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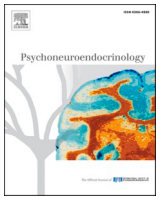

Altered coordination between sleep timing and cortisol profiles in night working female hospital employees

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ABSTRACT

Background: Cortisol typically peaks in the morning after waking up and declines throughout the day, reaching its lowest levels during nighttime sleep. Shift work can cause misalignment between cortisol levels and sleep-wake timing. We analyzed this misalignment in female shift workers focusing on the timing and extent of these changes.

Methods: We conducted a cross-sectional study involving 68 shift workers (aged 37 ± 10 years) and 21 non-shift workers (aged 45 ± 10 years) from a hospital. Shift workers were monitored through two day shifts and three night shifts, whereas non-shift workers were monitored during two day shifts. Each participant collected six to eight saliva samples (depending on their shift type) and provided sleep timing information, which was recorded via polysomnography and sleep diaries. Generalized additive mixed models were used to estimate shift-specific differences in cortisol smooth curves. Summary measures calculated for the cortisol smooth curves included cortisol awakening response, peak-to-bed slope, and total output.

Results: Between shift workers and non-shift workers, we observed similar diurnal cortisol profiles with a steep negative diurnal slope during day shifts. In shift workers on night shifts, a flattened U-shaped cortisol profile after the post-awakening maximum was observed, with a peak-to-bed slope close to zero. When comparing night to day shifts in the group of shift workers, mean cortisol levels were lower between 42 and 56 minutes and 1.8–11.9 hours after waking up, and higher between 14.9 and 22 hours after waking up.

Conclusion: Our findings indicate altered cortisol profiles in female hospital employees on night shifts. Specifically, cortisol levels were lower at night when higher levels would typically be necessary for work activities, and higher at bedtime after a night shift, when levels should normally be low.

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1. Introduction

In healthcare, shift and night work is essential to cover 24-hour patient care and emergency services (Costa et al., 2021). In contrast to significant societal benefits of shift and night work, there are, however, significant demands on worker health and safety. There is evidence for adverse health effects in night workers including cardiovascular problems, gastrointestinal disorders, cancer, metabolic diseases, as well as increased levels of depression, burnout, and anxiety (Boivin et al., 2021; Moreno et al., 2019). There is evidence for increased risk of accidents during night shifts (Folkard et al., 2005). One reason is that working at night forces employees to sleep during the day. Consequently, sleep is shorter, less restorative and of lower quality (Akerstedt, 2003; Chang and Peng, 2021). In addition, shift work alters light exposure, food intake, and exposure to physical and psychological stressors which have been shown to impact on the circadian system (Dibner, 2020).

Circadian cortisol rhythmicity is characterized by maximum levels within 30–45 minutes after awakening (i.e., cortisol awakening response, CAR), declining levels throughout daytime, a quiescent period of minimal secretory activity around midnight, and again an onset of increasing levels in anticipation of waking (Balbo et al., 2010; Copinschi and Challet, 2015). Cortisol production is under control of the hypothalamic-pituitary-adrenal (HPA) axis, which, in turn, is controlled by the suprachiasmatic nuclei, the main pacemaker of the circadian system (Oster et al., 2017). The circadian cortisol rhythm impacts on stress response, metabolism, cardiovascular and immune function, sleep-wake regulation, as well memory and learning processes (Oster et al., 2017).

Altered cortisol profiles in night-shift workers were shown, for example, in healthcare workers (Baba et al., 2015; Hung et al., 2016; Niu et al., 2015), police officers (Charles et al., 2016; Jensen et al., 2016b), oil rig personnel (Harris et al., 2010), and factory workers (Kudielka et al., 2007; Lac and Chamoux, 2004). In addition, laboratory studies also demonstrated changed cortisol rhythm (Weibel et al., 1996). Flattened diurnal cortisol slopes were shown to be associated with poorer physical (e.g., immune and inflammatory system dysregulation, fatigue, cancer, obesity) and mental health outcomes (e.g., depression symptoms and disorders) (Adam et al., 2017). A study in female hospital personnel demonstrated flattened cortisol profiles and lower total cortisol output (24-hour urinary cortisol) in night-shift workers (Hung et al., 2016). Charles et al. (2016) observed flattened cortisol profiles during night shifts compared to day shifts among police officers. Bracci et al. (2016) compared diurnal cortisol profiles in shift and daytime nurses working early morning shifts and observed lower morning cortisol levels in the shift-working nurses.

Research on cortisol in shift workers is challenging. One reason is that shift schedules are rarely comparable between studies. That means that differences in timing, duration, and intensity of night shifts affect the sleep-wake timing in the workers, resulting in different lengths of their waking periods. In addition, methodological incoherence troubles the comparison of different studies due to differences in number and timing of saliva samples and data analysis. Alteration or flattening of cortisol curves were described using different curve summary measures (e.g., peak-to-bed slope, wake-to-bed slope, AUC) (Abelson et al., 2023; Grosser et al., 2022; Sánchez et al., 2012). Further complicating is that individuals differ in the phase of entrainment of their circadian system which may be quantified as chronotype differences (Roenneberg et al., 2003) and which varies with age (Roenneberg et al., 2007). In addition to age and chronotype, night-work associated circadian misalignment has been suggested to affect menopause onset (Stock et al., 2019). The impact of shift work on the circadian system is not yet fully understood (Ritonja et al., 2019).

The present study aims to (i) compare cortisol profiles between day and night shifts in female hospital staff to help characterize alterations in cortisol levels with respect to timepoint and extent; (ii) compare diurnal cortisol patterns of two different day-shift schedules (i.e., regarding different shift starting times) between shift workers and non-shift workers; (iii) assess whether cortisol rhythm was shifted between two consecutive day and night shifts. For shift workers specifically, in addition, we analyzed cortisol profiles between night and day shifts with respect to participant age, chronotype, and menopausal status.

2. Materials and methods

2.1. Study population and design

Female hospital employees of the University Hospital Bergmannsheil Bochum, Germany, were recruited for a cross-sectional study. Participants were 25–60 years old and worked either day and night shifts or day shifts only. Exclusion criteria were current pregnancy, breast feeding in the last six months, ovarian stimulation, or a diagnosis of cancer. Between September 2012 and May 2015, a total of 100 women were recruited including 75 shift workers (68 nurses, seven laboratory staff) and 25 non-shift workers (nine nurses, eleven laboratory and, five administration staff). For cortisol analyses seven participants in total (six shift workers, one non-shift worker) had to be excluded: four participants due to glucocorticoid medication, two participants due to intake of antidepressants, and one participant due to severe obstructive sleep apnea (Supplementary Figure S1).

Shift workers were currently engaged in rotating shift work with night shifts. Their shift-work schedule was irregular, including three to five night shifts per month. The group of non-shift workers consisted of both never and former night-shift workers. The participants of this group worked regular day shifts without night shifts for at least two years prior to study start. Shift workers were studied on two consecutive day shifts and three consecutive night shifts. Day shifts lasted 8 hours and started between 06:00 and 07:00 in the morning. Night shifts lasted 9 hours and started between 21:00 and 22:00 in the evening. Non-shift workers were studied on two consecutive working days. These women worked an 8-hour day with shifts commencing at 6:00–6:59 h (N = 7 participants), 7:00–7:59 h (N = 14), 8:00–8:59 h (N = 4). The study protocol was designed that no participant worked night shifts within at least three days before each study period. All participants gave written informed consent prior to study start and received financial compensation (non-shift workers 100 €, shift workers 200 €). The study was approved by the Ruhr University Bochum Research Ethics Committee (No. 4450–12). More details of the study population are provided elsewhere (Burek et al., 2022; Rabstein et al., 2019).

2.2. Study procedures

Baseline evaluations took place after recruitment and included face-to-face interviews with anthropometric measurements. Women reported sociodemographic and lifestyle characteristics (age, sex, smoking status, etc.), menopausal status, length of menstrual cycle, irregular cycle lengths, a detailed shift-work history, job-related characteristics, health disorders, and current medication (including contraceptive use). Postmenopausal participants were identified by self-report (i.e., no menstrual bleeding for at least 12 months). Perimenopausal participants were identified either by self-report or as women aged from 40 to 49 years with irregular cycle lengths. All participants completed the Munich ChronoType Questionnaire for shift workers ($MCTQ_{shift}$) (Juda et al., 2013). Chronotype was based on an individual's mid-sleep, estimated as described by Rabstein et al. (2019) and categorized using the 25th percentile (3:11 h) and 75th percentile (4:47 h) as cut-offs for early, intermediate and late chronotypes. The day prior to study start (in most cases a Monday), participants received salivettes (Sarsted, Nuembrecht, Germany) to collect saliva samples, a diary about sleep, work/leisure times and momentary stress, and a SOMNOwatch™ plus the Rechtschaffen and Kales sensor module (SOMNOmedics GmbH, Randersacker, Germany) to record their sleep at home. For the current analysis, we only used the polysomnographically (PSG) recorded time of

wake up. More details of the PSG assessments are provided elsewhere (Burek et al., 2022). Beginning on Monday afternoon and for three following weekdays, participants filled their sleep times (bedtime, times of falling asleep and waking up, intra-sleep wake periods, time of light on, time of getting up), times of work (start and end times of each shift worked), and individual timepoints of extreme perceived levels of stress load using a 24-hour schedule template. Specifically, participants were advised to mark timepoints of stress load only in extreme, extraordinary stress situations during work or leisure time.

2.3. Saliva sampling

In each study group, participants collected saliva samples during two consecutive day shifts (Figs. 1A and 1B), and shift workers additionally collected saliva samples during three consecutive night shifts (Fig. 1C). Saliva sampling on day shifts started at waking up in the morning before the day shift schedule. Saliva sampling on night shifts started with the beginning of the night-work schedule. We defined saliva samples taken in reference to waketime: immediately at waking up (C1), 30 minutes after waking up (C2), at the start of the shift (C3), 2 hours after starting a shift (C4), in the middle of a shift (C5), 6 hours after starting shift (C6), at the end of work (C7), and immediately before going to bed (C8). During day shifts, C4 and C6 were not sampled. Participants were instructed to refrain from brushing their teeth and eating for at least 30 minutes prior to each sample collection. Participants marked saliva sampling times on the salivettes and recorded the sampling time in a log. Samples taken at home were kept in the fridge, samples taken at work were stored on cool packs until work time was finished. Study nurses from the research team collected the salivettes at the end of each shift.

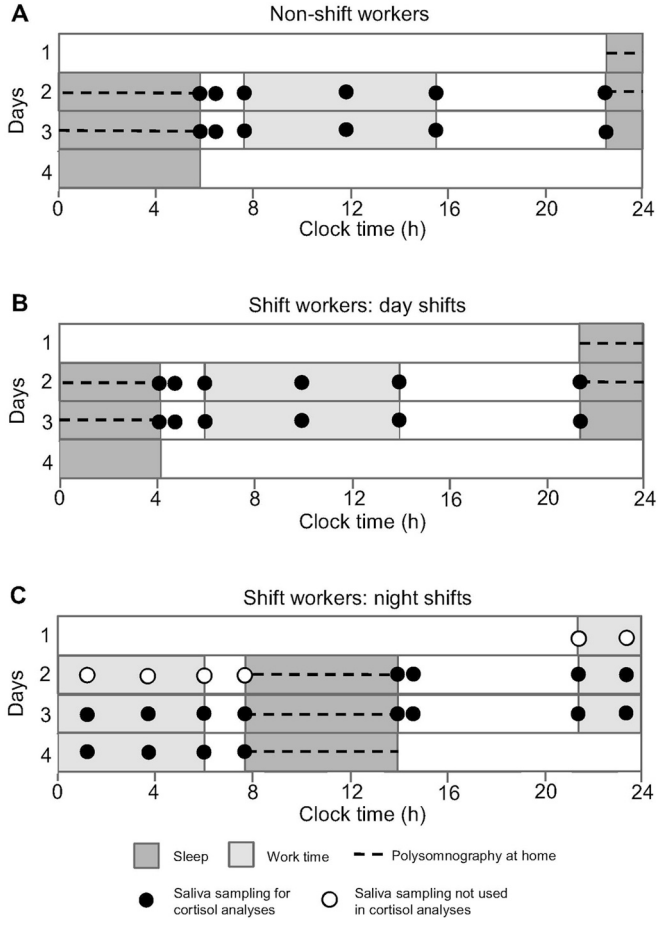

Fig. 1. Study protocol by group and shift for (A) non-shift workers, (B) shift workers on day shifts, and (C) shift workers on night shifts.

Saliva samples were immediately aliquoted and stored at −80 °C. Cortisol levels were determined employing a commercially available chemiluminescence assay (IBL-Hamburg, Hamburg, Germany). Intra-assay coefficients of variation ranged between 1.9% and 4.5%. Inter-assay coefficients of variation ranged between 3.6% and 4.3%.

2.4. Processing of "time since waking up"

Time of waking up was used as the reference point for the cortisol analysis. Times of saliva sampling were converted into time-since-waking-up (duration in hours). Time-since-waking-up relied on self-reported saliva sampling times, polysomnography recorded time of waking up from the SOMNOwatch, or, if SOMNOwatch data were not available for that day or of insufficient quality, the self-reported time of waking up from the sleep diary was used instead. For each collected saliva sample, the difference between sampling time and both times of waking up (SOMNOwatch and diary), was calculated. To ensure comparability of the cortisol profiles between participants, only study days with the first saliva sample taken within 0–15 minutes after waking up (verified by SOMNOwatch) were processed. If a first saliva sample at waking up was reported to have happened before waking up (from SOMNOwatch) or was taken later than 15 minutes, then the time of waking up from the sleep diary was considered. If a first saliva sample at waking up was reported to have happened before waking up from diary or was taken later than 15 minutes, all saliva samples collected on that day were omitted from the analysis. This procedure resulted in an exclusion of 439 saliva samples from the analysis (Supplementary Figure S1). In addition, we excluded 395 saliva samples collected during the first night shift, because time of waking up on the day before the first night shift was not recorded (Fig. 1C). For one woman, 22 saliva night-shift samples were excluded, because she had enrolled into the study on the fourth night shift instead of first night shift. Furthermore, 42 saliva samples were excluded due to missing saliva sampling times. Thus, the final analysis was performed on 216 saliva samples for non-shift workers on day shifts, 714 saliva samples for shift workers on day shifts, and 768 saliva samples for shift workers on night shifts (Supplementary Figure S1).

2.5. Statistical analyses

We applied generalized additive mixed models (GAMMs) (Wood, 2017) to analyze log-transformed cortisol levels as non-linear function of time since waking up. GAMMs are an extension of generalized linear mixed models with smooth functions $f(x)$ as part of the linear predictor. GAMMs have been previously applied to salivary cortisol data (Sánchez et al., 2012). Each smooth function $f(x)$ in a GAMM is defined as a weighted sum of k basis functions. Thin-plate regression splines (TPRS) (Wood, 2003) were applied as basis functions for the estimation of $f(x)$. The larger k, the wigglier is the fitted smooth function. Sufficient basis dimension k was chosen by using model diagnostics for a fitted GAMM. Fast restricted maximum likelihood (fREML) method was used to find the best fit of the smoothing parameter λ, which penalizes the "wiggliness" of the estimated smooth. Within-subject correlation needs to be considered because saliva samples were taken from the same participant over the time of wakefulness across two consecutive study days. GAMMs can work with missing observations, and inferences from the model are valid when the imbalance in the observations are missing at random or completely missing at random (Mundo et al., 2022). Depending on the fitted model, a random-effects structure (represented as random intercept, random slope, or random smooths) was included and was checked by model selection in advance. We fitted GAMMs with an ordered factor-predictor adjusted for confounders. Based on published literature, we adjusted for *a priori* defined confounders: age, chronotype, menopausal status, contraceptive use, extreme stress load, and current smoking status. We estimated smooth curves of cortisol for each level of the predictor (day shift, night shift), and then computed the non-linear

Table 1
Characteristics of study participants (N = 89) by study group and shift.

	Non-shift workers	Shift workers	*p*-value[a]
N	21	68	
Study days[b], n			
Day shift	37	120	
Night shift	NA	100	
Saliva samples, n			
Day shift	216	714	
Night shift	NA	768	
Age (years), mean (SD)	45.5 (10.1)	37.5 (10.3)	0.0032
Age group, N (%)			
25–34	3 (14.3)	34 (50.0)	
35–49	11 (52.4)	25 (36.8)	
50–60	7 (33.3)	9 (13.2)	0.0061
Working night shifts, N (%)			
Never	7 (33.3)	0	
Former	14 (66.7)	0	
Current	0	68 (100)	NA
Rotating shift work with night shifts (years), mean (SD)	9.2 (6.9)	14.4 (9.9)	0.0268
Time since last night shift (years), mean (SD)	10.0 (10.8)	NA	NA
Chronotype[c] (hh:mm), mean (SD)	3:13 (1:12)	4:15 (1:13)	0.0027
Chronotype group, N (%)			
Intermediate	9 (42.9)	14 (20.6)	
Early	9 (42.9)	32 (47.1)	
Late	1 (4.8)	22 (32.4)	0.0130
Missing	2 (9.5)	0	
BMI (kg/m^2), mean (SD)	28.2 (6.6)	26.2 (4.9)	0.2066
BMI group, N (%)			
<25 kg/m^2	9 (42.9)	37 (54.4)	
25–29.9 kg/m^2	5 (23.8)	17 (25.0)	
≥ 30 kg/m^2	7 (33.3)	14 (20.6)	0.4617
Current smoker, N (%)	2 (9.5)	23 (33.8)	0.0302
Menopausal status, N (%)			
Premenopausal	11 (52.4)	53 (77.9)	
Perimenopausal	1 (4.8)	6 (8.8)	
Postmenopausal[d]	4 (19.1)	8 (11.8)	
Surgical/other amenorrhea	5 (23.8)	1 (1.5)	0.0042
Contraceptive use[e], N (%)			
No	5 (45.5)	28 (52.8)	
Oral	4 (36.4)	19 (35.9)	
Other[f]	2 (18.2)	6 (11.3)	0.7272
Waketime (clock time), mean (SD)			
Day shift	05:12 (0:38)	04:37 (0:24)	
Night shift	NA	14:36 (1:29)	<0.0001
Bedtime (clock time), mean (SD)			
Day shift	22:31 (1:03)	22:12 (0:49)	
Night shift	NA	08:07 (1:01)	0.0561
Time since wake-up (hours), mean (SD)			
Day shift	17.1 (1.1)	17.6 (0.9)	
Night shift	NA	17.4 (2.1)	0.0090

Abbreviations: SD, standard deviation, NA, not available.
[a] *P*-values for Fisher's exact test for categorical variables or for two-sample t-test testing differences in means between non-shift and shift workers.
[b] Two consecutive study days in day shifts; two consecutive study days in night shifts
[c] Munich ChronoType Questionnaire for shift workers ($MCTQ_{shift}$)
[d] Self-report of natural menopause
[e] Among premenopausal
[f] Patch, implant, injection, or vaginal ring

difference between cortisol curves over time. Visualization of the smooth curves and their difference is needed to show how well the model fits the observed cortisol levels and to assess at which timepoints the smooth curves differ. 95% simultaneous confidence intervals (CI) were drawn around the estimated smooth curves and around the estimated difference between the curves (Marra and Wood, 2012; Wood, 2017). Time intervals of significant differences are timepoints at which the confidence interval for the estimated difference does not include the value zero.

GAMM was also applied to assess the difference in cortisol smooth curves between the two study days per study group and shift type (i.e., in non-shift workers, shift workers on day shift, and shift workers on night shift). We included a random intercept and slope per participant and study day in these three models. To examine the cortisol profiles in day and night shifts, we fitted the GAMM with fixed-effect and factor smooth per study group and shift type (i.e., in non-shift workers, shift workers on day shift, and shift workers on night shift) and random smooths for subject, group and shift type. In shift workers study group, we stratified analyses by age group (25−34, 35−49, 50−60), chronotype (early, intermediate, late), and menopausal status (premenopausal, perimenopausal, postmenopausal). For each variable, we fitted a GAMM with separate smooths of time since waking up by shift type and by each level of this variable. For example, in the GAMM for the age groups six cortisol smooths over the waking time were estimated, one smooth for each level of the interaction shift type by age group.

Data management and descriptive statistics were performed by SAS version 9.4. GAMM analyses were performed in the R statistical software (version 4.2.2; R Core Team 2022) using *bam* function from the package

'mgcv' (Wood, 2017). We used the package 'itsadug' (van Rij et al., 2020) for the visualization of fitted GAMMs and calculation of simultaneous CIs.

2.6. Summary measures of cortisol profiles

Cortisol smooth curves fitted from the GAMMs were additionally summarized (over the time interval [0,22] hours, in which the saliva samples were obtained), by CAR, peak-to-bed slope (Adam et al., 2017; Sánchez et al., 2012), and total area under the curve with respect to ground (AUC_G) (Pruessner et al., 2003). CAR describes the post-awakening rise in cortisol and was calculated as the area under the curve with respect to increase ($CAR_{AUC(I)}$) (Pruessner et al., 2003) from waketime to timepoint of post-awakening peak and as a difference (CAR_Δ) between cortisol levels estimated at timepoint of post-awakening peak and waketime. Peak-to-bed slope is the decline (for diurnal cortisol pattern) in cortisol levels from the timepoint of post-awakening peak to bedtime. AUC_G represents the total cortisol output and was estimated applying the trapezoidal rule over the time since waking up interval [0,22] hours (expressed as log(nmol/L) × hour). Simultaneous confidence intervals for the summary measures and their difference between the groups were calculated based on the posterior distribution from fitted GAMMs.

3. Results

3.1. Characteristics of the study participants

Table 1 shows characteristics of the 21 non-shift workers (eight nurses, ten laboratory and three administration staff) and 68 shift workers (61 nurses, seven laboratory staff). The group of shift workers was younger compared to non-shift workers (mean age 37.5 versus 45.5 years), included more women with premenopausal status (77.9% versus 52.4%), and more current smokers (33.8% versus 9.5%).

On day shifts the non-shift workers collected 216 saliva samples on 37 study days. Shift workers on day shifts collected 714 samples on 120 study days, and when on night shift they collected 768 saliva samples on 100 study days (Table 1). Supplementary Table 1 summarizes the number of saliva samples, saliva sampling times and cortisol levels by sampling timepoint, study group and work shift. In shift workers, mean cortisol levels before going to sleep after a night shift (C8) were at a comparable level to the mean morning-cortisol levels after sleep during days shifts (C1). We identified saliva samples taken during extreme levels of stress, which comprised about 4% of all samples in the final analysis (non-shift workers: n = 10 saliva samples, 4.6%; shift workers on day shifts: n = 29, 4.1%; shift workers on night shifts: n = 32, 4.2%).

3.2. Similar cortisol profiles between study days

Fig. 2 shows the salivary cortisol levels, fitted cortisol smooth curves for each study day, and the differences between the study days per group and work shift. Between the two study days, we observed similar cortisol

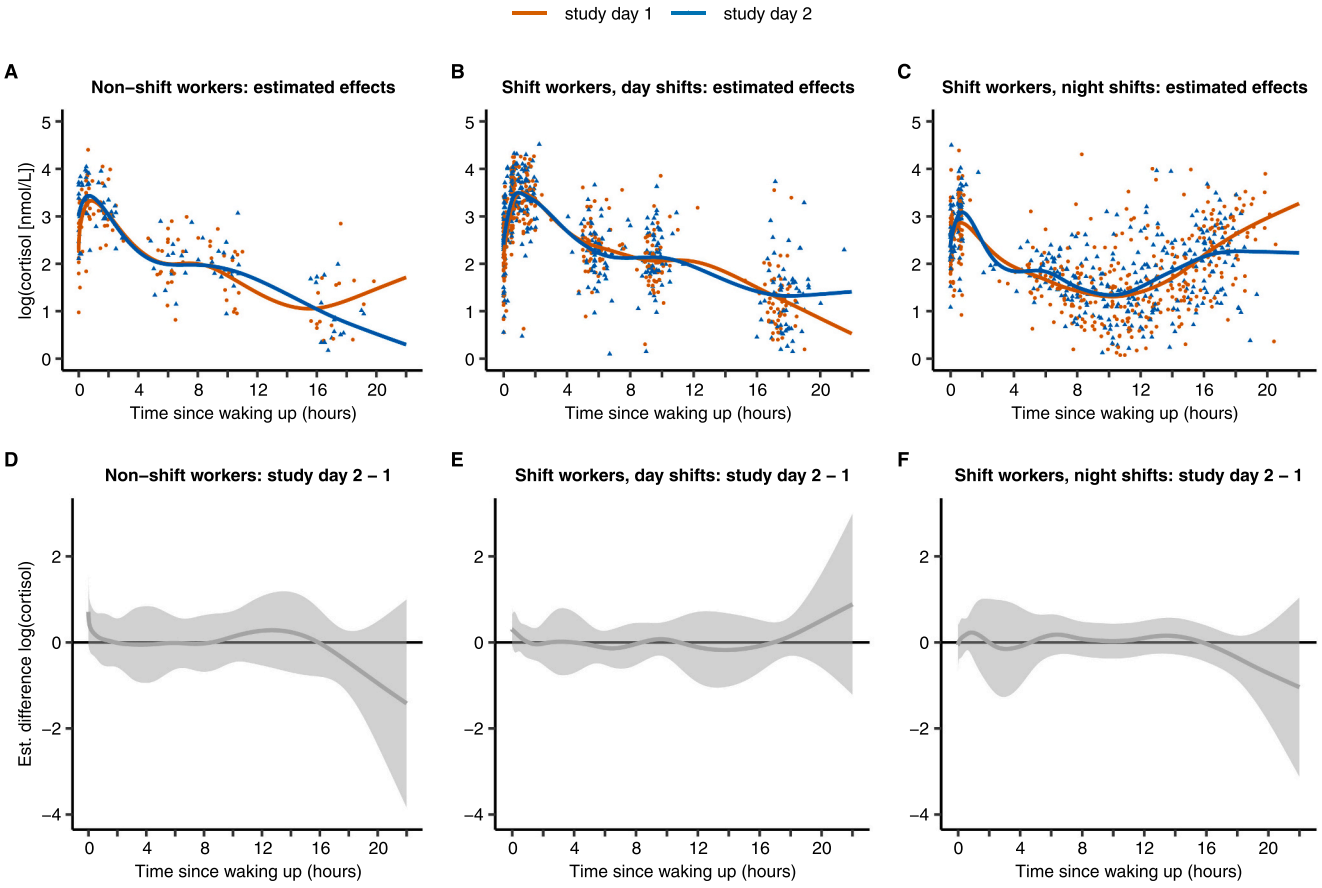

Fig. 2. Cortisol smooth curves by shift and study day, as well as the differences between study days. Individual salivary cortisol levels (points) and estimated cortisol smooth curves (with 95% simultaneous CI) across time since waking up plotted by study group and study day for (A) non-shift workers on day shifts, (B) shift workers on day shifts, and (C) shift workers on night shifts. Estimated difference and 95% simultaneous CI between study days in (D) non-shift workers on day shifts, (E) shift workers on day shifts, (F) in shift workers on night shifts. The random effects were set to zero. In panels D, E and F, the grey area represents durations of no difference (CI for the estimated difference include zero) between the curves (study day 2 compared to study day 1).

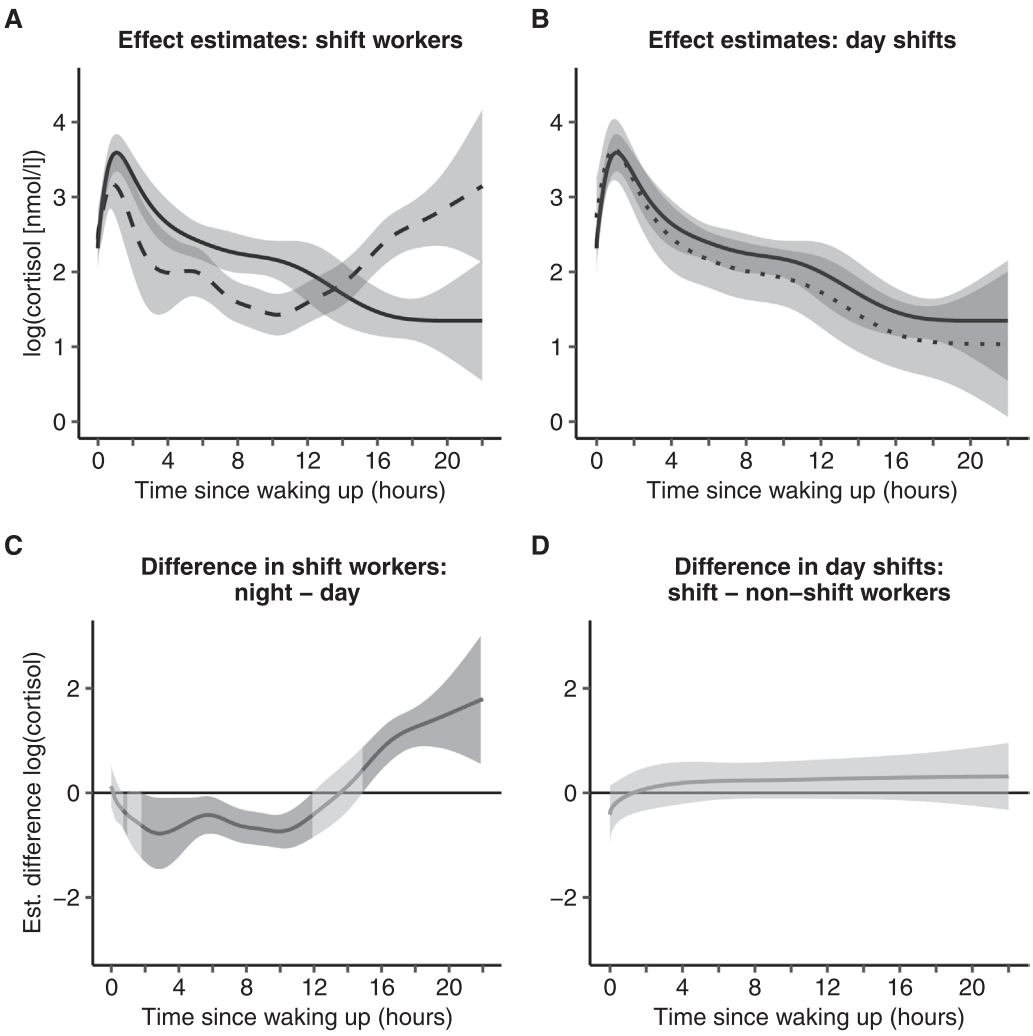

Fig. 3. Cortisol smooth curves by group and shift, and their differences between shifts. Estimated cortisol smooth curves with 95% simultaneous CI (A) on day shifts (——) and night shifts (- - -) of shift workers, and (B) on day shifts of shift workers (——) and non-shift workers (••••). Estimated difference and 95% simultaneous CI between (C) night and day shifts in shift workers, and (D) between day shifts in shift workers and non-shift workers. The random effects were set to zero. In panels C and D, the dark grey areas represent durations of difference between the curves, and the lighter grey areas represent durations of no difference between the curves.

patterns in all three comparison groups: in non-shift workers (Fig. 2A), shift workers on day shifts (Fig. 2B) and shift workers on night shifts (Fig. 2C). No statistically significant difference in cortisol patterns were observed because the simultaneous CIs of the difference curves cover the value zero over the entire waking time (Figs. 2D, 2E, 2F).

3.3. Altered cortisol profiles on day and night shifts

We observed different cortisol profiles (estimated cortisol smooth curves and their 95% simultaneous CI) comparing night to day shifts in the group of shift workers (Fig. 3A). In contrast, similar diurnal cortisol patterns during day shifts between shift workers and non-shift workers were observed (Fig. 3B). Mean cortisol levels in shift workers on night shifts were lower between 42 minutes to 56 minutes, and 1.8 hours to 11.9 hours after waking up compared to shift workers on day shifts (Fig. 3C). In shift workers on night shifts, mean cortisol levels were higher, with a change in trend starting around 10 hours after waking up, and statistically significant levels between 14.9 hours to 22 hours after waking up (before sleep after a night shift) (Fig. 3C). The timepoint of 14.9 hours after waking up corresponds to the second half of the night duty. There was no difference between cortisol smooth curves of shift and non-shift workers during day shifts (Fig. 3D). Supplementary Figure S2 shows cortisol smooth curves in shift workers on day and night shifts stratified by age, chronotype, and menopausal status (panels A, E, I). Panels B-D, F-H, J-L in Supplementary Figure S2 show the magnitude of the differences between night and day shift smooths for each stratum. We observed differences between night and day shift smooths in those 25–34 years (panel B; from 3.7 to 12.1 hours, and from 15.3 to 22 hours after waking up), intermediate types (panel G; 35 minutes to 11.8 hours, and 15.4–22 hours), and those with premenopausal status (panel J; 3.9–12.3, and 15.0–22). In the age group 50–60 (panel D) and for early types (panel F) there were differences between night and day shift smooths from 16.1 and from 14.0 hours after waking up, respectively.

3.4. Cortisol curves summary measures on day and night shifts

Table 2 shows the summary measures of the estimated cortisol smooth curves indicated in Fig. 3 (panels A and B). Cortisol levels at waketime in shift workers on day shifts were lower compared to cortisol levels at waketime in non-shift workers on day shifts (difference −0.41 log(nmol/L), 95% CI = −0.79, −0.02). We found higher CAR (both measures) among shift workers on day shifts in comparison to non-shift workers (difference in CAR_{Δ} = 0.37 log(nmol/L), 95% CI = 0.10, 0.63; difference in $CAR_{AUC(I)}$ = 0.29, 95% CI = 0.14, 0.43).

Table 2
Cortisol smooth curves summary measures and 95% simultaneous CIs by study group and shift.

	Non-shift workers	Shift workers		Difference[a] (95% CI)	Difference[b] (95% CI)
	Day shifts	Day shifts	Night shifts		
Waketime (95% CI) [log(nmol/L)]	2.73 (2.35, 3.10)	2.32 (2.10, 2.53)	2.44 (2.18, 2.69)	−0.41 (−0.79, −0.02)	0.12 (−0.18, 0.42)
CAR_{Δ} (95% CI) [log(nmol/L)]	0.91 (0.64, 1.17)	1.28 (1.11, 1.44)	0.73 (0.47, 0.99)	0.37 (0.10, 0.63)	−0.55 (−0.86, −0.24)
$CAR_{AUC(I)}$ (95% CI)	0.41 (0.24, 0.58)	0.70 (0.56, 0.84)	0.31 (0.15, 0.47)	0.29 (0.14, 0.43)	−0.39 (−0.60, −0.18)
Peak-to-bed slope (95% CI) [log(nmol/L)]	−2.96 (−3.75, −2.19)	−2.57 (−3.17, −1.98)	−0.02 (−0.82, 0.78)	0.40 (−0.21, 1.00)	2.54 (1.62, 3.49)
AUC_G(0–22 h) (95% CI) [log(nmol/L) × hour]	50.60 (45.85, 55.32)	53.29 (50.40, 56.12)	50.37 (46.97, 53.75)	2.68 (−2.18, 7.48)	−2.91 (−6.26, 0.48)

Abbreviations: CI, confidence interval; CAR_{Δ}, cortisol awakening response calculated as the difference between cortisol levels estimated at timepoint of post-awakening peak and waketime; $CAR_{AUC(I)}$, cortisol awakening response calculated as the area under the curve with respect to increase from 0 hours to timepoint of post-awakening peak; AUC_G(0–22 h), total area under the fitted cortisol smooth curve over the time since waking up from 0 to 22 hours (log(nmol/L) × hour). The summary measures described cortisol smooth curves showed in Fig. 3 and were estimated from generalized additive mixed model showed in Supplementary Table S2.

[a] Differences in summary measures between day shifts of shift workers and day shifts of non-shift workers (reference).

[b] Differences in summary measures between night shifts and day shifts (reference) within shift workers.

In shift workers, we found lower CAR (both measures) on night shifts compared to day shifts (difference in CAR_{Δ} = −0.55 log(nmol/L), 95% CI = −0.86, −0.24; and difference in $CAR_{AUC(I)}$ = −0.39, 95% CI = −0.60, −0.18). The peak-to-bed slope on night shifts was almost zero (−0.02, 95% CI = −0.82, 0.78) in comparison to the negative and steep peak-to-bed slope on day shifts in shift workers (−2.57, 95% CI = −3.17, −1.98). AUC_G across time since waking up from 0 to 22 hours was slightly lower on night shifts compared to day shifts in shift workers (difference in AUC_G(0–22 h) = −2.91 log(nmol/L) × hour, 95% CI = −6.26, 0.48).

Supplementary Table S4 shows $CAR_{AUC(I)}$, peak-to-bed slope, and AUC_G(0–22 h) of the estimated cortisol smooth curves in shift workers on day and night shifts stratified by age, chronotype, and menopausal status (Supplementary Figure S2; panels A, E, and I). For $CAR_{AUC(I)}$, differences between night and day shifts (meaning lower CAR on night compared to day shifts) were found in age group 25–34 (−0.55, 95% CI = −1.00, −0.08), late chronotypes (−0.72, 95% CI = −1.26, −0.19), and premenopausal women (−0.52, 95% CI = −0.95, −0.09). Difference in peak-to-bed slope was observed for all age groups, early and intermediate chronotypes, premenopausal and postmenopausal status. There were significant differences in AUC_G(0–22 h) for women 25–34 years (−12.55, 95% CI = −21.96, −3.26), intermediate chronotypes (−14.55, 95% CI = −23.83, −4.61), late chronotypes (−13.75, 95% CI = −24.9, −2.43), premenopausal women (−14.0, 95% CI = −23.0, −4.46) and postmenopausal women (−17.11, 95% CI = −29.74, −4.31).

4. Discussion

This study examined a possible misalignment between cortisol and sleep-wake timing in female shift workers with respect to timepoint and extent. We assessed cortisol profiles in a group of shift and non-shift workers from a German hospital during both day and night shifts.

In the group of shift workers, we observed differences in the shape of cortisol curves on day and night shifts. In women working on day shifts, a normal diurnal cortisol profile with a steep decline from the post-awakening peak to bedtime with a negative diurnal slope was observed. On night shifts, in contrast, a flattened U-shaped cortisol profile after the post-awakening maximum level was observed, with a peak-to-bed slope close to zero. Comparing cortisol profiles around bedtime between night and day shifts (on average 17.5 hours after waking up) suggests that the day sleep after night shifts was initiated at cortisol levels that usually occur during the circadian cortisol rise at night. These findings imply that day sleep after night shifts was initiated at cortisol levels higher than normal at bedtime. Previous studies in night workers have shown a higher prevalence of short sleep duration or poor sleep quality (Akerstedt, 2003; Boivin et al., 2021; Chang and Peng, 2021). Whether elevated cortisol levels prior and during day sleep after night shifts contribute to the reported sleep disturbances, cannot be answered from our data. The observation of flattened U-shaped cortisol profiles after the post-awakening maximum in night workers matches the results of a cross-sectional study in female hospital employees (Hung et al., 2016) and of an intervention study in male police officers (Jensen et al., 2016b). The authors of these two studies did not comment on the U-shape of the cortisol curves. However, Hung et al. (2016) concluded that the cortisol curve during night shifts was flattened, based on area under the curve measures over 24-hours. Other field studies found cortisol profiles during night shifts to be flattened, but not different in shape compared to cortisol profiles during day shifts (Charles et al., 2016; Kudielka et al., 2007). We, in turn, observed cortisol profiles during night shifts that were both flattened and different in shape.

Regarding the other cortisol summary measures among shift workers, we found lower CAR (calculated as CAR_{Δ}, and as $CAR_{AUC(I)}$) during night compared to day shifts. The result of lower CAR complements our previous work in the same study population, showing a negative association between night work and CAR_{Δ} (Burek et al., 2022). In our previous analysis, chronotype did not modify the association between night shift and CAR_{Δ}. In the present study, we found differences between chronotypes in their cortisol profiles at distinct timepoints after the post-awakening maximum, though. The chronotype-specific differences were driven by differences in cortisol levels collected during work at night. For example, the normal circadian rise in cortisol occurred earlier in early chronotypes compared to intermediate and late chronotypes. With respect to age, we found that cortisol levels in the age group 50–60 years differed solely around bedtime between night and day shifts in the group of shift workers. That basal cortisol levels tend to decrease in amplitude with increasing age has been previously shown (van den Beld et al., 2018). Comparing cortisol profiles between night and day shifts stratified by menopausal status, we found no differences in perimenopausal or postmenopausal women. This lack of difference in the cortisol profiles most likely results from flattened cortisol curves during night shifts in these groups of women.

Comparing cortisol levels on day shifts of shift and non-shift workers, we did not identify differences in diurnal cortisol profiles. However, when comparing curve summary measures, we observed lower waketime cortisol and higher CAR (CAR_{Δ} and $CAR_{AUC(I)}$) on day shifts in shift workers opposed to non-shift workers. These findings are consistent with observations of other studies in day workers (Bostock and Steptoe, 2013; Bracci et al., 2016; Karlson et al., 2006). In our previous work (Burek et al., 2022), comparing waketime cortisol and CAR after night sleep between female shift and non-shift working hospital staff, we suggested that the differences in CAR resulted from differences in the times of waking up, which resulted from taking cortisol samples at different circadian phases (i.e., times of different secretory activity).

One aspect in the discussion around adjustment to shift work (shift-work tolerance), is the aspect of circadian rhythmicity (Ritonja et al., 2019). An important factor is the number of consecutive night shifts

(Hennig et al., 1998; Jensen et al., 2016b, 2016a; Niu et al., 2015). Three consecutive night shifts (as in our study) are usually not expected to impact significantly on the circadian system (Jensen et al., 2016a). Previous research has shown that a minimum of five consecutive night shifts are needed to initiate phase shifts in cortisol rhythm (Hennig et al., 1998; Niu et al., 2015). Therefore, we think that our findings indicate a respective lack of circadian adjustment to the night shift in our population. For two reasons, these findings should have a major impact on shift and night workers: first, night workers need to sleep during the day, and if their day sleep is compromised, recovery from night work is reduced, leading to increased levels of fatigue (Wong et al., 2019). Second, incomplete circadian adjustment results in night work taking place during the quiescent period of cortisol secretion. This, in turn, affects the diurnal rhythmicity of the HPA axis which is discussed to help coping with demands and stressors, supporting the process of allostasis (McEwen, 1993; McEwen and Karatsoreos, 2015). But if demands and stressors occur during the cortisol quiescent period, i.e., if working night shifts, disruptions to metabolism, cardiovascular and immune function, sleep-wake regulation, as well memory and learning processes can be expected (Oster et al., 2017). As a result, shift workers experience less recovery from day sleep and potentially less coping capacity during night work, increasing the allostatic load (Minelli et al., 2021; Rao and Androulakis, 2019).

5. Strengths and limitations

One strength of our study is the modelling of cortisol profiles across two consecutive days of day and night shifts based on pre-timed saliva samples. Sampling times were processed to start between 0 and 15 minutes after an individual's time of waking up. This procedure allowed the intra- and inter-individual comparisons of cortisol profiles. Compared to other studies, we were able to describe the cortisol profiles in detail because of the following three reasons: first, we collected saliva samples at several timepoints during night shifts; second, we used the actual timepoints of the saliva sampling (instead of averaging per timepoint); and third, participants in our study population differed with respect to the timing of their night shifts, which resulted in different bed- and wake up times. The GAMM approach can help refine the design of future studies aiming to detect timepoints at which significant changes in secretion may be expected (Mundo et al., 2022).

Our study also has several limitations. Amplitude of a circadian rhythm can be only estimated based on full cycle data (Klerman et al., 2022). We did not collect full cycle data because we only sampled cortisol when participants were awake, but not during times of sleep. Therefore, we refrained from determining the cortisol amplitude, because no consensus regarding a definition of amplitude of the cortisol rhythm has been published yet. Our study population solely encompassed female healthcare workers, which limits the transfer of the results to male workers or other professions. For the analysis for the age group 50–60 years and for perimenopausal and postmenopausal women we had limited power, due to the small number of participants. Although we compared cortisol profiles between night and day shifts in the same individuals, we cannot exclude a potential bias from a healthy-worker effect in our study population (Moreno et al., 2019). It seems plausible that women who are better adapted to night work (e.g., less disrupted in their cortisol rhythm) continued working shifts compared to those less well-adapted (e.g., more disrupted in their cortisol rhythm) who could have opted out of shift work. Another source of potential selection bias in our study is the exclusion of mistimed saliva samples. If a first saliva sample was not taken within 0–15 minutes after waking up, then all saliva samples collected on that day were omitted from the analysis. The mistiming of the saliva samples may have arisen due to non-compliance to saliva sampling protocol or due to self-reported saliva sampling times (Stalder et al., 2016). In our previous work in this study population, we discussed the impact of the mismatch between time of waking up and first saliva sampling on CAR (Burek et al., 2022), and showed that applying a stricter margin of 5 minutes after waking up did not change the findings regarding the CAR. Also, we did not adjust for times of food intake or times of light exposure.

6. Concluding remarks

We found altered coordination between sleep and cortisol in our night-working participants. Their cortisol rhythm was not shifted to the same extent as their sleep-wake rhythm. The CAR in our shift workers remained aligned with the process of waking up, albeit reduced in amplitude, whereas the rest of the cortisol profile remained under the control of the circadian system. This resulted in two main issues: lower cortisol levels at night when high cortisol levels are needed for work, and higher cortisol levels after the night shift when needed for sleep. This misalignment between cortisol, sleep, and work may contribute to the potential of shift work to negatively impact health. Such a constellation not only puts the individual worker at risk but also, for example, affects patients who rely on healthy and alert staff, which is imperative in hospital settings (Behrens et al., 2019).

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Thomas Kantermann: Resources, Writing – review & editing. **Sylvia Rabstein:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – review & editing. **Katarzyna Burek:** Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing, Software. **Martin Lehnert:** Investigation, Writing – review & editing. **Rui Wang-Sattler:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Céline Vetter:** Resources, Validation, Writing – review & editing. **Thomas Behrens:** Conceptualization, Supervision, Writing – review & editing. **Thomas Brüning:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Karl-Heinz Jöckel:** Writing – review & editing. **Dirk Pallapies:** Conceptualization, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.107066.

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