, some authors have found a better objectively measured sleep quality and longer sleep duration in postmenopausal than in premenopausal women [10,15,16]. Because sleep is regulated via complex neurochemical systems including circadian rhythm regulators and hormones, it is not surprising that the underlying factors causing subjective and objective sleep disturbances in postmenopausal women are still not clear. One of the likely regulators of sleep is melatonin. Because melatonin production and secretion profiles are mainly regulated by the circadian cycle, and modified by the light–dark cycle, the plasma levels are at their lowest during the day, start to rise in the evening and peak at night. Specifically, a morning oscillator regulates melatonin offset time, and an evening oscillator its onset time, with sleep onset normally occurring approximately two hours after melatonin onset. Thus, melatonin is one of the best indicators of circadian rhythms in humans, including the sleep-wake cycle, and specifically regulates the timing of sleep.

Alterations in melatonin levels and secretion profile have been suggested as a possible underlying mechanism in the sleep disruption typical of many conditions, such as the delayed and advanced sleep phase syndromes, sleep disturbances related to jet lag and shift work, and, most likely, the sleep alterations typically found in the elderly [17]. Individuals with low endogenous melatonin secretion had longer sleep onset latency and REM latency, as well as lower sleep efficiency and REM percentage than normal secretors [18].

In 1982, Birkeland analyzed the secretion profile of nighttime melatonin in seven healthy men in relation to their sleep stages and found that melatonin levels were the highest during wake and the lowest during REM sleep [19]. Results of later studies are, however, inconsistent, showing either no associations, or highest and lowest melatonin levels in NREM and REM sleep, respectively [20–22]. Importantly, all these previous studies were conducted in men of different age groups. To the best of our knowledge, to date (PubMed search on 29 June 2020) no study has specifically examined the associations between melatonin secretion and sleep architecture, and the associations between melatonin, sleep stages and sleep stage transitions in women, and specifically in women of different reproductive states.

In this study we aimed to examine whether melatonin concentrations and secretion profile are related to sleep architecture, and whether melatonin concentrations are associated to the direction of sleep stage transitions. Because we have previously shown that melatonin concentrations were lower and the secretion profiles different in postmenopause than in perimenopause [23], we hypothesized that the associations, if any, are different between perimenopausal and postmenopausal women. We also hypothesized that these changes in melatonin secretion after the menopause are associated with worse sleep structure.

2. Methods

2.1. Subjects

This work was part of a larger study conducted at the Turku University Hospital and at the University of Turku (Finland), aimed to evaluate the effects of menopause on sleep and cognition. Altogether 17 perimenopausal (aged 43–51 years) and 18 postmenopausal (aged 58–71 years) women were recruited through advertisements in the local newspapers in the area of Turku. Perimenopausal status was defined by the serum follicle stimulating hormone (FSH) level (<23 IU/ml) and an ongoing regular or irregular menstrual cycle. Postmenopause was defined by age (≥58 years) and at least 12 months of amenorrhea.

Women experiencing a mental, cardiovascular (with the exception of drug-treated balanced hypertension), endocrine (with the exception of drug-treated balanced hyperlipidemia), pulmonary, neurological or specific sleep disorder (eg sleep apnea, narcolepsy or restless leg syndrome), or malignancies were excluded from the study. Additional exclusion criteria included alcohol abuse, smoking, excessive caffeine intake (>5 cups per day), use of other substances known to affect the central nervous system and other conditions possibly affecting sleep (eg fibromyalgia and anemia). All women had normal levels of blood hemoglobin, leukocytes, thrombocytes and serum thyrotropin. A washout period of at least 12 months was required for women who had previously used MHT (one perimenopausal and 13 postmenopausal women). Six of the perimenopausal and three of the postmenopausal women reported using Nonsteroidal anti-inflammatory drugs (NSAIDs); moreover, one perimenopausal and three postmenopausal women used beta-blockers. More details about the data collection and study design have already been described elsewhere [11]. After receiving oral and written information, all women gave written informed consent. The study was approved by the Ethics Committees of Turku University Hospital and of University of Turku, Finland.

All the participants kept a sleep diary during the three weeks before and one week after the study to verify their sleep-wake rhythms. All women had regular sleep-wake schedules (from 22:00–23:00 h to 06:00–07:00 h). One week before and during the study women were not allowed to travel abroad, as well as to use alcohol and caffeine. Coffee-drinkers were provided with decaffeinated beverages. The women spent three consecutive nights in the sleep laboratory: one adaptation night, the second night as a baseline night and the third night with repeated blood sampling. Every evening they arrived to the sleep laboratory at 19:30 h and the all-night polysomnography (PSG) recording was performed from 23:00 (lights-off) to 07:00 (lights-on). During the nights only red light was allowed for illumination if needed. Therefore, during the study period the women spent their time inside a building, in a dark room without windows, with strictly controlled nighttime illumination levels; this protocol limited the possible influence of different photoperiods in different subjects. The study was performed by similar timetable in all subjects and food was provided by the sleep laboratory. One perimenopausal woman was not willing to continue the study after the first night, and thus she was excluded from the current study.

2.2. Sleep architecture

The all-night PSG recordings consisted of continuous monitoring via electroencephalograms (EEG; channels C3/A2, C4/A1, O1/A2 and O2/A2), electro-oculograms (EOG), a mandibular electromyogram (EMG) and an electrocardiogram (ECG, Embla®, Medcare Flaga hf. Medical devices, Reykjavik, Iceland). Sleep stages were visually scored in 30-s epochs according to conventional criteria valid during the data collection (years 2001–2004) [24] by the same scorer (NK) and controlled by a senior scorer (PP–K). For the purpose of this study, three groups of sleep variables were considered as follows.

First, general sleep: sleep latency (period from lights-off to sleep onset, defined as the appearance of three consecutive epochs of S1 or the first epoch of any other stage); total sleep time (sum of time spent in Stage 1 (S1), Stage 2 (S2), SWS, REM sleep and movement time [MT]); sleep efficiency (the percentage of total sleep time out of time in bed); number of arousals (EEG α-activity for at least three seconds) [25]; total number of

awakenings (the number of times entering wake stage from each sleep stage); wake time after sleep onset (WASO) (the percentage of total time in bed, from lights-off to lights-on); and number of sleep stage transitions.

Second, NREM sleep: S1 percentage (the percentage of total time in bed, from lights-off to lights-on); S1 awakenings (the number of times entering wake stage from S1 sleep); S2 percentage (the percentage of total time in bed, from lights-off to lights-on); S2 awakenings (the number of times entering wake stage from S2 sleep); SWS latency (the time from sleep onset to the first 30 s of the SWS); SWS percentage (the percentage of total time in bed, from lights-off to lights-on); SWS awakenings (the number of times entering wake stage from SWS); and total slow wave activity in NREM-sleep (SWA, 0.75–4 Hz) [11].

Third, REM sleep: REM latency (the time from sleep onset to the first 30 s of the REM stage); REM percentage (the percentage of total time in bed, from lights-off to lights-on); and REM awakenings (the number of times entering wake stage from REM sleep).

2.3. Blood samples

On the evening before the third night an indwelling catheter was inserted into a forearm vein to permit a 24-h blood sampling at 20-min intervals, starting from 21:00 h. At night (from 21:00 h to 07:00 h) the catheter was connected to a plastic tube extending into an adjacent room, allowing repeated blood sampling without disturbing the woman's sleep. The catheter was kept patent with a slow heparinized saline infusion. Melatonin measurements were available for 20-min interval samples between 21:00 and midnight, and from 06:00 to 09:00; measurements on one-hour interval samples were available between midnight and 06:00. All perimenopausal women were examined in the beginning of their menstrual cycle (in the follicular phase). The blood samples were collected all throughout the year; in detail, 12 of the 16 perimenopausal women, and 10 of the 18 postmenopausal women were assessed during winter time (October to March).

The blood samples were drawn into EDTA tubes and placed in the refrigerator for 20 min. Thereafter, they were centrifuged to separate serum, which was frozen at 70 °C until assayed. For melatonin analyses the serum samples were first extracted with chloroform and then assayed by radioimmunoassay with an iodinated melatonin tracer and a melatonin-specific antiserum [26]. The lowest detectable concentration by the method was 1.3 pg/ml (5.7 pmol/l), and the intra-assay and inter-assay coefficients of variation were from 6.7 to 9.5% and from 9.8 to 12.5%, respectively.

The following five groups of melatonin indicators, two of concentration (#1-#2) and three of secretion (#3-#5), were derived: 1) the mean and median nighttime serum melatonin concentrations between lights-off (at 23:00 h) and lights-on (at 07:00 h); 2) the maximum and minimum levels of nighttime serum melatonin concentrations (between lights-off and lights-on); 3) the nighttime melatonin exposure level: after the interpolation of melatonin exposure level curve, the area under melatonin exposure curve (AUC, from lights-off to lights-on) was calculated for each individual; 4) the duration of nighttime melatonin secretion: the total amount of time (in hours) when serum melatonin levels (circulating melatonin) were $\geq$10 pg/ml, where 10 pg/ml is the usual threshold for melatonin onset; 5) the time from lights-off to melatonin peak time (in hours).

2.4. Questionnaire variables

The level of self-reported sleep disturbances during the previous three months was evaluated with the Basic Nordic Sleep Questionnaire (BNSQ) [27], and sum-scores for insomnia (difficulty falling asleep, nocturnal awakenings + their nocturnal frequency, early morning awakenings and sleep quality in the past three months, with the range of 5–25) were calculated accordingly, with a lower score indicating better sleep. Climacteric vasomotor symptoms during the previous six months were assessed with two questions inquiring into nocturnal hot flashes and sweating. The frequency of both symptoms (sum score, range 2–8) was determined on the basis of the following four-point scale: one ("seldom or never"), two ("approximately once a month"), three ("approximately once a week"), four ("almost every day"). The presence of depressive symptoms during the previous four weeks was assessed via the 21-item Beck Depression Inventory (BDI) [28], a sum score, with the range of 0–63.

2.5. Statistical analysis

The distribution of variables was tested via visual inspection of histograms and Shapiro–Wilk test.

A model with three sleep phases (wake, NREM, REM) was identified via multi-state models as the best fitting model for analyzing the PSG data. For the following analyses, only melatonin measurements between the first sleep onset after lights-off until lights-on were used. Median melatonin levels in each sleep phase were compared via Wilcoxon-rank-sum test in the whole sample, and separately in perimenopausal and postmenopausal women. By using linear mixed model regression analyses, we tested associations between repeated measurements of melatonin levels during sleep (between the first sleep onset after lights-off, until lights-on) and sleep phases (wake, NREM, REM). In these analyses, repeated melatonin measurements were used as the outcome variable, the individual as a random effect, and sleep phase and time of melatonin measurement as fixed explanatory variables. Interaction between time and sleep phases was tested using log-likelihood test. According to the best fitting model based on Akaike Information Criterion, measurement time was entered both as linear and quadratic terms, reflecting the melatonin secretion curve. Analyses were carried out separately in perimenopausal and postmenopausal women. In addition to the unadjusted model (Model 1), three multivariable models were performed to control for the effect of age and body mass index (BMI, kg/m^2) (Model 2), age, BMI and climacteric symptom score (Model 3), and age, BMI and BDI score (Model 4). Multi-state models with sleep phases (wake, NREM, REM) and melatonin concentrations (at each measurement), reproductive status (postmenopausal *vs.* perimenopausal), and time-dependent intensity for transitions as covariates were additionally performed to test the predictive values of covariates on sleep phases [29].

Pearson and Spearman analyses, as appropriate, were performed to examine the correlations between nighttime serum melatonin concentrations (mean, median, minimum and maximum), melatonin exposure level (AUC), duration of nighttime melatonin secretion and the time from lights-off to melatonin peak, *vs.* sleep variables, separately in perimenopausal and postmenopausal women. Univariable (Model 1) and progressively adjusted multivariable generalized linear models (Model 2: controlled for age and BMI; Model 3: controlled for age, BMI and climacteric vasomotor symptom score; Model 4: controlled for age, BMI and BDI score) were performed, separately in perimenopausal and postmenopausal women, to test the associations between melatonin and sleep variables.

The statistical analyses were performed using the R program (version 3.5.2) [30]. For all the analyses, the two-tailed *p*-values of <0.05 were considered statistically significant.

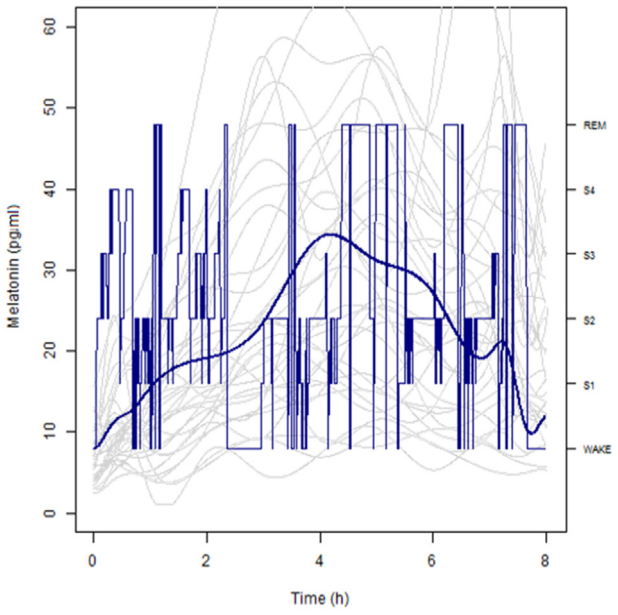

Fig. 1. An individual curve of sleep stages and melatonin levels (blue curves). Gray curves are melatonin level curves for each individual.

3. Results

Postmenopausal women had higher BNSQ insomnia score (mean = 16.0, SD = 4.0) and BDI score (mean = 6.3, SD = 4.9) compared to perimenopausal women (mean = 13.4, SD = 3.4, $p = 0.046$; and mean = 3.6, SD = 2.9, $p = 0.032$, respectively), showing a higher level of self-reported sleep disturbances and of depressive symptoms. Postmenopausal women had also higher BMI (mean = 27.6, SD = 5.0) than the perimenopausal ones (mean = 24.3, SD = 2.4, $p = 0.015$). However, the two groups did not differ with respect to their levels of climacteric symptoms (perimenopause: mean = 2.6, SD = 0.8; postmenopause: mean = 4.2, SD = 2.5, $p = 0.120$). Postmenopausal women had lower levels of mean melatonin (from lights off to light on; mean = 17.0, SD = 6.8) compared to those in perimenopause (mean = 24.0, SD = 10.0, $p = 0.026$).

3.1. Melatonin and sleep phases

Fig. 1 illustrates one individual example curve of overnight melatonin concentrations in relation to sleep stages. Median melatonin concentrations and interquartile ranges during each sleep phase (wake, NREM and REM) are reported in Table 1. Both in perimenopausal and in postmenopausal women, melatonin concentrations were the lowest during the NREM sleep, although the difference was not significant.

Results of linear mixed models showed that in perimenopausal women overnight melatonin concentrations tended to be lower during the NREM sleep compared to the wake phase ($p = 0.074$). The interaction between time and NREM sleep in predicting melatonin concentrations showed that the rate of linear change in melatonin concentrations during the night was faster, and the quadratic change slower during NREM sleep than during the wake phase ($\beta = 0.45$; 95% CI = 0.07 to 0.84; $p = 0.019$; $\beta = -0.05$; 95% CI = −0.09 to −0.003; $p = 0.038$). The results did not change after controlling for covariates (age, BMI, climacteric vasomotor symptom score and BDI score). No associations between melatonin concentrations and sleep phases were found in postmenopause (Table 2). Accordingly, the curves of predicted values of overnight melatonin concentrations varied according to sleep phases in perimenopausal, but not in postmenopausal women (Fig. 2). Predicted values for each woman are illustrated in Figs. S1 and S2.

The total number of sleep stage transitions is reported in Table 3. In multi-state models, higher point concentrations of melatonin were associated with higher odds of transitions from wake to NREM sleep. Postmenopausal state was associated with higher odds of transitions from wake to NREM, NREM to wake, and REM to NREM (Table 3).

3.2. Melatonin and sleep variables

In perimenopause higher minimum melatonin concentrations related to shorter SWS latency ($\rho = -0.55$, $p = 0.029$), and higher melatonin AUC related to higher amount of SWA ($r = 0.51$, $p = 0.043$). In postmenopausal women higher melatonin mean and maximum concentrations and AUC correlated with lower SWS percentage ($r = -0.48$, $p = 0.044$; $r = -0.52$, $p = 0.029$; and $r = -0.51$, $p = 0.032$, respectively). Additionally, higher mean, median, minimum and maximum melatonin concentrations correlated with more REM awakenings ($\rho = 0.51$, $p = 0.032$; $\rho = 0.52$, $p = 0.026$; $\rho = 0.50$, $p = 0.037$; and $\rho = 0.51$, $p = 0.032$, respectively) (Fig. 3, Fig. 4 and Fig. S3).

In generalized linear models no associations were found between melatonin peak time, melatonin AUC or duration of melatonin secretion and sleep variables, neither in perimenopausal nor in postmenopausal women. However, in postmenopausal women only, higher mean and maximum melatonin concentrations were (or tended to be) associated with lower SWS percentage in univariable ($\beta = -0.63$, 95% CI = −1.20 to −0.07, $p = 0.044$; and $\beta = -1.19$, 95% CI = −2.15 to −0.22, $p = 0.029$) and age- and BMI- adjusted models ($\beta = -0.62$, 95% CI = −1.25 to 0.001, $p = 0.071$; and $\beta = -1.18$, 95% CI = −2.23 to −0.13, $p = 0.046$) (Fig. 4). The associations were lost when further controlling for climacteric vasomotor symptom score ($p = 0.134$ and $p = 0.113$, respectively), or BDI score ($p = 0.143$, and $p = 0.084$, respectively).

Table 1
Median melatonin levels (pg/mL) and interquartile ranges (IQRs) across sleep phases.

	wake		NREM		REM		p-value[b]
	n[a]	median (IQR)	n[a]	median (IQR)	n[a]	median (IQR)	
All women	120	19.15 (12.10, 28.80)	231	18.40 (11.10, 30.25)	47	20.20 (14.00, 39.10)	0.314
Perimenopause	50	25.30 (15.10, 35.70)	115	21.60 (13.10, 37.55)	21	24.00 (14.30, 40.00)	0.812
Postmenopause	70	16.50 (11.70, 23.50)	116	13.80 (10.10, 24.80)	26	16.75 (13.87, 31.72)	0.123

NREM sleep phase includes stage 1, stage 2 and slow wave sleep.
[a] n refers to the total number of available melatonin observations for each sleep phase.
[b] p-value from Wilcoxon test.

Table 2
Linear mixed model analyses of associations between repeated measures of melatonin concentrations and wake (as reference), NREM and REM sleep phases.

	Model 1			Model 2			Model 3			Model 4		
	B	95% CI	*p*-value	β	95% CI	*p*-value	β	95% CI	*p*-value	β	95% CI	*p*-value
Perimenopausal women												
NREM sleep	−0.63	−1.33 to 0.06	0.074	−0.65	−1.34 to 0.05	0.069	−0.64	−1.34 to 0.06	0.072	−0.61	−1.31 to 0.09	0.088
REM sleep	−0.64	−2.17 to 0.89	0.41	−0.70	−2.23 to 0.83	0.37	−0.69	−2.22 to 0.84	0.38	−0.62	−2.16 to 0.92	0.43
Sampling time	0.40	0.07 to 0.73	0.018	0.40	0.06 to 0.73	0.020	0.40	0.07 to 0.73	0.019	0.41	0.08 to 0.74	0.016
Sampling time^2 (quadratic term)	−0.04	−0.08 to −0.004	0.029	−0.04	−0.08 to −0.004	0.031	−0.04	−0.08 to −0.004	0.030	−0.04	−0.08 to −0.01	0.026
NREM sleep * time interaction	0.45	0.07 to 0.84	0.019	0.46	0.08 to 0.84	0.018	0.46	0.08 to 0.84	0.018	0.44	0.06 to 0.83	0.023
REM sleep * time interaction	0.63	−0.15 to 1.41	0.11	0.66	−0.13 to 1.44	0.10	0.65	−0.13 to 1.44	0.10	0.62	−0.17 to 1.41	0.12
NREM sleep * time^2 interaction	−0.05	−0.09 to −0.003	0.038	−0.05	−0.09 to −0.003	0.035	−0.05	−0.09 to −0.003	0.036	−0.04	−0.09 to −0.001	0.044
REM sleep * time^2 interaction	−0.07	−0.16 to 0.02	0.12	−0.07	−0.16 to 0.02	0.11	−0.07	−0.16 to 0.02	0.11	−0.07	−0.16 to 0.02	0.12
Postmenopausal women												
NREM sleep	−0.04	−0.47 to 0.38	0.84	−0.04	−0.46 to 0.39	0.87	−0.03	−0.46 to 0.40	0.89	−0.03	−0.45 to 0.39	0.89
REM sleep	−0.87	−1.98 to 0.25	0.13	−0.86	−1.97 to 0.26	0.13	−0.84	−1.95 to 0.27	0.14	−0.92	−2.03 to 0.20	0.11
Sampling time	0.63	0.44 to 0.82	<0.001	0.63	0.44 to 0.82	<0.001	0.63	0.44 to 0.82	<0.001	0.63	0.44 to 0.82	<0.001
Sampling time^2 (quadratic term)	−0.07	−0.09 to −0.05	<0.001	−0.07	−0.09 to −0.05	<0.001	−0.07	−0.09 to −0.05	<0.001	−0.07	−0.09 to −0.05	<0.001
NREM sleep * time interaction	0.01	−0.22 to 0.24	0.95	0.002	−0.23 to 0.23	0.99	0.004	−0.23 to 0.24	0.98	0.002	−0.23 to 0.23	0.98
REM sleep * time interaction	0.38	−0.16 to 0.91	0.17	0.37	−0.16 to 0.90	0.17	0.37	−0.16 to 0.90	0.17	0.40	−0.14 to 0.93	0.15
NREM sleep * time^2 interaction	0.001	−0.03 to 0.03	0.91	0.002	−0.02 to 0.03	0.87	0.002	−0.02 to 0.03	0.89	0.002	−0.02 to 0.03	0.88
REM sleep * time^2 interaction	−0.03	−0.09 to 0.02	0.26	−0.03	−0.09 to 0.02	0.27	−0.03	−0.09 to 0.03	0.27	−0.03	−0.09 to 0.02	0.24

NREM sleep stage includes stage 1, stage 2 and slow wave sleep.
Model 1: a mixed linear model with sleep stage as predictor, sampling time (linear and quadratic terms) as fixed effect, and individual as the random effect.
Model 2: is Model 1 adjusted for age and BMI.
Model 3: is Model 1 adjusted for age, BMI and climacteric vasomotor symptom score.
Model 4 is Model 1 adjusted for age, BMI and BDI score.
Regression coefficients (β) indicate one SD unit change in melatonin concentrations per each sleep stage category (wake as the reference category), or per one unit change in continuous independent variables. 95% CI refers to 95% confidence interval.

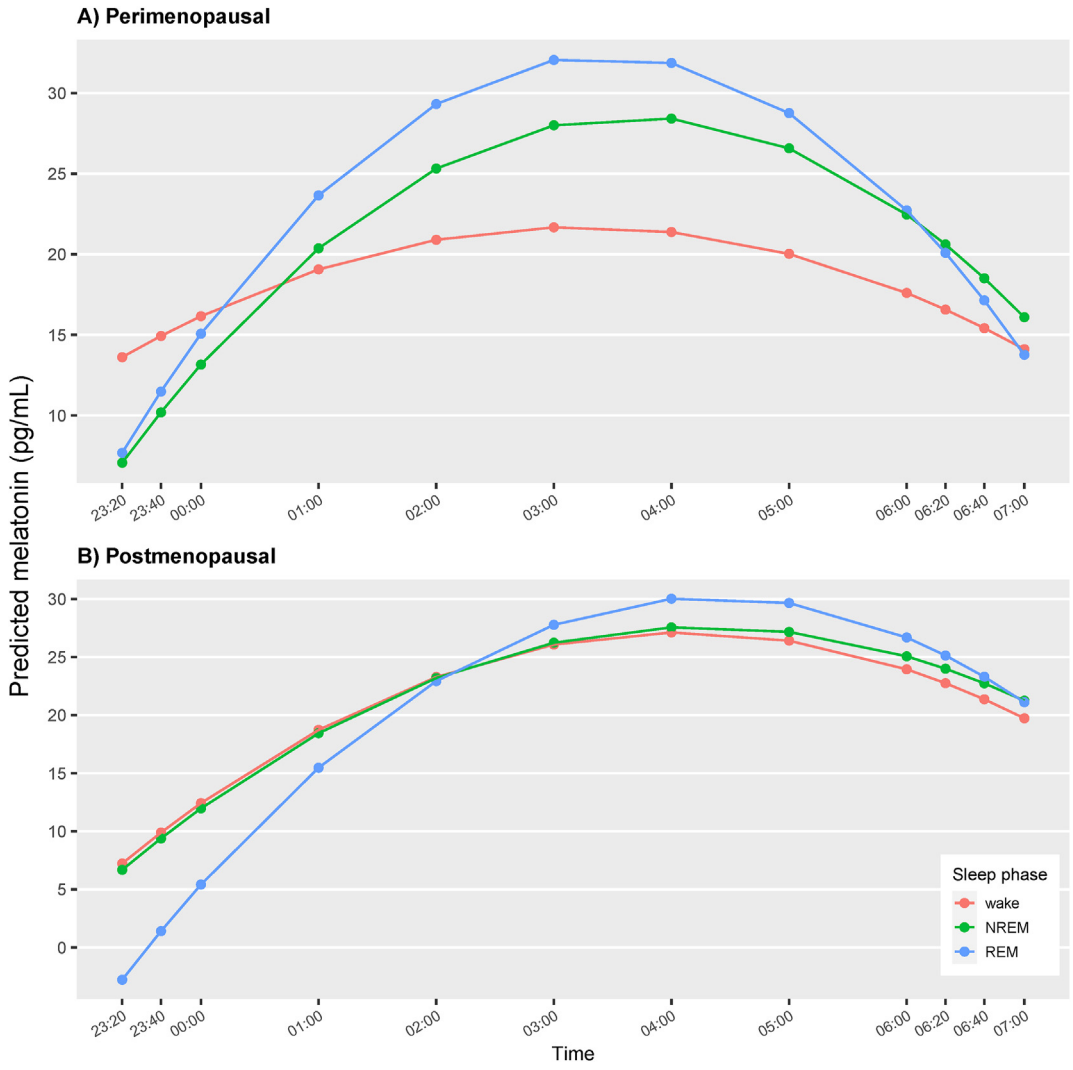

Fig. 2. Curve of predicted values of melatonin by sleep phases (wake, NREM, REM) in one perimenopausal (panel A) and postmenopausal (panel B) woman. Predicted values are from unadjusted linear mixed models.

Table 3
Associations between sleep phase transitions, time-point melatonin concentrations and postmenopausal *vs.* perimenopausal state.

Sleep phase transition	Number of transitions	Melatonin (pg/mL)		Postmenopausal *vs.* Perimenopausal	
		Hazard ratio	95% CI	Hazard ratio	95% CI
Wake → NREM	754	1.02	1.01 to 1.02	1.33	1.14 to 1.55
Wake → REM	51	1.02	1.00 to 1.05	1.48	0.73 to 3.01
NREM → Wake	653	1.00	1.00 to 1.00	1.28	1.08 to 1.51
NREM → REM	343	1.00	1.00 to 1.01	1.13	0.90 to 1.42
REM → Wake	142	1.01	0.99 to 1.02	1.01	0.70 to 1.44
REM → NREM	250	1.00	0.99 to 1.00	1.34	1.02 to 1.77

NREM sleep stage includes stage 1, stage 2 and slow wave sleep.

4. Discussion

We found associations between levels of melatonin and the NREM sleep, as well as different predicted curves of melatonin levels across sleep phases, only in perimenopausal women. In addition, in perimenopause indicators of high melatonin levels were related to better NREM sleep (shorter SWS latency and higher amount of SWA), whereas in postmenopause markers suggestive of higher melatonin levels were related to more disrupted NREM and REM sleep, as indicated by a lower SWS percentage and more awakenings from REM sleep, respectively. Consistently, postmenopausal status, but not melatonin concentrations, was a stronger predictor of sleep phase transitions.

This study is the first to assess the connections of nighttime melatonin concentrations and secretion with sleep architecture in women of two different reproductive phases. Even if the difference did not reach the significance level, median melatonin concentrations were the lowest during the NREM sleep, both in perimenopausal and in postmenopausal women. In addition, in perimenopausal women the NREM sleep tended to be associated

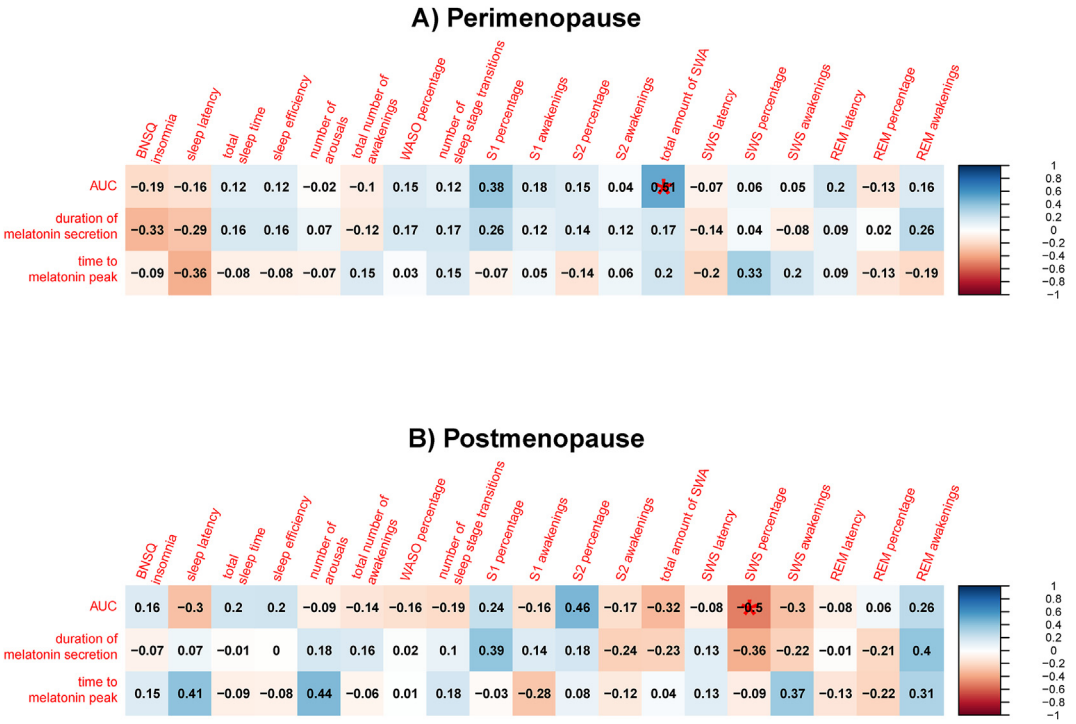

Fig. 3. Correlations between sleep variables and melatonin secretion profile in perimenopausal (A) and postmenopausal (B) women. Numbers indicate correlation coefficients. Significant correlations are marked in red (*$p = 0.043$ for both). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

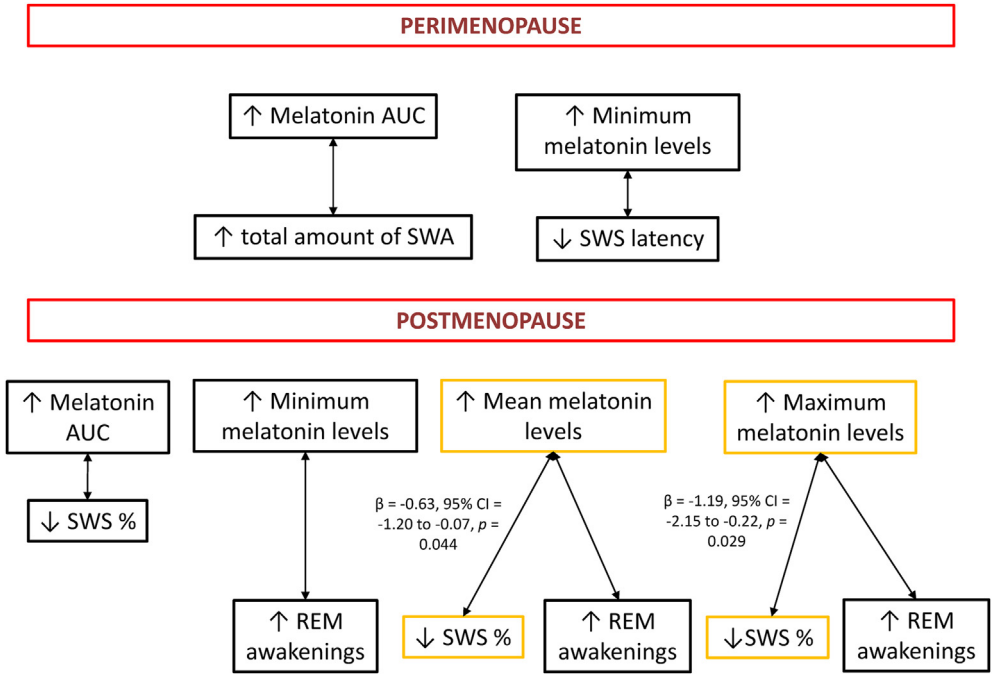

Fig. 4. Correlations and associations between melatonin concentration and secretion parameters, and sleep variables in perimenopausal and postmenopausal women. The figure displays significant correlations between melatonin and sleep variables. Results that were significant (or tended to be significant) also in unadjusted and age- and BMI-adjusted regression analyses are marked in orange. Numbers are β coefficients, 95% CIs and *p*-values from unadjusted models. Regression coefficients (β) indicate one unit change in melatonin mean and maximum levels (pg/ml) per one unit change in SWS percentage. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with lower melatonin concentrations compared to the wake phase, and the rate of change in melatonin concentrations was associated with the NREM sleep. Taken together, these results suggest that the NREM sleep may be the most sensitive to melatonin concentrations and secretion profile. This hypothesis seems to be confirmed by the observation that almost all of the connections between melatonin

indicators and sleep architecture measurements were related to the NREM sleep (SWS latency, SWS percentage, SWA). Interestingly, the connections between melatonin and NREM sleep were of opposite directions in perimenopausal and postmenopausal women. While in perimenopause higher melatonin concentrations were related to better NREM sleep (shorter SWS latency and higher amount of SWA), in postmenopause higher concentrations of melatonin were related to poorer NREM sleep, as indicated by lower SWS percentage. However, it is of note that most of these associations lost their significance after adjustment for age, BMI and vasomotor symptom scores or BDI scores, especially in perimenopause. In fact, factors such as age, BMI, depressive and climacteric symptoms are known to be connected to melatonin [31–33]. For example, melatonin concentrations decrease with advancing age [31], which in turn is associated with poorer sleep. Climacteric symptoms, on the other hand, are one of the causes of disrupted subjective and objective sleep in menopausal women [5,34]. It has been hypothesized that melatonin could reduce climacteric vasomotor symptoms, for example by suppressing the pulse generator responsible both for gonadotropin secretion and putatively for the generation of hot flushes; however, evidence supporting this hypothesis is rather contradictory [31,35].

On the basis of our results, it appears that melatonin concentrations and secretion profile are related to sleep phases differently before and after menopause, with NREM sleep being the most sensitive. Whether melatonin has a promotor effect on (NREM) sleep, or if the sleep phases influence the concentrations and profile of melatonin secretion, is not clear. It could be speculated that during perimenopause melatonin indeed contributes to maintain sleep. However, after menopause, when gonadal hormone levels change and reach a new balance, the melatonin-sleep regulation system may change. In this context, the lowered concentrations of melatonin and the shortened duration of secretion typical of the postmenopausal period [23] may not be able to positively regulate sleep. It could be similarly hypothesized that the SWS is differently effective in perimenopause and postmenopause: thus, before menopause, high melatonin levels are accompanied by lower amount of more effective SWS; on the contrary, during postmenopause, when the melatonin levels are lower, higher levels of less effective SWS are required to obtain effective sleep. Hence, our findings of an association between higher melatonin concentrations and poorer sleep (less SWS percentage and more REM awakenings) in postmenopause may reflect a compensatory mechanism, ie, the deteriorated sleep may itself induce a compensatory melatonin rise, possibly through a common mediating factor. In line with this hypothesis, we found a more fragmented sleep, as indicated by the higher hazard for sleep phase transitions (wake to NREM, NREM to wake and REM to NREM) in postmenopause compared to perimenopause, and for wake to NREM transitions in relation to higher levels of melatonin. These findings are partly consistent with those of a large community cohort study of over 5500 participants aged 40 years and older, showing that the overall and phase-specific (wake to NREM and NREM to wake) sleep phase transition rates predicted self-reported restless and light sleep, even after controlling for objective measures of sleep architecture [36]. Given their reciprocal interconnection, it is tempting to speculate that gonadal hormones (eg, the FSH), and the rate of change on their levels during postmenopause, may act as a key regulator, possibly affecting the direction of the association between melatonin and sleep. However, this hypothesis remains to be tested.

Our findings of lowest melatonin levels during the NREM sleep are in line with those reported by Luboshitzky et al., who studied six healthy and 23 hypogonadal young men, and found the lowest serum melatonin levels during SWS and the highest during REM sleep, irrespective of the testosterone levels [21]. On the contrary, when examining plasma melatonin levels in seven healthy men (22–49 years of age), Birkeland reported the highest levels during the wake stage and the lowest during REM sleep [19]. A study focused on plasma melatonin levels in relation to headache, found the lowest melatonin values during wake, intermediate levels in connection with the NREM sleep, and a peak during the REM stage in the 10 healthy male controls, but not in the 36 male patients (aged 26–67 years) [22]. On the contrary, Claustrat et al. found no associations between nocturnal melatonin levels and sleep stages in six healthy young men [20]. However, the comparability with such studies is extremely limited, since they all included only male and generally young populations. This is of crucial importance, since both sleep and melatonin differ across genders [37,38]. Additionally, gonadal hormones and reproductive phases are known to impact on women's sleep [39]. Recently, Obayashi et al. hypothesized that the changes in sex hormones typical of the menopausal period may account for their finding of lower urinary melatonin levels in old women than in old men ($\geq$60 years of age), irrespective of their exposure to light [40]. Nevertheless, the reciprocal relationships between melatonin, sleep stages, reproductive function and sex hormones in women needs to be further studied.

Taken together, our findings point to a relationship between melatonin and sleep stages, differently in perimenopausal and postmenopausal women; in particular, the NREM sleep, and its transition both from and to wake, seems to be the most sensitive both to melatonin and to reproductive phase. On this regard, it is important to notice that the two melatonin receptors, MT1 and MT2, are differently expressed in different brain regions, and mediate different effects of melatonin on sleep (a REM-sleep-promoting effect via MT1, and a NREM-sleep-promoting effect via MT2). It has been hypothesized that melatonin regulates the expression of its own receptors, thus resulting in the NREM/REM alternations typical of the sleep cycle [41]. The regulatory effect of melatonin on sleep may be blunted with age, which has been found to be associated with a reduction of MT1 density in the brain [42].

4.1. Strengths and limitations

The main limitation of this study is a small sample size. However, other previous studies on melatonin levels across sleep stages were conducted on similarly small or even smaller samples. In addition, as the study was carried out on a generally healthy population, the results cannot be generalized to larger populations with chronic diseases. Moreover, even though sleep disorders were not excluded on the basis of the PSG results, self-reported sleep disorders were one of our exclusion criteria. The melatonin sampling took place throughout the year, thus possibly influencing the results. However, during their visit in the sleep laboratory the women spent their time inside the building, slept in a room without windows and the night-time illumination levels were strictly controlled. Also, the high inter-individual variability in melatonin levels may have biased the results. Furthermore, the comparability with previous studies may be limited by the use of different tissue samples (plasma, urine, saliva) for measuring melatonin levels.

Even though the 1-h melatonin sampling may not fully capture sleep stage differences during the majority of the sleep episodes, the repeated serum sampling technique used for the melatonin assessment is the best technique to measure melatonin phase, duration and amplitude, in particular when frequent samples are taken [43]. Additional strengths of the study include the high-frequency collection of serum samples under strictly controlled sleep laboratory conditions, which ensured the good quality of the

samples. Furthermore, the strict exclusion criteria allowed us to eliminate several confounding factors.

5. Conclusions

The results of this study suggest that the relationship between melatonin and sleep architecture is different between perimenopausal and postmenopausal women. After menopause, higher concentrations of melatonin are associated with worse sleep, especially with worse SWS. Postmenopausal state seems to be related to more sleep phase transitions, especially to and from the NREM stage. Whether the different patterns of melatonin-to-sleep relationship before and after menopause are related to aging of the reproductive system, and whether they are causatively associated to menopausal sleep complaints, remain to be elucidated.

Funding

This work was partially supported (ET) by the Jane and Aatos Erkko Foundation (grant number 170062).

CRediT authorship contribution statement

Elena Toffol: Formal analysis, Data curation, Methodology, Writing - original draft, Visualization. **Nea Kalleinen:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Sari-Leena Himanen:** Investigation, Resources, Writing - review & editing. **Timo Partonen:** Conceptualization, Writing - review & editing. **Jari Haukka:** Formal analysis, Visualization, Writing - review & editing. **Päivi Polo-Kantola:** Conceptualization, Methodology, Validation, Supervision, Project administration, Writing - review & editing.

Acknowledgements

We would like to thank Dr. Olli Vakkuri for his invaluable contribution to performing melatonin laboratory analyses, and all the women who participated in the study.

Conflict of interest

The authors have no conflicts of interest to disclose.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2021.02.011.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2021.02.011.

References

[1] Kravitz HM, Joffe H. Sleep during the perimenopause: a SWAN story. Obstet Gynecol Clin N Am 2011;38(3):567–86. https://doi.org/10.1016/j.ogc.2011.06.002.
[2] Xu Q, Lang CP. Examining the relationship between subjective sleep disturbance and menopause: a systematic review and meta-analysis. Menopause 2014;21(12):1301–18. https://doi.org/10.1097/GME.0000000000000240.
[3] Hachul H, Frange C, Bezerra AG, et al. The effect of menopause on objective sleep parameters: data from an epidemiologic study in São Paulo, Brazil. Maturitas 2015;80(2):170–8. https://doi.org/10.1016/j.maturitas.2014.11.002.
[4] Polo-Kantola P, Erkkola R, Helenius H, et al. When does estrogen replacement therapy improve sleep quality? Am J Obstet Gynecol 1998;178(5):1002–9. https://doi.org/10.1016/s0002-9378(98)70539-3.
[5] Polo-Kantola P. Sleep problems in midlife and beyond. Maturitas 2011;68(3):224–32. https://doi.org/10.1016/j.maturitas.2010.12.009.
[6] Attarian H, Hachul H, Guttuso T, et al. Treatment of chronic insomnia disorder in menopause: evaluation of literature. Menopause 2015;22(6):674–84. https://doi.org/10.1097/GME.0000000000000348.
[7] Ohayon MM. Severe hot flashes are associated with chronic insomnia. Arch Intern Med 2006;166(12):1262–8. https://doi.org/10.1001/archinte.166.12.1262.
[8] Campbell IG, Bromberger JT, Buysse DJ, et al. Evaluation of the association of menopausal status with delta and beta EEG activity during sleep. Sleep 2011;34(11):1561–8. https://doi.org/10.5665/sleep.1398.
[9] Polo-Kantola P, Erkkola R, Irjala K, et al. Climacteric symptoms and sleep quality. Obstet Gynecol 1999;94(2):219–24. https://doi.org/10.1016/s0029-7844(99)00284-7.
[10] Young T, Rabago D, Zgierska A, et al. Objective and subjective sleep quality in premenopausal, perimenopausal, and postmenopausal women in the Wisconsin Sleep Cohort Study. Sleep 2003;26(6):667–72. https://doi.org/10.1093/sleep/26.6.667.
[11] Kalleinen N, Polo-Kantola P, Himanen S-L, et al. Sleep and the menopause: do postmenopausal women experience worse sleep than premenopausal women? Menopause Int 2008;14(3):97–104. https://doi.org/10.1258/mi.2008.008013.
[12] Xu M, Bélanger L, Ivers H, et al. Comparison of subjective and objective sleep quality in menopausal and non-menopausal women with insomnia. Sleep Med 2011;12(1):65–9. https://doi.org/10.1016/j.sleep.2010.09.003.
[13] Lampio L, Saaresranta T, Polo O, et al. Subjective sleep in premenopausal and postmenopausal women on workdays and leisure days: a sleep diary study. Menopause 2013;20(6):655–60. https://doi.org/10.1097/gme.0b013e31827ae954.
[14] Shaver JL, Woods NF. Sleep and menopause: a narrative review. Menopause 2015;22(8):899–915. https://doi.org/10.1097/GME.0000000000000499.
[15] Lampio L, Polo-Kantola P, Himanen SL, et al. Sleep during menopausal transition: a 6-year follow-up. Sleep 2017;40(7). https://doi.org/10.1093/sleep/zsx090. 10.1093/sleep/zsx090.
[16] Matthews KA, Kravitz HM, Lee L, et al. Does midlife aging impact women's sleep duration, continuity, and timing? A longitudinal analysis from the study of women's health across the Nation. Sleep 2020;43(4):zsz259. https://doi.org/10.1093/sleep/zsz259.
[17] Zawilska JB, Skene DJ, Arendt J. Physiology and pharmacology of melatonin in relation to biological rhythms. Pharmacol Rep 2009;61(3):383–410. https://doi.org/10.1016/s1734-1140(09)70081-7.
[18] Rahman SA, Marcu S, Kayumov L, et al. Altered sleep architecture and higher incidence of subsyndromal depression in low endogenous melatonin secretors. Eur Arch Psychiatry Clin Neurosci 2010;260(4):327–35. https://doi.org/10.1007/s00406-009-0080-7.
[19] Birkeland AJ. Plasma melatonin levels and nocturnal transitions between sleep and wakefulness. Neuroendocrinology 1982;34:126–31.
[20] Claustrat B, Brun J, Garry P, et al. A once-repeated study of nocturnal plasma melatonin patterns and sleep recordings in six normal young men. J Pineal Res 1986;3(4):301–10. https://doi.org/10.1111/j.1600-079x.1986.tb00753.x.
[21] Luboshitzky R, Lavi S, Lavie P. The association between melatonin and sleep stages in normal adults and hypogonadal men. Sleep 1999;22(7):867–74. https://doi.org/10.1093/sleep/22.7.867.
[22] Bruera O, Sances G, Leston J, et al. Plasma melatonin pattern in chronic and episodic headaches: evaluation during sleep and waking. Funct Neurol 2008;23(2):77–81.
[23] Toffol E, Kalleinen N, Haukka J, et al. Melatonin in perimenopausal and postmenopausal women: associations with mood, sleep, climacteric symptoms, and quality of life. Menopause 2014;21(5):493–500. https://doi.org/10.1097/GME.0b013e3182a6c8f3.
[24] Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring systems for sleep stages of human subjects. Los Angeles, CA: Brain Information Service/Brain Research Institute, UCLA; 1968.
[25] A preliminary report from the sleep disorders atlas task force of the American sleep disorders association. Sleep 1992;15(2):174–84. https://doi.org/10.1093/sleep/15.2.174.
[26] Vakkuri O, Leppäluoto J, Vuolteenaho O. Development and validation of a melatonin radioimmunoassay using radioiodinated melatonin as tracer. Acta Endocrinol 1984;106(2):152–7. https://doi.org/10.1530/acta.0.1060152.
[27] Partinen M, Gislason T. Basic Nordic Sleep Questionnaire (BNSQ): a quantitated measure of subjective sleep complaints. J Sleep Res 1995;4(S1):150–5. https://doi.org/10.1111/j.1365-2869.1995.tb00205.x.
[28] Beck AT, Ward CH, Mendelson M, et al. An inventory for measuring depression. Arch Gen Psych 1961;4:561–71.
[29] Jackson CH. Multi-state models for panel data: the msm package for R. J Stat Softw 2011;38:1–29. Available: http://www.jstatsoft.org/v38/i08/.
[30] R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2018. URL, https://www.R-project.org/.
[31] Gursoy AY, Kiseli M, Caglar GS. Melatonin in aging women. Climacteric 2015;18(6):790–6. https://doi.org/10.3109/13697137.2015.1052393.
[32] Parry BL, Meliska CJ, Sorenson DL, et al. Increased melatonin and delayed offset in menopausal depression: role of years past menopause, follicle-stimulating hormone, sleep end time, and body mass index. J Clin Endocrinol Metab 2008;93(1):54–60. https://doi.org/10.1210/jc.2006-2853.

[33] Meliska CJ, Martínez LF, López AM, et al. Relationship of morningness-eveningness questionnaire score to melatonin and sleep timing, body mass index and atypical depressive symptoms in peri- and post-menopausal women. Psychiatr Res 2011;188(1):88–95. https://doi.org/10.1016/j.psychres.2010.12.010.
[34] de Zambotti M, Colrain IM, Javitz HS, et al. Magnitude of the impact of hot flashes on sleep in perimenopausal women. Fertil Steril 2014;102(6):1708–15. https://doi.org/10.1016/j.fertnstert.2014.08.016.
[35] Pines A. Circadian rhythm and menopause. Climacteric 2016;19(6):551–2. https://doi.org/10.1080/13697137.2016.1226608.
[36] Laffan A, Caffo B, Swihart BJ, et al. Utility of sleep stage transitions in assessing sleep continuity. Sleep 2010;33(12):1681–6. https://doi.org/10.1093/sleep/33.12.1681.
[37] Mongrain V, Lavoie S, Selmaoui B, et al. Phase relationships between sleep-wake cycle and underlying circadian rhythms in Morningness-Eveningness. J Biol Rhythm 2004;19(3):248–57. https://doi.org/10.1177/0748730404264365.
[38] Zhang B, Wing Y-K. Sex differences in insomnia: a meta-analysis. Sleep 2006;29(1):85–93. https://doi.org/10.1093/sleep/29.1.85.
[39] Manber R, Armitage R. Sex, steroids, and sleep: a review. Sleep 1999;22(5):540–55.
[40] Obayashi K, Saeki K, Tone N, et al. Lower melatonin secretion in older females: gender differences independent of light exposure profiles. J Epidemiol 2015;25(1):38–43. https://doi.org/10.2188/jea.JE20140035.
[41] Gobbi G, Comai S. Differential function of melatonin MT1 and MT2 receptors in REM and NREM sleep. Front Endocrinol 2019;10:87. https://doi.org/10.3389/fendo.2019.00087.
[42] Wu YH, Zhou JN, Van Heerikhuize J, et al. Decreased MT1 melatonin receptor expression in the suprachiasmatic nucleus in aging and Alzheimer's disease. Neurobiol Aging 2007;28(8):1239–47. https://doi.org/10.1016/j.neurobiolaging.2006.06.002.
[43] Benloucif S, Burgess HJ, Klerman EB, et al. Measuring melatonin in humans. J Clin Sleep Med 2008;4(1):66–9.