Original article

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Night shift work and other determinants of estradiol, testosterone, and dehydroepiandrosterone sulfate among middle-aged nurses and midwives

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Objectives The aims of our study were to (i) investigate the association between rotating night shift work and blood concentrations of estradiol, testosterone and dehydroepiandrosterone sulfate (DHEAS) and (2) evaluate the role of their non-occupational determinants.

Methods A cross-sectional study was conducted on 345 premenopausal and 187 postmenopausal nurses and midwives (263 women working rotating night shifts and 269 women working during days). Data from in-person interviews were used, anthropometric measurements were performed, and body mass index (BMI) and waist-to-hip ratio were calculated. Morning blood and spot urine samples were collected. Multiple linear regression models were fitted with hormone concentrations as dependent variables, and night shift work characteristics and demographic, reproductive, lifestyle and anthropometric determinants as independent variables. Modification of the effect by chronotype was examined.

Results Among postmenopausal women, we observed a statistically significant positive association between the total duration of night shift work >15 years and estradiol level ($P<0.05$ when compared to night work duration <5 years). Night shift work characteristics were significantly associated with estradiol among morning-type postmenopausal women. The well-established associations between hormones and their major determinants, such as age and BMI, were confirmed.

Conclusions The findings of our study imply that prolonged night shift work may be associated with increased estradiol levels among postmenopausal women, especially among the morning-type postmenopausal women.

Key terms midwife; sex hormone; women.

Sex hormones that serve essential functions in women's physiology are also known to play a role in the etiology of common diseases such as osteoporosis and bone fractures (1), breast cancer (2, 3), and cardiovascular diseases (1). Both experimental and observational studies confirmed the role of estrogens in the etiology of breast cancer (4). Strong epidemiological evidence for the association between circulating estrogens and breast cancer was established among postmenopausal women, although this relationship was less convincing among premenopausal women (5, 6). Also, increased circulating concentrations of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS) and testosterone were associated with breast cancer risk among women (5, 7). A pooled analysis of data performed by the Endogenous Hormones and Breast Cancer Collaborative Group provided strong evidence that plasma sex hormone levels predict breast cancer risk (2, 7), and the Breast Cancer Prevention Collaborative Group recommends measuring these hormones in plasma to assess the risk of breast cancer (8).

Shift work causing circadian disruption was linked to the risk of breast cancer, and the International Agency for Research on Cancer (IARC) has classified night shift work which disrupts the circadian rhythm as probably carcinogenic to humans (9). Working at night and

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exposure to artificial light at night have been proposed as potent circadian rhythm disruptors (10), which could contribute to physiological disturbances and – in a long run – to the etiology of various chronic diseases. There have also been a number of epidemiological studies focusing on an association between night shift work and breast cancer risk. The most recent meta-analysis based on 16 epidemiological studies reported a 9% increase in breast cancer risk per five years of night work based on case–control but not cohort studies (11). Still the most recent cohort study in Sweden showed breast cancer risk that increased by 77% among long-term female night workers (12).

One of the proposed mechanisms underlying the increased risk of breast cancer among night shift workers was a decrease in melatonin level and an increase in reproductive hormone synthesis in response to light-at-night (13). Moreover, night workers were more likely than day workers to become obese (14, 15) and to have unhealthy lifestyle (16) such as smoking (17), unhealthy diet (16) or low physical activity (18), which may contribute to the risk of cancer and affect hormonal milieu and sex hormone metabolism (19, 20).

There has been a limited number of observational studies examining the potential alterations in sex hormones or their precursor DHEA among women working night shifts (13, 21–27). In a study investigating the association between the duration of night shift work and estradiol level among 663 postmenopausal women, a significant positive relationship was found (21). Another study among postmenopausal women, comparing hormones among those who ever worked graveyard versus those working during the days (25), did not reveal differences, although the number of women classified as night workers who ever worked the graveyard shift was small (N=7). No statistically significant difference in estradiol level by night shift work status among postmenopausal women was noted in the study from Spain, but adjusted mean of estradiol was higher among night than day workers (24.3 pg/ml versus 15.04 pg/ml) (27). Two recent analyses have shown a positive link between estradiol and night work among premenopausal women when the hormone was measured in the follicular phase (24, 27) or when the analysis was adjusted for the menstrual phase (27). Statistically significant associations between the duration of night shift work and estradiol were observed in crude analysis among 82 Canadian premenopausal nurses with mean night work history of 11 years but not in an adjusted analysis (23). Another study among premenopausal nurses (N=79) selected from NHSII cohort showed no association between the number of night duties during two weeks before blood draw and estradiol, testosterone and DHEAS concentrations, but the majority of nurses in this study (82%) reported no night duties during this period (21). Given these sparse data as well as the relatively small scale of the studies and the differences in the exposure definition used, no definite conclusions regarding the relationships between night shift work and estradiol, testosterone or DHEAS could so far be drawn.

Factors such as age, menopausal transition and the phase of menstrual cycle in premenopausal women are well-recognized determinants of the circulating levels of sex hormones, but other possible modifying factors have not been fully identified. The largest epidemiological study thus far, which was based on a population of over 6000 postmenopausal women from 13 studies, demonstrated several associations between sex hormones and, for example, obesity, smoking and alcohol consumption, while the findings did not confirm associations for other factors (7).

We used data from a cross-sectional study on Polish nurses and midwives to examine night shift work and other determinants of blood concentrations of estradiol, testosterone and DHEAS among women, taking their well-evidenced role in breast carcinogenesis into consideration. Since the hormonal milieu differs substantially before and after menopause, we performed separate analyses for pre- and postmenopausal women.

Methods

This cross-sectional study was described elsewhere (28). Carried out between 2008–2011, the study included nurses and midwives, aged 40–60 years, who were currently employed in public healthcare in Lodz, Poland. Of 1117 nurses and midwives randomly selected for the study from the registry run by the Chamber of Nurses and Midwives in Lodz, 924 (83%) were contacted, and 866 had their current employment confirmed. As many as 725 agreed to participate in the study, and blood samples were obtained from 710 subjects. The following categories of women were excluded from the present analysis, those who: (i) reported using oral contraceptives or hormone replacement therapy (N=70); (ii) had the uterus removed before the age of 50 years (mean age of menopause) (N=18); and (iii) had the uterus removed after 50 years of age but had estradiol concentrations >50 pg/ml (upper limit for postmenopausal women) (N=8). In addition, 18 premenopausal women for whom no information could be obtained on the date of their last menstruation or the time lapse since their last period exceeded 60 days were also excluded. Two women had unusually high concentrations of estradiol or testosterone and were consequently excluded as outliers. The final dataset referred to 594 women. To avoid the potential confounding from the short lapse of time since the last period among premenopausal women,

we excluded 62 women who had reported having their last period within a year before the interview. Thus, the analyses included as many as 345 premenopausal and 187 postmenopausal women (263 women were currently working on rotating night shifts and 269 worked only during the days).

Nurses currently working night shifts were employed according to the fast rotating system, with typically a night shift followed by a day off. At hospitals, night duties usually start at 19:00 hours and last for 12 hour, while day shifts run from 07:00–19:00 hours, with no evening shifts. The working schedules vary depending on the organization and also on a month-to-month basis within the same organization, thus no typical roster could be described. The most frequent working schedule was 6–7 night duties per month (76.8% of current night workers); 30 women working night shifts (11.4%) had from 2–5 night duties per month, 27 (10.3%) had 8 night duties, and 4 (1.5%) had >9 duties, within a month, and the maximum was 14 duties reported by a single woman. There were no permanent night workers in the study population.

Day workers worked mostly in the outpatient clinics and the shift usually lasted 7.5 hours, from 07:00–16:00 hours.

Among the subjects currently working day shifts, the majority had some history of night shift work, but most of them (83%) had resigned from night shifts more than five years before the onset of the present study.

A structured questionnaire was administered during in-person interviews to elicit information on demographics, medical and reproductive history, hormone use, physical activity [according to the International Physical Activity Questionnaire (IPAQ)] (29), smoking and alcohol use, diet, sleep quality [using the Pittsburgh Sleep Quality Index (PSQI)] (30) and occupational history, including specific questions on current job and previous jobs held for ≥6 months.

The question that we used in our study to identify respondent's chronotype was partly adapted from the Horne-Ostberg Questionnaire – self assessment final question (31). The actual question asked was: "Which type of person do you consider yourself to be?" with responses "lark" or "owl".

The study participants completed a 7-day sleep and work diary, recording the start and end time of work for each day before blood sample collection.

Women were classified as premenopausal if they reported having menstrual periods.

Blood sample and spot morning urine sample were collected on average 1–2 weeks after the interview. Directly before blood collection, a short interview was conducted to elicit, among others, information on the date of the last menstrual period. Based on this date and the date of sample collection, the current stage of menstrual cycle was determined for the premenopausal women. Three categories were distinguished, namely the follicular (0–10 days), mid-cycle (11–16 days) and luteal (≥17 days) stage.

In spot morning urine samples, a biomarker of melatonin [ie, 6-sulfatoxymelatonin (creatinine adjusted)] was measured as described in our previous paper (28).

Body weight, height, waist and hip circumference were measured, and body mass index (BMI) and waist-to-hip ratio (WHR) were calculated.

The ethical institutional review board at the Nofer Institute of Occupational Medicine in Lodz, Poland, approved the study, and a signed informed consent was obtained from each study participant.

Blood sample collection and sex hormone analysis

Blood samples were collected into S-Monovette® heparinized test tubes (Sarstedt AG & Co, Nümbrecht, Germany) between 06:00–10:00 hours. For night workers, blood samples were drawn at the end of their night shift (which was after a day off except for 14 women who had a day shift work preceding the night shift), and for day workers, before or at the beginning of their work. Trained nurses collected the samples, which transferred to the laboratory. Estradiol (E2) concentration was determined in fresh plasma samples during the data collection phase of the study, ie, between 2008–2010. The remaining samples were frozen at -80°C and stored until DHEAS and testosterone (T) analyses, which were performed in 2010. The electrochemiluminescence immunoassay method (ECLIA, Cobas®, Roche, Diagnostics, Elecsys 2010) was used according to the manufacturer's instructions on determining DHEAS, E2 and T concentration in blood plasma. Detection limits were 5.0 pg/ml for E2, 0.025 ng/ml for T, and 0.100 µg/dl for DHEAS.

In 36 women, E2 concentration was below the sensitivity of the assay and, in 10 women, T concentration was below the detection level. In these cases, the sensitivity level was entered. As many as 10% of the samples were analyzed twice, and a high correlation was obtained between the two measurements: 0.99 for T, 0.98 for DHEAS, and 0.94 for E2 ($P<0.001$ for each correlation).

Statistical analysis

Arithmetic means with standard deviations and frequencies of the basic characteristics were calculated.

To satisfy the assumptions of the linear regression model, hormone concentrations were log transformed and then retransformed to obtain adjusted geometric means. Linear regression models were fitted with each hormone concentration as the dependent variable.

With regard to the rotating night shift work charac-

teristics, we examined the current night shift work status (yes/no), night shift work frequency (2–7, 8–14 night shifts per month), and the total duration of employment involving night shift work (≤5, >5–≤15, >15–≤ 25, >25 years).

For this part of the analysis, the list of potential confounders tested included age, BMI, age at menarche, number of full-term births, age at first full-term birth, years since and age at menopause (in postmenopausal women), menstrual stage (in premenopausal women), calendar season of the year, hour of blood collection, chronotype, sleep quality index, usual sleep duration, total physical activity, recreational activity, alcohol drinking and smoking cigarettes. Only these variables that turned out to be significant (P<0.05) in the multivariate models were retained in the final analysis. The list of the covariates included in the final (basic) models varied depending on the hormone and menopausal status (see table 2).

To examine the potential role of BMI, smoking and total physical activity, we carried out the analyses with all these factors incorporated in the models.

We also examined a potential effect of MT6s by introducing this parameter into the basic model and the one extended with lifestyle-related factors. The results remained substantially unchanged and thus are not presented.

Of the non-occupational determinants of the examined hormones, the following were considered: age (ranges 40–<45, ≥45–<50, ≥50–<55 and ≥55–60 years; the two extreme age groups: younger of postmenopausal and older of premenopausal women, being combined due to a small number of subjects), BMI (-<25, ≥25–<30, ≥30kg/m^2), WHR (≤0.85, >0.85), age at menarche (<12, ≥12–≤13, ≥14 years), number of full-term births (1, 2, ≥3), age at first full-term birth (≤25, >25 years), total physical activity expressed in MET[1]×hour per week (in tertiles: ≤179.3; >179.3–≤248.6, and >248.6), recreational activity (any, none), smoking (never, past-, current) and years since menopause in postmenopausal women (<1, 1–<5 , ≥5–<10, and ≥10 years). When examining the associations between hormones and reproductive and lifestyle factors, such covariates as age (continuous), calendar season of the year (October-March versus April–September), BMI (continuous) and phase of menstrual cycle (follicular, mid-cycle, and luteal, in premenopausal subjects) were included in the regression models. In order to avoid over adjustment, age was not included in the multivariate analysis of age groups, and BMI was not included in the adjusted analysis of BMI and WHR. In T analysis, we also controlled for the time of blood drawing. (Neither E2 nor DHEAS concentration was associated with the hour of blood draw.)

Given that the sleep behavior could be affected during 24 hours before the drawing of blood, depending on whether the woman worked or had a day off, we additionally determined these data based on the sleep and work 7-day diaries. As many as 83 day workers had their blood drawn after a day off, and 14 night workers reported to have day shift and following night shift. Neither of these characteristics was found to impact on hormone concentrations in the univariate analyses [β statistically insignificant (P>0.15) for each hormone] and thus they were not included in further analyses (data are not presented).

The tests for trends were conducted by entering the categories of the specific characteristics, ie, occupational characteristics, age groups, and categories of body composition, and reproductive and lifestyle factors as ordinal values.

Current rotating night shift work was examined as a potential confounder of the associations between hormones and age, BMI, reproductive or lifestyle factors and included into the models as a covariate. It was also tested as a potential modifier. Statistical significance of the effect modifier was determined using the likelihood ratio test to compare models with and without interaction term for specific characteristics and current system of work (32).

We also examined chronotype as a potential confounder or modifier in the analyses of associations for night shift work characteristics, using the approach described above for current night shift work.

All the analyses were run separately for pre- and postmenopausal women, using STATA 11 (StataCorp LP, College Station, TX, USA).

Results

The basic characteristics of the study population by current night work within the strata by menopausal status are presented in table 1. The mean age of the rotating night workers was similar to that of day worker both in the group of pre- and postmenopausal women (~46 years among premenopausal and ~55 years among postmenopausal women). Postmenopausal night workers tended to be heavier than day workers. Current smoking was more frequent among night workers than day workers in both pre- and postmenopausal women. Total physical activity was higher among night workers, while this group reported being engaged in recreational activity less frequently than day workers. Blood samples were more frequently collected between April and September among night than day workers. Postmenopausal day

[1] Metabolic equivalent ratio of the metabolic rate to a standard resting metabolic rate of 1, MET=1 resting metabolic rate during quiet sitting ≈ 3.5 ml O_2 kg^{-1} min^{-1} in adults.

Table 1. Selected characteristics of the study population by menopausal status and work system. [BMI=body mass index; DHEHS= dehydroepiandrosterone sulfate; MET=metabolic equivalent; PSQI=Pittsburgh Sleep Quality Index; SD=standard deviation]

Characteristics	Premenopausal women (N=345)								Postmenopausal women (N=187)							
	Night shift work (N=191)				Day work (N=154)				Night shift work (N=72)				Day work (N=115)			
	Mean	SD	N	%	Mean	SD	N	%	Mean	SD	N	%	Mean	SD	N	%
Age (years)	45.9	4.0			46.4	3.9			54.5	2.8			55.1	2.9		
BMI (kg/m^2),	26.2	4.4			26.8	4.5			29.4	5.2			28.0	5.1		
Waist-to-hip ratio	0.8	0.1			0.8	0.5			0.8	0.1			0.8	0.1		
Age at menopause (post-menopausal women) (years)									48.8	3.3			49.5	3.8		
Smoking																
Current			62	32.5			39	25.3			30	41.7			38	33.0
Past			44	23.0			43	27.9			16	22.2			36	31.3
Non-smoker			85	44.5			72	46.8			26	36.1			41	35.7
Total physical activity (MET hours per week)	268.8	111.8			209.2	103.8			240.0	104.5			192.5	82.6		
Recreational activity [a]																
Any			132	69.1			111	72.1			51	70.8			89	77.4
None			58	30.4			43	27.9			21	29.2			26	22.6
Alcohol consumption (drinks per week)	0.57	0.77			0.67	0.88			0.48	0.50			0.55	0.60		
Season when blood samples were collected																
October-March			101	52.9			101	65.6			36	50.0			70	60.9
April-September			90	47.1			53	34.4			36	50.0			45	39.1
PSQI (score)	6.0	3.0			6.1	3.4			7.4	2.9			7.7	3.7		
Chronotype																
Morning chronotype - Larks			103	53.9			89	57.8			34	47.2			58	50.4
Evening chronotype - Owls			88	46.1			65	42.2			38	52.8			57	49.6
Average time of blood collection (hr:min.)	7:10				8:35				7:25				8:20			
Average duration of night work in years	23.4	5.8			11.0	7.5			30.4	8.2			12.9	9.1		
Geometric means																
Estradiol (pg/ml)	153.5	131.4			154.2	117.3			30.4	31.0			27.5	28.1		
Testosterone (ng/ml)	0.29	0.13			0.23	0.13			0.22	0.14			0.19	0.13		
DHEAS (µg/dl)	173.1	86.6			161.3	82.8			125.3	66.9			112.7	69.6		

[a] A missing information for recreational activity for one premenopausal woman – night worker

workers had higher PSQI scoring than night workers; no such difference was observed among premenopausal women. Proportionally more night than day workers classified themselves as an evening or "owl" type. There was also a one-hour difference in the time of blood collection (earlier hour in night workers).

The average total duration of night work was longer among night than day workers (23.4 years and 30.4 years versus 11.0 and 12.9 among pre- and postmenopausal women, respectively). For each of the hormones, blood concentrations were higher among the premenopausal than postmenopausal women, with higher concentrations of every geometric mean for night workers compared to day workers, except for E2 among premenopausal women.

Association between rotating night shift work characteristics and hormones (table 2)

There was no significant difference in the circulating sex hormone concentrations between current night shift workers and day workers (both in pre- and postmenopausal women). Also the frequency of night shift work did not determine hormone concentrations. Total duration of night work tended to be positively associated with E2 concentration. Among postmenopausal women, the mean values rose from 13.8 pg/ml among women for whom the total duration of night shift work was not >5 years, to 20.7pg/ml among women who worked night shifts for >25 years. When compared to reference (≤5 years), the mean concentrations were significantly higher for two categories of total night work >15 years: 15–25 years and >25 years (P<0.05) (data not shown), but the trend was only of borderline significance (P=0.051). The mean geometric concentration of DHEAS tended to be higher among postmenopausal nurses and midwives who had longer duration of night work, however, the P-value for the trend was of borderline significance (P=0.082). None of the mean DHEAS concentrations were significantly different from reference (data not shown).

In the group of premenopausal women, the relationships for night shift work duration and E2 and DHEAS

Table 2. Adjusted geometric mean serum concentrations of sex hormones by rotating night shift work characteristics among premenopausal and postmenopausal nurses and midwives. [95% CI=95% confidence interval]

	Adjusted geometric mean (95%CI)					
	Premenopausal [a]			Postmenopausal [b]		
	Mean	95% CI	P-trend	Mean	95% CI	P-trend
Estradiol (pg/ml)						
Current day workers (N=269)	115.9	98.7–133.1	0.333	19.1	16.0–22.2	0.619
Night shift workers (N=263)	105.1	91.2–119.0		20.4	16.3–24.6	
Frequency of NSW			0.141			0.474
Day workers	115.8	98.6–132.9		19.1	16.0–22.3	
2–7 per month	108.7	93.6–123.7		19.8	15.4–24.2	
≥8 per month	77.3	45.0–109.6		23.9	12.0–35.8	
Duration of NSW (years)			0.683			0.051
≤5	111.0	78.4–143.6		13.8	9.3–18.2	
>5–≤15	110.7	87.8–133.6		18.7	14.2–23.2	
>15–≤25	103.9	88.2–119.7		24.2	17.8–30.5	
>25	124.1	94.0–154.3		20.7	16.4–24.9	
Testosterone (ng/ml)						
Current day workers (N=269)	0.23	0.21–0.25	0.199	0.17	0.15–0.20	0.655
Night shift workers (N=263)	0.25	0.23–0.27		0.16	0.13–0.19	
Frequency of NSW			0.245			0.868
Day workers	0.23	0.21–0.25		0.17	0.15–0.20	
2–7 per month	0.25	0.23–0.27		0.16	0.13–0.19	
≥8 per month	0.25	0.20–0.31		0.18	0.10–0.25	
Duration of NSW (years)			0.138			0.748
≤5	0.22	0.19–0.26		0.16	0.11–0.21	
>5–≤15	0.24	0.21–0.27		0.17	0.13–0.20	
>15–≤25	0.24	0.22–0.26		0.16	0.12–0.19	
>25	0.27	0.23–0.30		0.17	0.14–0.20	
DHEAS (µg/dl)						
Current day workers (N=269)	144.8	132.6–156.9	0.381	94.9	83.3–106.5	0.264
Night shift workers (N=263)	152.2	140.9–163.5		106.1	90.1–122.1	
Frequency of NSW			0.810			0.229
Day workers	144.7	132.6–156.9		95.0	83.3–106.6	
2–7 per month	155.2	143.0–167.3		104.5	87.1–121.8	
≥8 per month	127.7	97.4–156.9		114.5	72.3–156.7	
Duration of NSW (years)			0.263			0.082
≤5	142.6	119.2–166.1		85.1	63.9–106.3	
>5–≤15	145.5	129.4–162.6		97.0	79.5–114.6	
>15–≤25	147.8	135.1–160.4		93.6	75.0–112.2	
>25	159.9	138.0–181.2		110.9	93.6–128.2	

[a] Adjusted for Estradiol: age (continuous), menstrual cycle phase (follicular, mid cycle, and luteal); Testosterone: age (continuous), time of blood collection (continuous), BMI (continuous), sleep quality (continuous), smoking (non-smoker, past, current); DHEAS: age (continuous), BMI (continuous), sleep quality (continuous), smoking (non-smoker, past, current).

[b] Adjusted for Estradiol: age (continuous), age at menopause (continuous); Testosterone: age (continuous), time of blood collection (continuous), recreational activity (none vs. any), smoking (non-smoker, past, current); DHEAS: age (continuous), season when blood samples were collected (October-March vs. April-September).

concentration followed the direction observed among postmenopausal women, but no statistically significant results were observed.

When these associations were investigated with the models that included BMI, smoking and total physical activity in addition to the covariates in the basic models, the estimates remained generally unchanged (table S1 and table S2 in appendix www.sjweh.fi/index.php?page=data-repository). The P values for trends were slightly attenuated.

Chronotype was not found to be a confounder of the associations, but it was a significant modifier of the association for E2 among postmenopausal women (P for heterogeneity <0.05 for each night work characteristics). A positive association was observed between E2 and current night work status, frequency of night duties and night work duration among morning type or "lark" persons but not "owls" (table S3). When the model was extended by including BMI, total physical activity and smoking, no major effect on the estimates of mean E2 concentration could be noted (table 3), although the P-values for trends became insignificant for current night work status and frequency. For the night work duration, the trend remained highly significant (P<0.001). No heterogeneity of the associations by chronotype was observed among premenopausal women and for associations of T and DHEAS among postmenopausal (data not shown).

Associations between selected non-occupational characteristics and sex hormone levels

Inverse associations between age and E2, T and DHEAS concentrations were found in both pre- and postmenopausal women, although they were stronger among premenopausal subjects (P<0.01) (table 4). E2 tended to increase significantly with increasing BMI among postmenopausal women, while in premenopausal women, BMI was positively associated with T, and DHEAS concentrations. Abdominal obesity, as marked by WHR>0.85, was associated with higher T only among premenopausal women (P=0.006). Of the reproductive factors, only an older age at first full-term birth showed a significant association with E2 (positive) and DHEAS (inverse), this effect was observed only among premenopausal women. Current smoking status was related to higher concentrations of T (P=0.040) and DHEAS (P=0.001) in premenopausal women. Recreational physical activity tended to be linked to lower concentration of T (P -0.005) and DHEAS (P=0.016) in post- but not premenopausal women. In postmenopausal women, the time since last menopause was associated with a decreasing concentration of each of the hormones under study.

The current night shift work status was not found to be a confounder of the associations between hor-

Table 3. Adjusted [a] geometric mean serum concentrations of estradiol by rotating night shift work characteristics and chronotype among postmenopausal nurses and midwives. [95% CI=95% confidence interval

Night work characteristics	Larks (N=92)			Owls (N=95)		
	Mean (pg/ml)	95%CI	P-trend	Mean (pg/ml)	95%CI	P-trend
Current day workers	19.3	15.0–23.7	0.105	20.4	15.3–25.5	0.221
Night shift workers	26.3	19.0–33.5		15.8	11.0–20.6	
Frequency of night shifts per month						
Day workers	19.2	14.9–23.6	0.082	20.4	15.2–25.5	0.249
2–7 per month	25.3	17.6–32.9		15.9	10.6–21.1	
≥8 per month	31.9	10.5–53.4		15.4	3.3–27.5	
Duration of night shift work (years)						
≤5	12.4	6.8–18.0	<0.001	15.4	8.3–22.6	0.604
>5–≤15	15.5	10.6–20.5		23.3	14.9–31.8	
>15–≤25	26.0	18.6–33.5		20.6	10.3–30.8	
>25	28.8	20.7–36.8		16.1	11.2–21.0	

[a] Adjusted for: age (continuous), age at menopause (continuous), BMI (continuous), physical activity (continuous) and smoking (non-smoker, past, current).

mones and non-occupational determinants; the estimates remained substantially unchanged when it was introduced as a covariate to the models. As for the modification, the only statistically significant P-value for the heterogeneity of results by current night work status among premenopausal women was recorded for the association between recreational activity and E2 (P-heterogeity=0.042). However, the former was not found to be a significant determinant of E2 either among night shift or day workers. In postmenopausal women, age at menarche was inversely associated with DHEAS among night shift (P=0.022) but not day workers, with a significant heterogeneity (P heterogeneity=0.036).

Discussion

In this cross-sectional study on nurses and midwives, we examined associations between the concentrations of three sex hormones: E2, T and DHEAS, and rotating night shift work characteristics as well as various non-occupational factors related to reproduction and lifestyle.

The major finding of our study was the significant association between total duration of night work >15 years and higher E2 levels among postmenopausal women. This observation is consistent with the previously reported findings among nurses in the US (21).

Interestingly, associations of either current characteristics of night work and its lifetime duration with E2 were confined to women with the morning-type or "lark" personality. To our knowledge, chronotype has not been investigated as an effect modifier in previous studies examining association between night work and sex hormones. The strong effects that we observed between every night work metrics we analyzed and extradiol among postmenopausal women with morning preference, supports the view that earlier chronotypes should be affected more by working at night (33, 34). However, no consensus has as yet been reached which chronotype is more vulnerable. For example, the work-at-night tolerance has been found to be poorer among morning types (35). Also, the association between night work and breast cancer risk was most evident among women with the morning chronotype (36). The results for susceptibility of night workers with respect to melatonin synthesis by their chronotypes have been varying. One study reported lower melatonin levels among subjects with the morning preference when compared to those with the evening preference (37). On the other hand, two other studies indicated that circadian pattern of melatonin production was better maintained among morning-type night shift workers (38, 39).

The mechanism explaining why some steroid hormones can increase due to night work remains to be investigated. Night work and artificial light at night may affect human physiology, introducing changes in the regulation of the circadian rhythm, with a reduction of melatonin synthesis and changes in the profiles of other hormones. Some speculations point to an interrelation between melatonin and E2, which results in decreased melatonin synthesis leading to an increase in E2 concentration (40–42). It has been hypothesized that women working night shifts for many years may have up-regulated hypothalamic gonadal axis (with increase of circulating estrogenes) as a result of decreased melatonin (43). We made crude tests for this hypothesis by including MT6s concentration in spot morning urine sample into the adjusted analysis. Since MT6s had practically no effect on the estimates, its mediating role was not demonstrated.

It is worth noting that in our study the significant effect we observed referred to postmenopausal women, in whom ovaries, a key component of the hypothalamic-hypophysis-gonadal axis, are inactive with respect to

Table 4. Association between non occupational characteristics and estradiol, testosterone and DHEAs concentrations among pre and postmenopausal women. [BMI=body mass index; MET=metabolic equivalent]

Characteristics	ß-coefficient [a]					
	Estradiol (pg/ml)		Testosterone (ng/ml)		DHEAs (µg/dl)	
	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
Age (years)						
<45/<50						
45–<50/50–<55	-0.127	-0.041	-0.150 [b]	0.036	-0.200 [c]	0.103
50–60/55–60	-0.378 [c]	-0.287	-0.336 [c]	-0.201	-0.305 [c]	-0.211
BMI (kg/m^2)						
<25						
≥25–<30	-0.139	0.391[b]	0.135 [b]	0.042	0.054	0.144
≥30	0.047	0.405 [b]	0.222 [c]	0.039	0.154 [b]	0.024
Wait-to-hip ratio						
≤0.85						
>0.85	-0.010	0.165	0.188 [c]	0.065	0.097	-0.053
Age at menarche [yr]						
<12						
12–13	0.124	-0.314	-0.032	0.005	-0.064	0.010
≥14	0.077	-0.202	-0.074	-0.046	-0.016	-0.072
Number of full-term births						
0						
1	- 0.160	0.126	-0.116	0.019	-0.164	-0.012
2	-0.113	0.257	-0.047	0.048	-0.130	0.084
3 or more	-0.148	0.367	-0.180	0.617	-0.105	0.622 [b]
Age at first full-term birth						
<25						
≥25	0.214 [b]	-0.100	-0.047	0.013	-0.164 [c]	-0.099
Total physical activity (MET hours per week)						
≤179.3						
>179.3–≤248.6	-0.121	-0.028	-0.014	-0.127	0.021	-0.011
>248.6	-0.158	-0.192	0.081	-0.138	0.047	-0.075
Recreational activity						
None						
Any	-0.016	-0.031	0.010	-0.344 [c]	-0.036	-0.265 [b]
Smoking						
Never						
Past	-0.015	-0.096	0.091	-0.036	0.112	-0.133
Current	0.003	0.099	0.131 [b]	0.165	0.232 [c]	-0.004
Years since menopause [d] (years)						
<1						
>1–<5		-0.349 [b]		-0.176		-0.153
5–<10		-0.578 [c]		-0.172		-0.124
≥10		-0.468		-0.443 [b]		-0.3721 [b]

[a] Adjusted for age (continuous), menstrual cycle phase (follicular, mid cycle, and luteal - among premenopausal women), calendar season (October-March versus April-September), BMI (continuous), and time of blood collection (in the analysis of testosterone); in the analysis of BMI and waist-to-hip ratio, all the covariates listed above except for BMI; in the analysis of age categories, age was not included as a covariate.

[b] P<0.05.

[c] P<0.01.

[d] Analysis includes 62 women who reported last menstrual period within a year since interview.

estrogenes synthesis, as opposed to premenopausal women. The major source of E2 in women after menopause is the fat tissue, where the aromatization of T into E2 or conversion from estrone to E2 takes place (44). Peripheral synthesis of E2 is dependent on the level of androgenic precursors. The expression of aromatase may be regulated by many factors including gonadotropins, prostaglandin E_2, cytokines: IL-6, IL-11, oncostatin M, and tumor necrosis factor – TNFα (45). Moreover, melatonin was found to suppress aromatase, but this effect was observed only in MCF-7 human breast cancer cells (46), while cortisol was reported to increase aromatase activity (46). Given that the increase in E2 concentration was accompanied by some increase in DHEAS, we may speculate that an increased production of E2 substrates, which takes place in the adrenal glands, may play a major role. If prolonged night work acts as a chronic stressor, the up-regulation of activity of the hypothalamic–pituitary–adrenal (HPA) axis may be expected. Although the literature on this subject is limited, we can still find some support for this thesis. An experiment in monkeys demonstrated increased DHEAS concentration

after chronic stress (47). Increased cortisol in hair, as a biomarker of chronic stress, was also observed among shift workers, but only those <40 years of age (48).

In our study, we did not find significant associations with night work among premenopausal women, although the mean concentration of the hormones tended to be higher among women with longer night shift duration, just as we observed among postmenopausal women. Previous studies in this field were not numerous and the association between night work and E2 was found only in two (24, 27) of the five studies conducted among premenopausal women (21, 23, 26). The lack of consistent findings may have been due to the high variability of E2 concentration during the menstrual cycle in premenopausal women, and a relatively small scale of the studies. A larger single study or a pooled collaborative analysis from individual studies are warranted in the future to further clarify the associations of night shift work with sex hormone concentrations among premenopausal women.

The outcomes of our study confirmed several of the previously reported relationships for non-occupational factors. As expected, age turned out to be the strongest determinant for each of the hormones. BMI, smoking, and recreational activity, which we tested as the potential mediators of night work showed associations with hormones in the direction reported by other researchers (49). Body weight reflects adipose tissue, which is a source of estrogens and testosterone (50). For smoking, the suggested mechanisms may involve stimulation of hormones synthesis by the adrenal glands (2) through up-regulation of the hypothalamic-pituitary-adrenal axis (3). As for the physical activity, the changes in endogenous sex hormones may be related to modification of body weight (adiposity) (50).

Generally, current night shift work neither confounded nor modified the observed associations. The significant heterogeneity of the results by current night work status we observed for the association between E2 and recreational activity among premenopausal women, and between DHEAS and age at menarche among postmenopausal women, may have rather been due to chance, given the multiple comparisons and the lack of consistency across menopausal strata.

In our study, BMI, physical activity and smoking were found to contribute to some extent to the examined associations between E2 or other hormones and night work metrics. When the results were obtained through the analysis carried out with or without these variables, we observed only negligible changes of the adjusted geometric means. However, the effect was more pronounced for the statistical significance of the estimates that appeared to be insignificant in the analysis of two night shift work metrics, but not night work duration, among morning type postmenopausal women. While the role of these three factors was controlled for, the role of other lifestyle-related factors, such as diet, could not be ruled out.

Our study has several strengths. It was conducted on a well-characterized female population. The response rate was relatively high, and 97% women who agreed to participate in our study provided biological samples for analysis. We demonstrated high levels of reproducibility of measurements for each of the hormones studied. Detailed information on both current work characteristics and lifetime occupational history was elicited via a face-to-face interview by trained interviewers. All the major confounding factors were evaluated.

A potential limitation of the study relates to the lack of standardization with respect to the menstrual phase among premenopausal women. However, we controlled for the menstrual phase by determining the phase cycle based on the first day of the last menstrual period, like did the other investigators (23).

The study could have been limited by a recall bias, which is typical for most questionnaire-based studies.

We also acknowledge that the biological material was collected only once; therefore, we could not assess the intrasubject variations. While satisfactory reproducibility was noted for DHEAS and T (51), the reproducibility of E2 measurements during the luteal phase – when the two consecutive measurements were taken a year apart – was found to be poor (51).

We also could not exclude some uncontrolled confounding by the circadian stage, although support from the literature for the circadian variation could be found only for T (52, 53), and all the analyses that we ran for T were adjusted for the hour of blood collection. For E2 secretion, the literature on the circadian rhythmicity is inconsistent and tends not to support the circadian pattern (54). There is no circadian rhythmicity of DHEAS (55).

The conclusions are also limited to the nurses and midwives working rotating night shifts. Nurses in Poland do not work permanent nights; therefore, it was impossible to address permanent night work in our research.

We also note that the Roche Elecsys electrochemiluminescence immunoassay method that we used to determine steroid hormone concentrations in plasma is inferior when compared to gas chromatography, especially in the case of low hormone concentrations. In previous studies, Pearson's correlation coefficient for E2 concentration in serum in postmenopausal women, as measured by the immunoassay and gas chromatography-tandem mass spectrometry (GC-Ms/MS) equaled r = 0.57 (56). Moreover, we did observe the well-established associations for the hormones, which supports the validity of our study.

We also acknowledge that the self-assessment of chronotype by respondents was a rough method. We classified women into a given chronotype category using

a simple question about the morning or evening preference. We acknowledge that such a crude assessment could have resulted in some misclassification. Future studies using better tools for the assessment of chronotype are needed to verify our findings.

In conclusion, our study revealed that a higher total number of years of night shift work was associated with higher E2 concentrations. This association was not mediated by BMI, smoking or recreational physical activity. Both current night shift work and total lifetime duration of night work were associated with higher E2 among postmenopausal women with the morning type personality. The role of the major determinants of sex hormones in women: reproductive and lifestyle-related, was also confirmed.

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