



UNIVERSITY OF HELSINKI

<https://helda.helsinki.fi>

Worse sleep architecture but not self-reported insomnia and sleepiness is associated with higher cortisol levels in menopausal women

Sahola, Nima; Toffol, Elena; Kalleinen, Nea; Polo-Kantola, Päivi

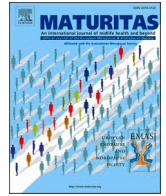
2024-09

Elsevier Ireland Ltd.

<http://hdl.handle.net/10138/585296>

Sahola, N, Toffol, E, Kalleinen, N & Polo-Kantola, P 2024, 'Worse sleep architecture but not self-reported insomnia and sleepiness is associated with higher cortisol levels in menopausal women', *Maturitas*, vol. 187, 108053. <https://doi.org/10.1016/j.maturitas.2024.108053>

Downloaded from Helda, University of Helsinki institutional repository. <https://helda.helsinki.fi>
This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.
Please cite the original version.



Original article

Worse sleep architecture but not self-reported insomnia and sleepiness is associated with higher cortisol levels in menopausal women

Nima Sahola^{a,1}, Elena Toffol^{b,*}, Nea Kalleinen^{c,d}, Päivi Polo-Kantola^{c,e}

^a Department of Obstetrics and Gynecology, University of Turku, FI-20014 Turku, Finland

^b Department of Public Health, University of Helsinki, PO BOX 20, 00014 Helsinki, Finland

^c Sleep Research Centre, Department of Pulmonary Diseases and Clinical Allergology, University of Turku, FI-20014 Turku, Finland

^d Heart Center, Turku University Hospital and University of Turku, FI-20014, University of Turku, Turku, Finland

^e Department of Obstetrics and Gynecology, Turku University Hospital and University of Turku, FI-20014, University of Turku, Turku, Finland

ARTICLE INFO

Keywords:

Cortisol

Sleep

Polysomnography

Sleep architecture

Menopause

Climacteric

ABSTRACT

Objective: Worsening of sleep quality during menopause is well recognized. However, the underlying hormonal regulation is insufficiently described. In this study, we evaluated associations between sleep and cortisol levels. **Study design:** Seventeen perimenopausal and 18 postmenopausal women were enrolled in a three-night sleep study. Diurnal blood sampling was performed during the third night and the following day.

Main outcome measures: Self-reported insomnia and sleepiness were evaluated with the Basic Nordic Sleep Questionnaire and sleep architecture with all-night polysomnography. Diurnal cortisol samples were collected at 20-min intervals. Correlation analyses and generalized linear models adjusted by age, body mass index, vasomotor symptoms and depressive symptoms were conducted.

Results: In correlation analyses, self-reported insomnia and sleepiness were not associated with cortisol levels. Lower sleep efficiency, slow-wave sleep and stage 1 percentages, number of slow-wave sleep and of rapid-eye-movement (REM) periods, longer slow-wave sleep latency and higher wake after sleep onset percentage were associated with higher cortisol levels (all $p < 0.05$). Further, lower slow-wave sleep percentage and longer slow-wave sleep latency correlated with steeper daytime cortisol slope (i.e. day cortisol decrease, both $p < 0.05$). In adjusted generalized linear models, lower sleep efficiency and number of rapid-eye-movement periods as well as higher wake after sleep onset percentage correlated with higher cortisol levels; lower slow-wave sleep percentage correlated with higher cortisol awakening response.

Conclusions: Worse sleep architecture but not worse self-reported insomnia and sleepiness was associated with higher cortisol levels. This is important for understanding sleep in women, especially during the menopausal period.

1. Introduction

Worsening in sleep quality during the climacteric is well described [1]. Although the exact underlying mechanisms are uncertain, other climacteric symptoms, especially concomitant vasomotor symptoms have been suggested to strongly account for the sleep symptoms [1,2]. Furthermore, somatic and mental symptoms, as well as chronic diseases

and medications have shown to be important [1–3].

One of the possible related factors for sleep impairment is cortisol. Cortisol secretion follows a circadian rhythm, with high levels at wake, a surge in 30 to 40 min after awakening, followed by a steep decline and thereafter a slower decline to a nadir around bedtime [4]. With age, cortisol levels increase especially in women [5]. This increase is particularly evident for night levels, nocturnal nadir and morning

Abbreviations: AUC, area under curve; BDI, Beck depression inventory; BMI, body mass index; BNSQ, Basic Nordic Sleep Questionnaire; CAR, cortisol awakening response; ECG, electrocardiogram; EEG, electroencephalogram; EMG, electromyogram; EOG, electro-oculogram; HPA, hypothalamic-pituitary-adrenal; NREM, non-rapid eye movement; N1, stage 1 sleep; N2, stage 2 sleep; REM, rapid eye movement; SWA, slow wave activity; SWS, slow wave sleep; VMS, vasomotor symptoms; WASO, wake after sleep onset; WHQ, Women's Health Questionnaire.

* Corresponding author at: Department of Public Health, Clinicum, University of Helsinki, Helsinki, Finland.

E-mail addresses: nima.n.sahola@utu.fi (N. Sahola), elena.toffol@helsinki.fi (E. Toffol), nea.kalleinen@utu.fi (N. Kalleinen), paivi.polo@utu.fi (P. Polo-Kantola).

¹ Equal contribution.

<https://doi.org/10.1016/j.maturitas.2024.108053>

Received 31 October 2023; Received in revised form 10 June 2024; Accepted 12 June 2024

Available online 18 June 2024

0378-5122/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

cortisol acrophase [5]. These age-related higher levels of cortisol are attributable to a reduction of the nocturnal inhibition of cortisol secretion. The nocturnal inhibition is considered to function as a sort of natural recovery of the hypothalamic-pituitary-adrenal (HPA) axis from the cortisol awakening response (CAR) induced by the circadian pacemaker in the suprachiasmatic nuclei of the hypothalamus. A decreased resiliency of the HPA axis to such endogenous morning challenge plausibly explains the age-related decreased nocturnal inhibition [5]. However, no notable differences in cortisol levels between perimenopausal and postmenopausal women have been shown [6,7].

Abnormal cortisol levels and secretion have shown to be associated with several adverse health outcomes [8]. In addition, as a mediator of stress response, cortisol secretion is particularly relevant for sleep, since by activating the sympathetic nervous system, it induces high alertness in the body [9], and thus, possibly interferes with sleep [10]. Increase in diurnal cortisol levels, specifically of evening levels [11], as well as enhanced CAR [12] have shown to be related to chronic insomnia. Furthermore, some studies have reported an association between poor sleep quality and either blunted or increased CAR and slower cortisol decline over the day, yielding in increased total diurnal cortisol output [13], although others have reported no associations [14,15].

Only a few studies have considered the associations between subjective sleep quality and cortisol in women, and especially in menopausal women, with inconsistent results. Gerber et al. [16] found that more frequent sleep problems were associated with higher salivary cortisol levels at bedtime in midlife women. Huang et al. [17] found no differences in salivary cortisol levels between postmenopausal good and poor sleepers, with the only exceptions of higher bedtime cortisol rise in good sleepers, and slower late cortisol decline in poor sleepers. In that study [17], taken sleep quality as a continuous variable, slower late cortisol decline was associated with general shorter sleep duration, lower sleep efficiency, poorer sleep quality and more daytime dysfunction, and women with long sleep latency had a reduced CAR. On the contrary, other authors reported associations between difficulties falling asleep and lower overnight urinary cortisol excretion in perimenopausal and postmenopausal women [18], or no associations at all [19,20]. Katainen et al. [6] found that peri- and postmenopausal women with more climacteric sleep symptoms had lower serum cortisol levels during the night (1.30 a.m.–4. a.m.) and higher CAR.

To date, only limited studies have evaluated the associations between sleep architecture and cortisol. Two sleep deprivation studies, with 12 males [21], and later, with 31 males [22], found no effect of sleep deprivation on nocturnal cortisol profile. Of note is that no studies of the association between diurnal cortisol secretion and sleep architecture in menopausal women have been published. Thus, the aim of our study was to examine the relationship between subjective sleep quality (as self-reported insomnia and sleepiness) and sleep architecture and diurnal cortisol secretion in perimenopausal and postmenopausal women. We hypothesized that self-reported insomnia and sleepiness, and deterioration of sleep architecture are attributed to higher cortisol secretion.

2. Methods

2.1. Subjects

This study was a part of a larger menopausal sleep study, conducted in the Sleep Research Center of Turku University, Turku, Finland. Altogether 35 women were enrolled, of which 17 (aged 45–51 years) were perimenopausal (verified by low serum follicle stimulating hormone [<23 IU/ml] and ongoing menstrual cycle) and 18 (aged 58–71 years) were postmenopausal (verified by chronic amenorrhea of at least one year). Before inclusion, serum thyroid stimulating hormone, blood hemoglobin, leucocyte and thrombocyte levels were measured to ensure that they fell into normal ranges. Exclusion criteria included smoking, current use of menopausal hormone therapy (MHT), positive drug

screen test, pre-existing neurological, cardiovascular (apart from treated hypertension), endocrinological (apart from treated hyperlipidemia) and mental diseases, diagnosed sleep apnea and insomnia, malignancies, abuse of alcohol and medications and excessive consumption of caffeine (> 5 cups/day). The use of antioxidants, hormones, and medications with central nervous system effects (including sleeping pills) was prohibited during the study with a minimum washout time of three months (in case of previous MHT use, the wash-out period was at least 12 months). During the sleep studies and for one week prior the use of alcohol as well as traveling across time zones was prohibited. Before inclusion, a gynecological examination with transvaginal ultrasound was performed and body mass index (BMI kg/m^2) was calculated. Vasomotor symptoms (VMS) were evaluated with the vasomotor score drawn from the Women's Health Questionnaire (WHQ) [23] and depressive symptoms were assessed with the Beck Depression Inventory (BDI) [24]. The sleep-wake schedule was assessed during a phone interview before acceptance to participate in the study, and only women with a regular sleep-wake schedule (10–11 p.m. to 6–7 a.m.) were enrolled. In addition, to ensure a regular sleep-wake schedule, the subjects kept a sleep diary for three weeks before and one week after the sleep studies.

2.2. Procedure

The study procedure is described in detail elsewhere [6,25]. Shortly, the women spent three nights in the sleep laboratory (Fig. 1). The women spent the time allotted for sleep (11 p.m. – 7 a.m., lights off – lights on) in bed in a dark room. On the third evening, an intravenous catheter was placed in the forearm at 7 p.m., and blood samples were collected at 20-min intervals for 24 h, starting at 9 p.m. The catheter was connected to a plastic tube running through a soundproof lock into an adjoining room to allow repeated blood sampling with a minimal disturbance to sleep. During the night, the forearm of the woman was loosely attached to the bed supporting the extension of the forearm to ensure the blood sample collection. The catheter was kept patent with a slow saline infusion containing a small dose of heparin. All the perimenopausal women were studied during the follicular phase of the menstrual cycle. The PSG indicated that 19 women woke up before lights-on (5.15–6.59 a.m.), but, in line with the study protocol, they stayed in bed until lights-on. Women were not allowed to exit the windowless sleeping room during the nighttime (11 p.m. – 7 a.m.), and only red light was allowed for illumination in the night when necessary. There was a bucket toilet in the sleeping room. During the night of blood collection, three women urinated once and two women twice. None of the women had need to defecate during the night.

2.3. Subjective sleep evaluation

Subjective sleep quality during the past three months was evaluated by the Basic Nordic Sleep Questionnaire (BNSQ) [26], including questions about general sleep quality, different insomnia symptoms (difficulties falling asleep, nocturnal awakenings [per week and per night] and too early morning awakenings), and sleepiness symptoms (morning sleepiness, daytime sleepiness, compulsive falling asleep at work, compulsive falling asleep at leisure time and napping). The questions were rated with a 5-point Likert scale ranging from 1 = 'never or less than once a month' to 5 = 'daily or almost daily', except in the question of frequency of nocturnal awakenings per night, which was rated 1 = 'none', 2 = 'once', 3 = 'twice', 4 = 'three to four times', 5 = 'at least five times'. Both insomnia score and sleepiness score were calculated and used in the analyses; total scores range between 5 and 25, higher scores indicating more severe symptoms.

2.4. Sleep architecture measurements

The polysomnography included data from four

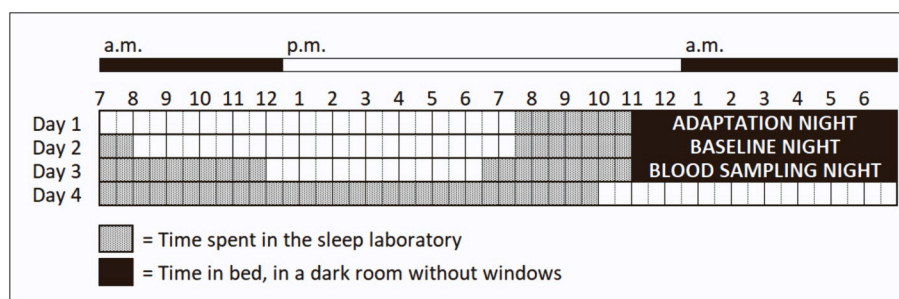


Fig. 1. Study procedure. The women spent three consecutive nights in the sleep laboratory: adaptation night, baseline night and blood sampling night. The women went to bed at 23:00 h (lights-off) and were woken-up (lights-on) at 07:00 h. Women were not allowed to exit the windowless sleeping room during the nighttime (23:00–07:00 h), and only red light was allowed for illumination overnight when necessary. There was a bucket toilet in the sleeping room. The schedule and content of the days, including nutrition, were similar for each participant. The third evening, an IV catheter was placed in the forearm at 7 p.m., and blood samples were collected at 20-min intervals for 24 h, starting at 9 p.m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

electroencephalograms (EEG; C3/A2, C4/A1, O1/A2 and O2/A1), a mandibular electromyogram (EMG), two electro-oculograms (EOG) and an electrocardiogram (ECG, three channels) (Embla, Medcare Flaga hf. Medical devices, Reykjavik, Iceland). The sleep stages were visually scored in 30 s intervals (N.K. and rescored by senior scorer P.P.-K.) according to conventional criteria during the study [27] (stage 1 [N1], stage 2 [N2], slow wave sleep [SWS], rapid eye movement [REM] and wake time after sleep onset [WASO]). Non-REM (NREM) sleep consisted of N1, N2 and SWS. Slow wave activity (SWA, 0.75–4 Hz) of NREM sleep episodes was quantified with spectral analysis. Sleep cycles were defined according to the rules of Feinberg and Floyd [28]. Sleep onset was considered as an appearance of three consecutive epochs of N1 or the first epoch of any other stage. The following sleep variables were included in the current study, as described elsewhere [25]: sleep efficiency, sleep latency, SWS- and REM-latencies, the percentage of the time in bed (from lights off to lights on) in each sleep stage, total slow wave activity in NREM-sleep, number of awakenings and of sleep stage transitions.

2.5. Cortisol measurements

The blood samples were drawn into Li-Heparin tubes, placed in the refrigerator (4–8 °C) for 20 min, centrifuged, and frozen immediately. The serum cortisol levels were measured using AutoDELFIA (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) solid-phase, two-site fluorometric assays. The assays were based on competitive binding of europium-labeled cortisol and sample cortisol to a limited amount of monoclonal antibodies. The analytical sensitivity for cortisol was 15 nmol/l and the interassay coefficient of variation was 1.9 % for cortisol at a concentration of 212 nmol/l.

Cortisol mean, maximum, minimum and area under curve (AUC) were computed for daytime (7.20 a.m. – 9 p.m.), nighttime (11 p.m. – 7 a.m.) and diurnal (9 p.m. – next day 9 p.m.) periods. The trapezoidal rule was employed to calculate the AUCs. The cortisol awakening response (CAR) was defined as the maximum increase of cortisol during the next 60 min after waking up (baseline), and calculated as the difference between the highest cortisol level during the 60 min following polysomnography-determined wake, and cortisol level at the sampling time closest to polysomnography-determined wake [29]. With few participants, this change in cortisol was negative. In addition, cortisol night slope (from lights off to lights on) and daytime slope (from morning maximum to 9 p.m.) were calculated by dividing the change in cortisol by the change in time.

2.6. Statistical analysis

The distribution of variables was tested with Shapiro-Wilk test and

by visually evaluating histograms and Q-Q plots. We have previously published sleep and cortisol secretion data from the same population from the same three-night study and showed that neither sleep architecture [25] nor serum cortisol levels (the same night measurement) [6,7] differed between perimenopausal and postmenopausal women; and thus, data of perimenopausal and postmenopausal women were combined for the current analyses. First, Pearson correlation coefficient (in normally distributed variables) or Spearman's rank correlation coefficient were used to evaluate correlations between self-reported and objective sleep variables, and cortisol variables. Thereafter, for significant correlations, generalized linear models were carried out to test the associations between sleep variables and cortisol levels. In addition to unadjusted analyses, an adjusted model was conducted controlling for age, BMI, VMS and BDI. Statistical analyses were performed with IBM SPSS statistics 27 and R version 4.2.3 [30].

2.7. Ethics

The study was approved by the Ethical Committee of Turku University Central Hospital. All study subjects gave their written informed consent after receiving oral and written study information.

3. Results

All of the 35 women completed the study. Because of incomplete

Table 1

General characteristics of 34 perimenopausal and postmenopausal women participating in the study.

	n	Mean (SD, range)
Age (years)	34	55.8 (7.9, 45–70)
BMI (kg/m ²)	34	26.0 (4.4, 20.6–38.1)
Education years	33	14.6 (4.3, 7.0–23)
VMS score	34	1.97 (0.94, 1–4)
BDI score	34	5 (3.8, 0–15)
N (%)		
Marital status	29	
Single		2 (6.9)
Married/cohabiting		20 (69.0)
Divorced/widowed		7 (24.1)
Employment	29	
Employed		13 (44.8)
Unemployed		4 (13.8)
Retired		12 (41.4)
Smoking status	27	
Never		25 (92.6)
Occasionally		2 (7.4)

BDI, Beck Depression Inventory; BMI, Body Mass Index; VMS, vasomotor symptoms.

data, one perimenopausal woman was excluded, leaving data from 34 women for the analyses (Basic characteristics in Table 1). On average, women slept 360 min, with total sleep time ranging between 185 min (one participant) to over 450 min (three participants). Self-reported and objective sleep data is shown in Table 2 and cortisol data in Table 3. In spite of moderate inter-individual variation, the diurnal cortisol secretion curve showed a characteristic morning increase after awakening, followed by a steep morning decline and a more flattened afternoon decline with a nadir at bedtime (Fig. 2).

3.1. Sleep and cortisol correlation analyses

The correlogram is presented in Fig. 3. No significant correlations were found between self-rated sleep variables (insomnia and sleepiness scores) and cortisol levels. Lower sleep efficiency correlated with higher cortisol levels (daytime mean, night mean and maximum, diurnal mean and AUC; $r/p = -0.34$ to -0.39 ; $p < 0.05$). Furthermore, higher WASO% related to higher cortisol levels (night mean, maximum and AUC, as well as diurnal mean and AUC; $r/p = 0.36$ to 0.43 ; $p < 0.05$).

Of associations with NREM sleep, poorer NREM sleep correlated with higher cortisol levels (Fig. 3). Specifically, higher cortisol levels related to lower SWS% (night minimum, diurnal maximum and minimum and CAR; $r/p = -0.36$ to -0.52 ; $p < 0.05$ to <0.01), with lower number of SWS periods (daytime mean and AUC, night mean and minimum, diurnal mean, minimum and AUC; $r/p = -0.35$ to -0.41 ; $p < 0.05$), lower amount of SWA (night minimum, $r/p = -0.35$, $p < 0.05$) and longer SWS latency (CAR, $r/p = 0.47$, $p < 0.01$). In addition, steeper daytime cortisol slope (i.e., daytime cortisol decrease) correlated with less SWS% ($p = 0.42$, $p < 0.05$) and longer SWS latency ($p = -0.37$, $p < 0.05$). Higher cortisol levels were associated with less N1% (night mean, $r/p = -0.34$, $p < 0.05$) (Fig. 3).

As for the REM sleep, fewer REM periods were related to higher cortisol levels (daytime mean and AUC, night mean, maximum, minimum and AUC and diurnal mean, minimum and AUC ($r/p = -0.36$ to -0.44 ; $p < 0.05$ to <0.01)).

3.2. Sleep and cortisol linear regression analyses

The results of the unadjusted regression analyses are described in Supplementary Table (Table S1), and the results of regression analyses adjusted for age, BMI, BDI and VMS scores are shown in Fig. 4.

Unadjusted regression analyses confirmed the associations of lower sleep efficiency and higher cortisol levels (night mean and maximum; B

Table 3

24-h serum cortisol levels and secretion characteristics in the 34 perimenopausal and postmenopausal women participating in the study.

	n	Mean nmol/l	SD nmol/l	Minimum nmol/l	Maximum nmol/l
Serum cortisol level					
Daytime mean	34	214.6	43.3	152.6	346.4
Daytime maximum	34	494.2	88.6	347.0	719.2
Daytime minimum	34	87.7	27.6	36.3	147.0
Daytime AUC	34	2908.6	593.6	2062.2	4716.8
Night mean	34	206.2	41.1	144.3	299.1
Night maximum	34	451.5	82.5	347.8	738.1
Night minimum	34	51.2	26.1	19.9	138.7
Night AUC	34	1629.4	338.9	1046.1	2435.6
Diurnal mean	34	202.4	36.7	148.4	314.6
Diurnal maximum	34	515.9	88.4	368.8	738.1
Diurnal minimum	34	50.7	24.9	19.9	133.1
Diurnal AUC	34	4881.3	890.6	3471.8	7673.8
CAR	33	143.9	110.2	-15	452.1
Serum cortisol secretion					
Daytime slope ^a	32	-29.00	7.01	-42.9	-17.07
Night slope ^b	33	34.5	11.9	10.7	58.4

AUC, Area Under Curve; CAR, Cortisol Awakening Response.

^a From morning maximum to 9.00 pm.

^b From lights off to lights on.

= -0.128 and B = -0.076; $p < 0.05$), as well as of higher WASO% and higher cortisol levels (night mean and maximum, night AUC; B = 0.017 to 0.141, $p = 0.008$ to 0.023) (Table S1). The results remained the same after adjustment for covariates (Fig. 4).

In unadjusted regression analyses, higher cortisol levels were associated with lower SWS% (diurnal maximum and CAR; B = -0.019 and B = -0.021, $p = 0.046$ and 0.005), with lower number of SWS periods (daytime mean and AUC, diurnal mean and AUC; B = -0.0009 to -0.013, $p = 0.028$ to 0.034) and with longer latency to SWS (CAR; B = 0.054, $p = 0.040$). Additionally, steeper (negative) daytime slope was associated with lower SWS% (B = 0.267, $p = 0.022$) (Table S1). However, in adjusted analyses, only higher CAR levels remained related to lower SWS% (B = -0.020, 95%CI = -0.037 to -0.004, $p = 0.025$) (Fig. 4).

Both unadjusted (Table S1) and adjusted regression analysis (Fig. 4) confirmed the associations between fewer REM periods and higher cortisol levels, with the exceptions of those with night and diurnal minimum cortisol levels and daytime AUC.

4. Discussion

To the best of our knowledge, our study is the first to evaluate associations between sleep architecture and cortisol secretion in menopausal women. We found reciprocal associations between worse sleep architecture and higher cortisol levels. Specifically, fewer REM periods consistently correlated with higher cortisol levels, and more fragmented sleep particularly with cortisol night levels. The associations between worse NREM sleep and higher cortisol levels were less robust and lost significance after correction for covariates, with the exception of less SWS in relation with higher CAR. Self-rated insomnia and sleepiness, on the contrary, were not associated with cortisol secretion.

Cortisol levels are low during the first part of the night, when SWS is dominant, while episodes of cortisol secretion become more frequent in the second part of the night, when REM sleep is more common, and peak with the morning CAR. Results of the current study additionally show specific and distinct associations of SWS with CAR compared to other cortisol parameters. CAR is induced by a stimulus originating from the circadian pacemaker in hypothalamus [5], a primary regulator of the sleep-wake cycle, including sleep architecture such as the number and duration of NREM episodes. Thus, it is plausible that impaired NREM sleep, as indicated by reduced SWS, may eventually contribute to

Table 2

Characteristics of self-reported and polysomnography -recorded sleep on a night of continuous blood sampling in the 34 perimenopausal and postmenopausal women participating in the study.

	n	Mean	SD	Min	Max
Sleep variable					
BNSQ Insomnia	34	14.76	3.89	5	22
BNSQ Sleepiness	34	10.74	3.62	5	21
Sleep efficiency (%)	34	74.98	15.28	38.53	96.02
Sleep latency (min)	34	18.07	12.54	3	58
N1%	34	9.26	3.26	3.36	16.15
N2%	34	37.63	10.40	8.02	53.53
SWS %	34	11.16	4.92	0.73	18.83
Number of SWS periods	34	2.82	1.49	0	6
Total NREM SWA	33	91.43	47.14	28.69	223.14
SWS latency (min)	33	17.62	15.23	1	61.5
REM latency (min)	33	91.46	65.14	38	372.5
REM %	34	16.9	7.0	2.1	30.9
Number of REM periods	34	6.09	2.79	1	12
Number of sleep stage transitions	34	167.32	43.23	101	275
WASO %	34	21.28	14.45	1.31	57.52
Number of awakenings	34	22.71	9.91	4	50

BNSQ, Basic Nordic Sleep Questionnaire; REM, Rapid Eye Movement; SWA, Slow Wave Activity; SWS, Slow Wave Sleep; WASO, Wake After Sleep Onset.

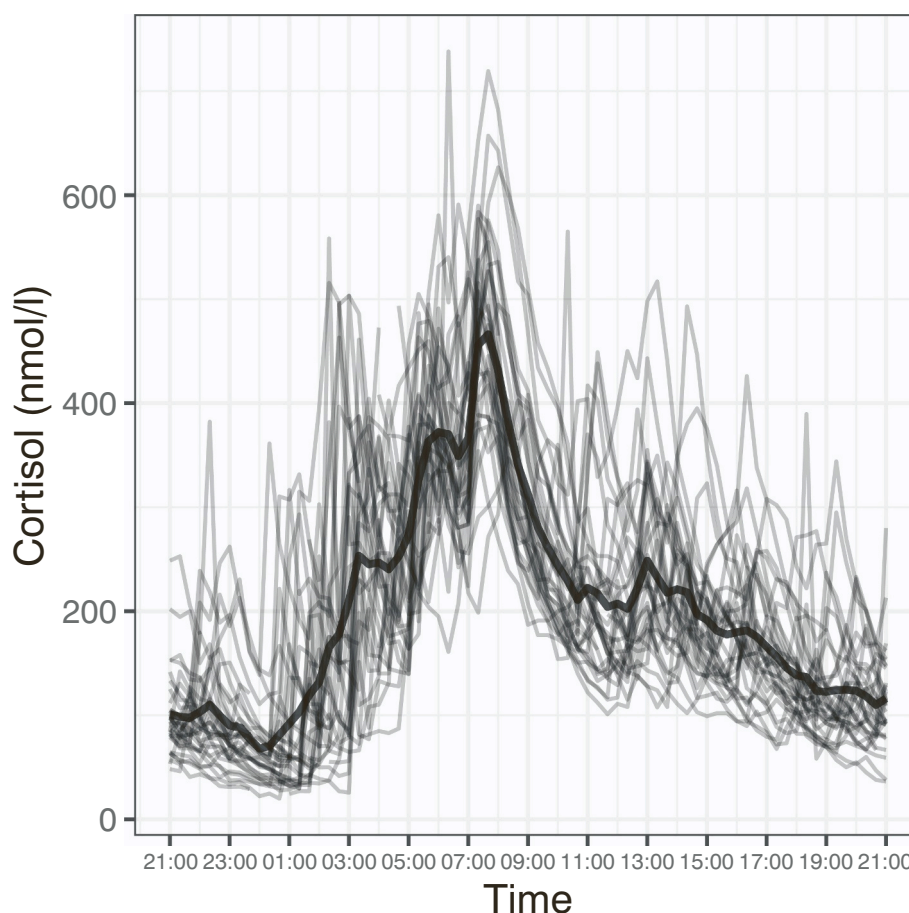


Fig. 2. 24-h serum cortisol curve for the study population. The women went to bed (lights-off) at 23:00 h and were woken-up (lights-on) at 07:00 h. Black line indicates the mean cortisol curve, grey lines indicate individual curves.

enhancing the CAR. The physiological meaning of the CAR is not completely known, but it probably relates with higher morning perceived arousal [31], and has been hypothesized to be associated with anticipation of the upcoming day and the subsequent demands required of the individual [32]. In this context, poor and unrefreshing sleep may act as a triggering signal to enhanced morning and subsequent day arousal (i.e., to be more alert, active, energetic).

Our findings of fewer REM periods and more fragmented sleep (as indicated by higher WASO) related to higher night and, to a lesser extent, day and diurnal cortisol levels are largely supported by the available literature [10,33,34]. For example, a previous study reported cross-sectional associations between more fragmented sleep, with longer WASO, and higher salivary day cortisol levels in a sample of nearly 700 66–78 years old subjects [35]. Other authors found that shorter sleep duration and poorer sleep efficiency related to flatter cortisol decline during the day and increased CAR even with lower waking cortisol levels [12,13,17].

As for self-rated sleepiness and insomnia, we found no associations with cortisol secretion, suggesting that cortisol levels or changes in secretion pattern with age and/or menopause plausibly do not contribute to sleep disturbances. This finding is in line with that of our previous study conducted in the same population of perimenopausal and postmenopausal women ($n = 35$), who had their climacteric symptoms (vasomotor symptoms, sleep problems, depressive symptoms, anxiety/fears, cognitive difficulties, sexual functioning, and menstrual and somatic symptoms) assessed with the Women's Health Questionnaire. In that study only few correlations were found between more climacteric sleep problems and serum cortisol, such as higher CAR and lower night levels [6]. Similarly, other previous studies reported no associations

between sleep difficulties and overnight urinary cortisol excretion measured on a single morning urine sample [19,20]. However, two previous studies using salivary cortisol measurements found a few associations between sleep disturbances and cortisol levels. One study of 209 postmenopausal women measured salivary cortisol five times over a day, and found higher bedtime cortisol rise in good sleepers, slower late cortisol decline in poor sleepers, and reduced CAR in women with long sleep latency [17]. In the other study, salivary cortisol in 109 midlife women was measured four times a day (morning and evening), and higher cortisol levels at bedtime were observed among those who reported trouble sleeping [16]. We used the BNSQ to evaluate self-reported insomnia and sleepiness symptoms. Even though the BNSQ is validated [26] and widely used also in studies with menopausal populations, it is plausible that it is not as sensitive as other sleep questionnaires like the Pittsburgh Sleep Quality Index, Insomnia Severity Index, Athens Insomnia Scale or Epworth Sleepiness Scale. Therefore, future cortisol studies should utilize these other questionnaires.

The main limitation of our study was the small number of participants, which, however, was relatively high compared to most of other laboratory sleep studies. We collected blood samples for only 24 h, less than desirable because of the daily fluctuations in cortisol levels. However, samples were collected at 20-min intervals, allowing us to assess the cortisol profile and the total secretion during an entire day. As most of the previous studies are based on one or few selected, mostly urinary or salivary cortisol samples over a single day, our data, with 20-min interval 24-h serum samples, represent a major strength of this study. Even though sleeping in a new environment in itself is associated with poorer sleep, the adaptation night likely minimized this effect. Also, the possible stress caused by being in the sleep laboratory could have

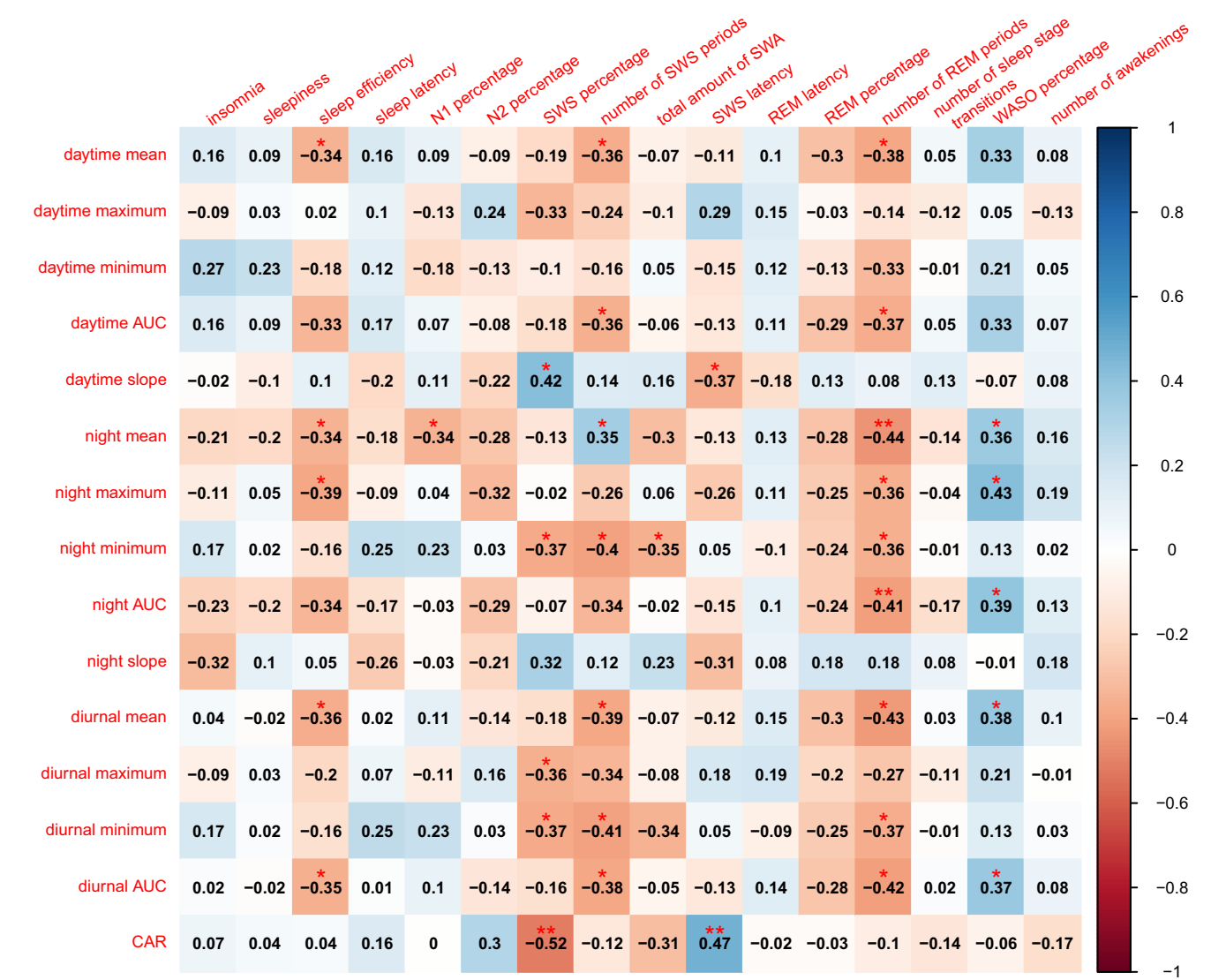


Fig. 3. Correlations between sleep variables and cortisol levels and secretion profile in the 34 women included in the analyses. Numbers indicate correlation coefficients. Significant correlations are marked with red * ($p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). AUC, Area Under Curve; CAR, Cortisol Awakening Response; REM, Rapid Eye Movement; SWS, Slow Wave Sleep; WASO, Wake After Sleep Onset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

affected the sleep and cortisol levels. Previous studies [36–39], including ours [36], have shown that nocturnal blood sampling set up acts as an external sleep disturbance resulting in poorer objective sleep quality. In addition, some previous studies have shown that the nocturnal indwelling catheter can increase cortisol levels to some extent [36,38]. Despite the inclusion criterion of a regular sleep-wake schedule (10–11 pm to 6–7 am) in our study, the circadian phase varied quite much in our sample and it is possible that, in some women, the sleeping time imposed in the laboratory setting (11 pm–7 am) have interfered with the relationship between sleep and cortisol secretion. Additionally, the comparability of our results to previous studies is limited by the use of different methodologies, especially in cortisol measuring. The cross-sectional study-design does not allow any conclusion on the direction of the associations. Although only self-rated insomnia and sleepiness were assessed, and no other validated measures of self-reported sleep quality were available, the BNSQ is a valid and widely used instrument to measure self-rated sleep complaints. Finally, the study was carried out in a healthy population, preventing the generalization of the results to populations with common diseases.

5. Conclusion

Our findings reflect an intrinsic complexity of the reciprocal relationship between sleep and cortisol in menopausal women. Poor sleep, as indicated by fewer REM periods and more wake episodes, was related to higher diurnal cortisol levels, while worse SWS was associated with higher morning cortisol response. Further studies are needed to examine specific relations between distinct sleep stages and cortisol levels and secretion profiles.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.maturitas.2024.108053>.

Contributors

Nima Sahola was a principal investigator and main writer of the paper. Elena Toffol was a principal investigator and main writer of the paper. Nea Kalleinen was a co-investigator and co-writer of the paper.

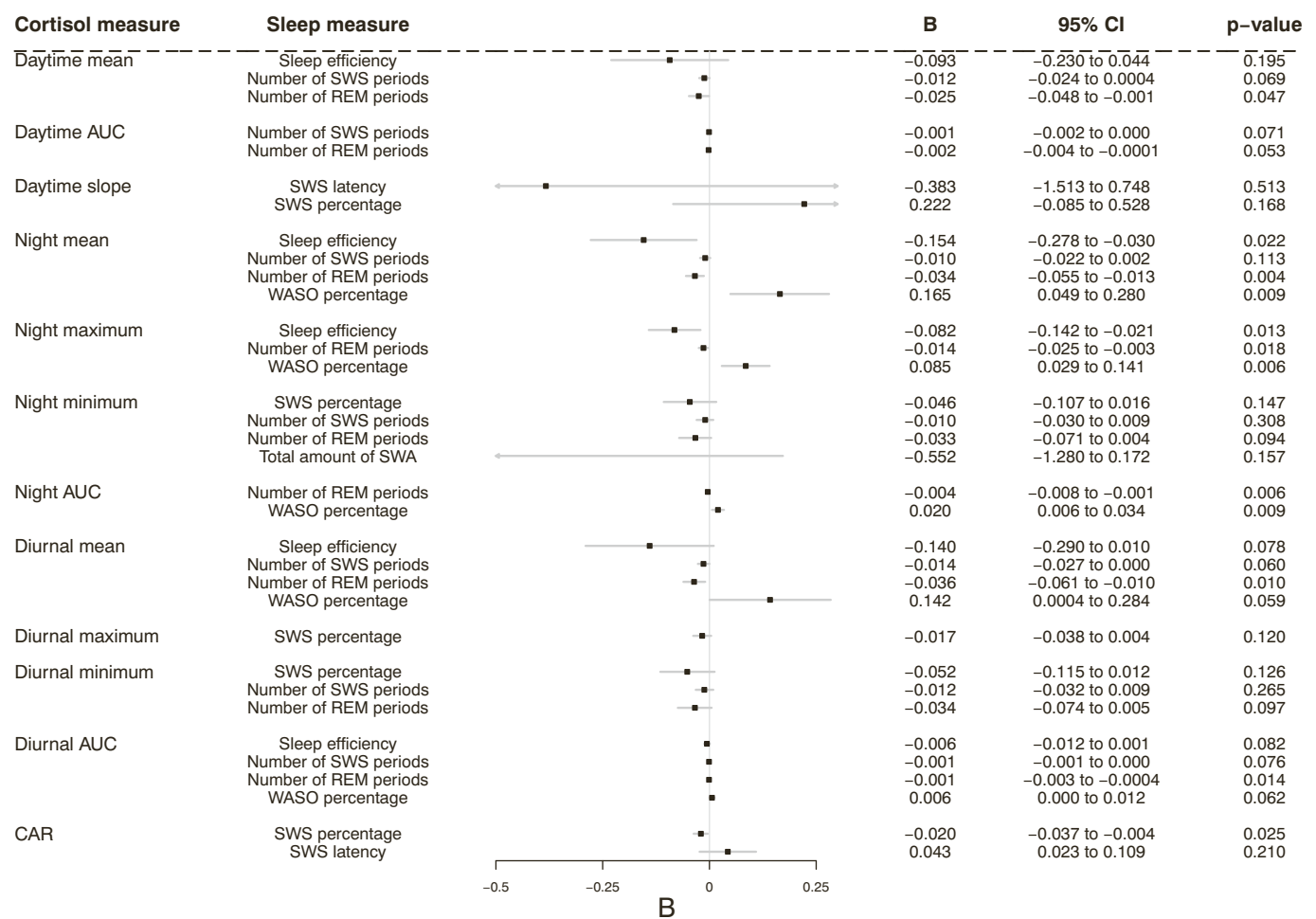


Fig. 4. Associations between sleep variables and cortisol levels in the 34 women included in the analyses. Results are expressed as B coefficients with 95 % Confidence Intervals. The model is adjusted for age, BMI, VMS and BDI. AUC, Area Under Curve; BDI, Beck Depression Inventory; BMI, Body Mass Index; CAR, Cortisol Awakening Response; REM, Rapid Eye Movement; SWS, Slow Wave Sleep; VMS, Vasomotor Symptoms; WASO, Wake After Sleep Onset.

Päivi Polo-Kantola was the leader of the study, a co-investigator, and co-writer of the paper.

Funding

The study was financially supported by a European Commission grant (QLK6-CT-2000-00499).

Ethical approval

The study was approved by the Ethical Committee of Turku University Central Hospital. All study subjects gave their written informed consent after receiving oral and written study information.

Provenance and peer review

This article was not commissioned and was externally peer reviewed.

Data sharing and collaboration

There are no linked research data sets for this paper. The authors do not have permission to share data.

Declaration of competing interest

The authors declare that they have no competing interest.

References

[1] P. Polo-Kantola, Sleep problems in midlife and beyond, *Maturitas* 68 (2011) 224–232, <https://doi.org/10.1016/j.maturitas.2010.12.009>.

[2] H.M. Kravitz, X. Zhao, J.T. Bromberger, E.B. Gold, M.H. Hall, K.A. Matthews, M. R. Sowers, Sleep disturbance during the menopausal transition in a multi-ethnic community sample of women, *Sleep* 31 (2008) 979–990.

[3] P. Polo-Kantola, A. Laine, M. Aromaa, P. Rautava, J. Markkula, T. Vahlberg, M. Sillanpää, A population-based survey of sleep disturbances in middle-aged women—associations with health, health related quality of life and health behavior, *Maturitas* 77 (2014) 255–262. doi: <https://doi.org/10.1016/j.maturitas.2013.11.008>.

[4] E.K. Adam, M. Kumari, Assessing salivary cortisol in large-scale, epidemiological research, *Psychoneuroendocrinology* 34 (2009) 1423–1436. <https://doi.org/http://doi.org/10.1016/j.psyneuen.2009.06.011>.

[5] E. Van Cauter, R. Leproult, D.J. Kupfer, Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol, *J. Clin. Endocrinol. Metab.* 81 (1996) 2468–2473, <https://doi.org/10.1210/jcem.81.7.8675562>.

[6] R. Katainen, N. Kalleinen, S. Teperi, T. Vahlberg, U. Turpeinen, E. Hämäläinen, L. Nelimarkka, P. Polo-Kantola P, The relationship between diurnal cortisol secretion and climacteric-related symptoms, *Maturitas* 115 (2018) 37–44. doi: <https://doi.org/10.1016/j.maturitas.2018.06.007>.

[7] N. Kalleinen, P. Polo-Kantola, K. Irjala, T. Porkka-Heiskanen, T. Vahlberg, A. Virkki, O. Polo, 24-hour serum levels of growth hormone, prolactin, and cortisol in pre- and postmenopausal women: the effect of combined estrogen and progestin treatment, *J. Clin. Endocrinol. Metab.* 93 (2008) 1655–1661, <https://doi.org/10.1210/jc.2007-2677>.

- [8] E.K. Adam, M.E. Quinn, R. Tavernier, M.T. McQuillan, K.A. Dahlke, K.E. Gilbert, Diurnal cortisol slopes and mental and physical health outcomes: a systematic review and meta-analysis, *Psychoneuroendocrinology* 83 (2017) 25–41, <https://doi.org/10.1016/j.psyneuen.2017.05.018>.
- [9] L. Thau, J. Gandhi, S. Sharma, Physiology, Cortisol, in StatPearls, Aug. 2022, Treasure Island (FL): StatPearls Publishing, 2023. <https://www.ncbi.nlm.nih.gov/books/NBK538239/>.
- [10] J. Born, E. Späth-Schwalbe, H. Schwachenhofer, W. Kern, H.L. Fehm, Influences of corticotropin-releasing hormone, adrenocorticotropin, and cortisol on sleep in normal man, *J. Clin. Endocrinol. Metab.* 68 (1989) 904–911, <https://doi.org/10.1210/jcem-68-5-904>.
- [11] A.N. Vgontzas, C. Tsigos, E.O. Bixler, C.A. Stratakis, K. Zachman, A. Kales, A. Vela-Bueno, G.P. Chrousos, Chronic insomnia and activity of the stress system: a preliminary study, *J. Psychosom. Res.* 45 (1998) 21–31, [https://doi.org/10.1016/S0022-3999\(97\)00302-4](https://doi.org/10.1016/S0022-3999(97)00302-4).
- [12] J.G. Abell, M.J. Shipley, J.E. Ferrie, M. Kivimäki, M. Kumari, Recurrent short sleep, chronic insomnia symptoms and salivary cortisol: a 10-year follow-up in the Whitehall II study, *Psychoneuroendocrinology* 68 (2016) 91–99, <https://doi.org/10.1016/j.psyneuen.2016.02.021>.
- [13] M. Kumari, E. Badrick, J. Ferrie, A. Perski, M. Marmot, T. Chandola, Self-reported sleep duration and sleep disturbance are independently associated with cortisol secretion in the Whitehall II study, *J. Clin. Endocrinol. Metab.* 94 (2009) 4801–4809, <https://doi.org/10.1210/JC.2009-0555>.
- [14] M.N. Rao, T. Blackwell, S. Redline, N.M. Punjabi, E. Barrett-Connor, T.C. Neylan, K.L. Stone, Osteoporotic fractures in men (MrOS) study group, association between sleep duration and 24-hour urine free cortisol in the MrOS sleep study, *PloS One* 8 (2013) e75205, <https://doi.org/10.1371/journal.pone.0075205>.
- [15] J. Zhang, R.C. Ma, A.P. Kong, W.Y. So, A.M. Li, S.P. Lam, S.X. Li, M.W. Yu, C.S. Ho, M.H. Chan, B. Zhang, Y.K. Wing, Relationship of sleep quantity and quality with 24-hour urinary catecholamines and salivary awakening cortisol in healthy middle-aged adults, *Sleep* 34 (2011) 225–233, <https://doi.org/10.1093/sleep/34.2.225>.
- [16] L.M. Gerber, L.L. Sievert, J.E. Schwartz, Hot flashes and midlife symptoms in relation to levels of salivary cortisol, *Maturitas* 96 (2017) 26–32, <https://doi.org/10.1016/j.maturitas.2016.11.001>.
- [17] T. Huang, E.M. Poole, C. Vetter, K.M. Rexrode, L.D. Kubzansky, E. Schernhammer, N. Rohleder, F.B. Hu, S. Redline, S.S. Tworoger, Habitual sleep quality and diurnal rhythms of salivary cortisol and dehydroepiandrosterone in postmenopausal women, *Psychoneuroendocrinology* 84 (2017) 172–180, <https://doi.org/10.1016/j.psyneuen.2017.07.484>.
- [18] N.F. Woods, E.S. Mitchell, Sleep symptoms during the menopausal transition and early postmenopause: observations from the Seattle midlife Women's health study, *Sleep* 33 (2010) 539–549, <https://doi.org/10.1093/sleep/33.4.539>.
- [19] N.F. Woods, M.C. Carr, E.Y. Tao, H.J. Taylor, E.S. Mitchell, Increased urinary cortisol levels during the menopausal transition, *Menopause* 13 (2006) 212–221, <https://doi.org/10.1097/01.gme.0000198490.57242.2e>.
- [20] N.F. Woods, E.S. Mitchell, K. Smith-DiJulio, Cortisol levels during the menopausal transition and early postmenopause, *Menopause* 16 (2009) 708–718, <https://doi.org/10.1097/gme.0b013e318198d6b2>.
- [21] M. Follenius, G. Brandenberger, J.J. Badesapt, J.P. Libert, J. Ehrhart, Nocturnal cortisol release in relation to sleep structure, *Sleep* 15 (1992) 21–27, <https://doi.org/10.1093/sleep/15.1.21>.
- [22] L. Redwine, R.L. Hauger, J.C. Gillin, M. Irwin, Effects of sleep and sleep deprivation on Interleukin-6, growth hormone, cortisol, and melatonin levels in humans, *J. Clin. Endocrinol. Metab.* 85 (2000) 3597–3603, <https://doi.org/10.1210/JCEM.85.10.6871>.
- [23] I. Girod, L. Abetz, C. de la Loge, C. Fayol-Paget, M.S. Hunter, Women's health questionnaire: user manual, Lyon MAPI res. Institute, 2004, pp. 1–55. <http://178.23.156.107:8085/Instruments/files/USERS/whq.pdf>.
- [24] A.T. Beck, C.H. Ward, M. Mendelson, J. Mock, J. Erbaugh, An inventory for measuring depression, *Arch. Gen. Psych.* 4 (1961) 561–571, <https://doi.org/10.1001/archpsyc.1961.01710120031004>.
- [25] N. Kalleinen, O. Polo, S.L. Himanen, A. Joutsen, P. Polo-Kantola, The effect of estrogen plus progestin treatment on sleep: a randomized, placebo-controlled, double-blind trial in premenopausal and late postmenopausal women, *Climacteric* 11 (2008) 233–243, <https://doi.org/10.1080/13697130802112033>.
- [26] M. Partinen, T. Gislason, Basic nordic sleep questionnaire (BNSQ): a quantitated measure of subjective sleep complaints, *J. Sleep Res.* 4 (1995) 150–155, <https://doi.org/10.1111/j.1365-2869.1995.tb00205.x>.
- [27] A. Rechtschaffen, A. Kales (Eds.), *A Manual of Standardized Terminology, Techniques and Scoring Systems for Sleep Stages of Human Subjects*, National Institute of Health, Washington DC, 1968.
- [28] I. Feinberg, T.C. Floyd, Systematic trends across the night in human sleep cycles, *Psychophysiol* 16 (1979) 283–291, <https://doi.org/10.1111/j.1469-8986.1979.tb02991.x>.
- [29] A. Clow, F. Hucklebridge, L. Thorn, The cortisol awakening response in context, *Int. Rev. Neurobiol.* 93 (2010) 153–175, [https://doi.org/10.1016/S0074-7742\(10\)93007-9](https://doi.org/10.1016/S0074-7742(10)93007-9).
- [30] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2023. <https://www.R-project.org/>.
- [31] L. Thorn, F. Hucklebridge, P. Evans, A. Clow, The cortisol awakening response, seasonality, stress and arousal: a study of trait and state influences, *Psychoneuroendocrinology* 34 (2009) 299–306, <https://doi.org/10.1016/j.psyneuen.2008.11.005>.
- [32] E. Fries, L. Dettenborn, C. Kirschbaum, The cortisol awakening response (CAR): facts and future directions, *Int. J. Psychophysiol.* 72 (2009) 67–73, <https://doi.org/10.1016/j.ijpsycho.2008.03.014>.
- [33] E. Van Cauter, R. Leproult, L. Plat, Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men, *JAMA* 284 (2000) 861–868, <https://doi.org/10.1001/jama.284.7.861>.
- [34] J. Born, W. Kern, K. Biebrer, G. Fehm-Wolfsdorf, M. Schiebe, H.L. Fehm, Night-time plasma cortisol secretion is associated with specific sleep stages, *Biol. Psychiatry* 21 (1986) 1415–1424, [https://doi.org/10.1016/0006-3223\(86\)90333-1](https://doi.org/10.1016/0006-3223(86)90333-1).
- [35] E. Morgan, L.P. Schumm, M. McClintock, L. Waite, D.S. Lauderdale, Sleep characteristics and daytime cortisol levels in older adults, *Sleep* 40 (2017) zsx043, <https://doi.org/10.1093/sleep/zsx043>.
- [36] I. Virtanen, P. Polo-Kantola, U. Turpeinen, E. Hämäläinen, N. Kalleinen N, Effect of external sleep disturbance on sleep architecture in perimenopausal and postmenopausal women, *Climacteric* 26 (2023) 103–109. doi: <https://doi.org/10.1080/13697137.2022.2158727>.
- [37] K.E. Moe, L.H. Larsen, M.V. Vitiello, P.N. Prinz, Estrogen replacement therapy moderates the sleep disruption associated with nocturnal blood sampling, *Sleep* 24 (2001) 886–894, <https://doi.org/10.1093/sleep/24.8.886>.
- [38] P. Prinz, S. Bailey, K. Moe, C. Wilkinson, J. Scanlan, Urinary free cortisol and sleep under baseline and stressed conditions in healthy senior women: effects of estrogen replacement therapy, *J. Sleep Res.* 10 (2001) 19–26, <https://doi.org/10.1046/j.1365-2869.2001.00236.x>.
- [39] A. Caufriez, R. Leproult, M. L'Hermite-Balériaux, M. Kerkhofs, G. Copinschi, Progesterone prevents sleep disturbances and modulates GH, TSH, and melatonin secretion in postmenopausal women, *J. Clin. Endocrinol. Metab.* 96 (2011) E614–E623, <https://doi.org/10.1210/jc.2010-2558>.