Research Article

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Increased and Mistimed Sex Hormone Production in Night Shift Workers

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Abstract

Background: Night shift work has been associated with an increased risk for breast and prostate cancer. The effect of circadian disruption on sex steroid production is a possible underlying mechanism, underinvestigated in humans. We have assessed daily rhythms of sex hormones and melatonin in night and day shift workers of both sexes.

Methods: We recruited 75 night and 42 day workers, ages 22 to 64 years, in different working settings. Participants collected urine samples from all voids over 24 hours on a working day. Urinary concentrations of 16 sex steroid hormones and metabolites (estrogens, progestagens, and androgens) and 6-sulfatoxymelatonin were measured in all samples. Mean levels and peak time of total and individual metabolite production were compared between night and day workers.

Results: Night workers had higher levels of total progestagens [geometric mean ratio (GMR) 1.65; 95% confidence intervals (CI), 1.17–2.32] and androgens (GMR: 1.44; 95% CI, 1.03–2.00), compared with day workers, after adjusting for potential confounders. The increased sex hormone levels among night shift workers were not related to the observed suppression of 6-sulfatoxymelatonin. Peak time of androgens was significantly later among night workers, compared with day workers (testosterone: 12:14 hours; 10:06-14:48 vs. 08:35 hours; 06:52-10:46).

Conclusions: We found increased levels of progestagens and androgens as well as delayed peak androgen production in night shift workers compared with day workers.

Impact: The increase and mistiming of sex hormone production may explain part of the increased risk for hormone-related cancers observed in night shift workers. *Cancer Epidemiol Biomarkers Prev; 24(5); 854–63. ©2015 AACR.*

Introduction

Night shift work has been associated with cancer risk in humans, especially after long-term exposure (1). The strongest epidemiologic evidence, to date, is for female night shift workers and breast cancer (2–4), but there is also limited evidence on other hormone-related cancers such as prostate (5, 6) and endometrial cancer (7). Several mechanisms have been proposed to explain the association between night shift work and cancer risk, including light-induced melatonin suppression, sleep disturbances, and circadian disruption (8–10). An increase in sex hormones after night shift work has been a long discussed, though not confirmed, hypothesis, particularly relevant for hormone-dependent tumors (11, 12).

The circadian timing system is closely related to the endocrine system. A functional master clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus is necessary for rhythmic steroid synthesis and excretion (13, 14). It has been hypothesized that exposure to light at wrong times, such as experienced during night shift work, can disrupt normal melatonin synthesis which in turn may increase estrogen production (11). Some observational studies reported higher plasma estrogen levels related to long-term exposure to night shift work in women (12, 15, 16). Although melatonin has potential antiestrogenic effects (17, 18), an inverse association between endogenous melatonin and estrogens has not yet been confirmed in humans (15, 19–21). Lifetime exposure to higher levels of estrogens and androgens may increase breast cancer risk, while recent evidence also indicates that progesterone is an important hormone in breast cancer etiology (22–24). The possible effect of night shift work on estrogen, androgen, and progestagen production is largely underinvestigated, especially in men, and might explain in part the increased risk of breast and prostate cancer observed among female and male night shift workers, respectively.

We assessed daily rhythms of urinary sex hormones and metabolites including estrogens, progestagens, and androgens in permanent night and day workers of both sexes. We also examined

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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the interrelations between urinary 6-sulfatoxymelatonin and sex hormones. We hypothesized that permanent night shift work would increase 24-hour sex hormone levels and alter the peak time of their production.

Materials and Methods

Participants in this study were female and male night and day workers from four companies in Barcelona: two hospitals, a car industry, and a train company. The selection procedures used for participant recruitment were the same in all participating companies. Study participation was offered to all workers in permanent day or night shifts through the Health and Safety departments of each company using leaflets and personal contacts. Enrolment was voluntary and there was no compensation for participating. All those initially agreeing to participation were enrolled and because of this selection procedure, we cannot report participation rates. Our study sample was balanced across the four companies in terms of numbers of participants in each shift; however, the proportion of participants per company is not the same because these companies were very different in size and structure. One hundred and seventeen workers (42 day and 75 night workers) of both sexes (63 men and 54 women) ages 22 to 64 years, participated in the study. All but 2 women were hospital nurses and assistants. All but 4 men worked in the car industry and train company. Night shift nurses worked 10-hour shifts (21:00–07:00 hours) on a short (2 days off-2 nights work-3 days off) and a long (2 nights work- 2 days off-3 nights work) working week alternately. Night shift car industry workers and train employees performed 8-hour shifts (22:00–06:00 hours) on a schedule including 3 and 5 consecutive nights per week, respectively. All day workers were engaged in fixed 8-hour morning shifts 5 days a week with starting times varying from 05:45 to 07:00 hours depending on the working sector. Night workers were engaged in night shift work for at least 1 year and, although some day workers had a history of night shift work, this did not occur within a year before participation. Recruitment took place from March to June 2011. Subjects were not eligible if they had a history of cancer and for females if they had been pregnant in the previous 6 months or were currently taking oral contraceptives or hormonal therapy. Filter questions were included in the study questionnaire to ensure that the exclusion criteria were fulfilled. The study was approved by the local ethics board and all participants signed an informed consent.

We collected information on sociodemographics, lifestyle habits, night shift history, health symptoms, and medication through a personal interview. Occupation information included start and stop times, number of shifts per week, main tasks and activities, and years worked at night. Questions related to sleep duration, physical activity, smoking, and alcohol and caffeine consumption were asked for both habitual use as well as for the 24 hours before study enrolment. For women, information on reproduction history including parity and age of first full-term birth was collected. Menopausal status was based upon cessation of periods and menstrual cycle phase on the days since last period (follicular phase: <11 days, mid-cycle phase: 11–16 days, luteal phase: $\geq$17 days). Diurnal preference was assessed using the Morningness-Eveningness Questionaire (25). Participants were asked to collect one urine sample from each natural void over a 24-hour working day or night and store them at 4°C. Samples were transported on ice and frozen at −80°C until analysis. Four subjects with less than 3 urine samples collected were excluded from the statistical analysis assuming that these individuals were most likely to have missed samples and therefore data would be incomplete. The final study population consisted of 72 night and 41 day workers.

Concentrations of 16 steroid hormones and metabolites were measured in a total of 899 urine samples, using gas chromatography and mass spectrometry (GC-MS). Analyses were performed by the Bioanalysis Research Group at IMIM, in Barcelona, Spain. Hormones and metabolites included estrogens (estradiol, estrone, and estriol), progestagens (pregnanediol, pregnanetriol, and 16-androstenol), and androgens [testosterone, epitestosterone, dehydroepiandrosterone (DHEA), androsterone, etiocholanolone, 11β-OH-androsterone, androstenedione, 6α-OH-androstenedione, 3α,5α-androstanediol, and 3α,5β-androstanediol). The procedure for preparing the samples was based upon routinely used screening methods in doping control analysis (26). Briefly, 2.5 mL of urine were hydrolysed, extracted and derivatized, and analyzed by GC-MS. The method used was validated following internationally accepted criteria. The validation results proved linearity of the method from 1 ng/mL to 400 ng/mL for most steroids ($r > 0.99$). For androsterone, etiocholanolone, pregnanediol, pregnanetriol, and 16-androstenol, linearity was demonstrated up to 10,000 ng/mL. Adequate inter-assay precision (coefficients of variation < 19%) was found for the quantification of all steroids at the assayed concentrations (Supplementary Table S1). The validity of the results for the samples was assessed by verifying the proper concentrations for all steroids in the quality control sample analyzed in the same analytical batch. Undetected values were replaced by half of the limit of detection of each of the following metabolites: estradiol ($N = 45$), estrone ($N = 10$), estriol ($N = 21$), epitestosterone ($N = 10$), testosterone ($N = 5$), 3α,5α-androstanediol ($N = 11$), 3α,5β-androstanediol ($N = 9$), androstenedione ($N = 23$), and 6α-OH-androstenedione ($N = 31$). For 16-androstenol ($N = 5$) and androsterone ($N = 1$), undetected values were due to the presence of interfering peaks at the retention times of the analytes that did not allow their proper quantification and were left as nondetected. Urinary 6-sulfatoxymelatonin (aMT6s) concentrations, the major melatonin metabolite, were measured by the Chronobiology Group, University of Surrey, Guildford, United Kingdom (Stockgrand, Ltd.) using a radioimmunoassay (27) The intra-assay variability was 5.7% at 3.3 ng/mL, 7.8% at 15.5 ng/mL, and 6.1% at 28.3 ng/mL and the limit of detection was 0.2 ng/mL. Inter-assay variability was 8.7% at 2.6 ng/mL, 7.9% at 17.6 ng/mL, and 10.3% at 31.3 ng/mL. Creatinine levels were determined in all urine samples by the same laboratory using the manual picric acid, sodium hydroxide colorimetric method (Randox Laboratories Ltd.). Limit of detection of the assay was 25.1 mg/dL and inter-assay variability was 7.6% at 87.4 mg/dL and 9.9% at 198.3 mg/dL. All metabolite values were creatinine standardized and quoted as ng/mg creatinine.

Statistical analysis

Individual cosinor analysis was used to evaluate the rhythm of 6-sulfatoxymelatonin, individual sex hormone metabolites, and total estrogen, progestagen, and androgen production. Cosinor analysis is a curve fitting procedure used in the analysis of rhythms with a cyclic nature and an approximate 24-hour period (28). The acrophase (peak time) and mesor (24-hour mean) of the

metabolites were derived for each subject. The validity of the cosinor-derived parameters was determined using the percentage variability accounted for the cosine curve (100% indicates that all data points fall on the cosine curve). The 24-hour mean levels (geometric mean and standard deviation) and peak time (geometric mean and 95% confidence interval; CI) were described for all metabolites in day and night workers and by sex and menopausal status. The correlation between aMT6s and sex steroid metabolites (24-hour mean levels and peak time) was evaluated using Pearson correlations for the log-transformed variables. Generalized linear models were used to examine associations between shift work and log-transformed 24-hour mean levels and peak time. Log transformation was applied to achieve a normal distribution of the variables. For 24-hour mean levels, regression estimates were back-transformed and presented as the geometric mean ratio (GMR) of levels in night versus day workers. Peak time difference was estimated as the geometric mean difference (GMD) of the predicted acrophases in night workers compared with day workers and expressed in hours. We used directed acyclic graph (DAG; Supplementary Fig. S1) to select confounders a priori for each of the outcomes (mean levels, peak time). We included age, sex, education, diurnal preference, menopausal status, and menstrual cycle phase, in basic models. We additionally adjusted models for variables (potential confounders and intermediate variables) that were significantly associated with at least one of the metabolites (list of variables shown in Supplementary Fig. S1). To account for the possible effect of melatonin on steroid production, we adjusted in separate models for aMT6s levels or aMT6s peak time. In sensitivity analyses, subjects with reported chronic health symptoms, any drug use, and women with irregular menstrual cycles were excluded and results remained unchanged (data not shown). Statistical analysis was performed using the statistical package Stata version 12.1.

Results

Night shift workers were older, heavier, and reported more chronic symptoms and drug use, compared with day workers in both sexes but differences were not statistically significant (Table 1). Night shift workers had significantly lower

Table 1. Sociodemographic, lifestyle, and reproductive characteristics of day and night workers by sex

	Men		Women	
	Day ($N = 21$) *N* (%)	Night ($N = 39$) *N* (%)	Day ($N = 20$) *N* (%)	Night ($N = 33$) *N* (%)
Age, y; mean (SD)	38.4 (9.2)	39.9 (9.8)	44.9 (8.8)	49.5 (8.7)
BMI (kg/m^2); mean (SD)	26.0 (3.4)	26.6 (4.0)	24.4 (4.7)	24.6 (4.3)
Highest education				
High school	18 (85.7)	27 (82.1)	6 (30.0)	11 (33.3)
University	3 (14.3)	7 (17.9)	14 (70.0)	22 (66.7)
Working sector				
Hospitals	0 (0)	4 (10.3)	19 (95.0)	32 (97.0)
Car factory	12 (57.1)	18 (46.2)	0 (0)	0 (0)
Train company	9 (42.9)	17 (43.6)	1 (5.0)	1 (3.0)
Working h/wk	37.3 (1.5)	32.8 (1.2)[a]	37.4 (1.7)	35.2 (0.5)
Nights worked over last 2 wks; mean (SD)	0	6.8 (0.4)[a]	0	5.2 (0.3)[a]
Consecutive nights worked; mean (SD)	0	2.8 (1.4)	0	2.0 (0.4)
Total years of night work; mean (SD)	2.1 (3.0)	10.8 (8.3)[a]	2.2 (3.1)	15.4 (11.3)[a]
Morningness-eveningness score; mean (SD)	55.2 (6.1)	48.4 (9.1)[a]	50.4 (8.5)	46.7 (8.0)[a]
Diurnal preference				
Evening	1 (4.8)	8 (20.5)	4 (20.0)	8 (24.2)
Neither	15 (71.4)	27 (69.2)	13 (65.0)	22 (66.7)
Morning	5 (23.8)	4 (10.3)	3 (15.0)	3 (9.1)
Current smoker	9 (42.9)	13 (33.3)	6 (30.0)	10 (30.0)
Any alcohol use last 24 h	3 (14.3)	13 (33.3)	3 (15.0)	6 (18.2)
Any physical activity last 24 h	8 (38.1)	15 (38.5)	10 (50.0)	13 (39.4)
Any sleep problems	20 (95.2)	38 (97.4)	17 (85.0)	30 (90.9)
Caffeinated drinks last 24 h; mean (SD)	2.8 (1.7)	2.6 (1.7)	2.9 (2.4)	2.3 (1.8)
Sleep duration last 24 h; mean (SD)	5.6 (0.8)	6.4 (1.7)[a]	6.4 (0.9)	6.2 (1.9)
Hours of sunlight last 24 h; mean (SD)	13.6 (0.6)	13.8 (0.9)	13.7 (1.3)	13.2 (1.3)
Time spent outdoors last 24 h				
1–2 h	10 (47.6)	26 (66.7)	11 (55.0)	24 (72.7)
$\geq$3 h	11 (52.4)	13 (33.3)	9 (45.0)	9 (27.3)
Chronic health problems	5 (23.8)	17 (43.6)	6 (30.0)	19 (57.6)
Any drug use	4 (19.1)	13 (33.3)	7 (35.0)	22 (66.7)
Premenopausal women			16 (80.0)	16 (48.5)
Menstrual cycle phase[b]				
Follicular			8 (50.0)	8 (50.0)
Mid-cycle			5 (31.2)	3 (18.8)
Luteal			3 (18.8)	5 (31.2)
Irregular menstrual cycle			4 (20.0)	10 (30.3)
Ever parous			16 (80.0)	30 (90.9)
1 or 2 children			13 (65.0)	22 (66.7)
3 or more			3 (15.0)	8 (24.2)
Age at first full-term birth; mean (SD)			22.8 (12.6)	25.8 (9.9)

[a]$P_{difference} < 0.05$ for the two-sided χ^2 for categorical and *t* test or Wilcoxon rank-sum test for continuous variables.
[b]Percentages calculated among premenopausal women.

Table 2. Mean 24-h metabolite levels (mesor) in day and night workers by sex and menopausal status[a]

	Men		Premenopausal women		Postmenopausal women	
	Day workers ($N = 21$)	Night workers ($N = 39$)	Day workers ($N = 16$)	Night workers ($N = 16$)	Day workers ($N = 4$)	Night workers ($N = 17$)
Metabolite[a]	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)
Melatonin						
6-Sulfatoxymelatonin	14.0 (1.8)	10.5 (1.9)	17.9 (2.2)	10.4 (2.0)[b]	14.5 (2.7)	12.5 (1.7)
Estrogens						
Estradiol	1.0 (2.0)	1.1 (1.6)	3.9 (2.4)	4.2 (4.5)	1.2 (2.0)	1.2 (4.3)
Estrone	3.0 (1.6)	2.7 (1.8)	7.5 (2.0)	8.5 (3.6)	1.4 (2.0)	3.3 (2.4)
Estriol	1.6 (2.6)	2.0 (2.0)	9.1 (2.4)	11.2 (6.4)	4.2 (2.2)	4.3 (2.2)
Total estrogens	6.1 (1.5)	6.1 (1.6)	21.3 (2.2)	26.3 (4.3)	7.3 (1.8)	9.4 (2.3)
Progestagens						
Pregnanediol	96.9 (3.3)	125.5 (2.0)	372.0 (3.0)	1,094.1 (3.2)[b]	228.8 (1.8)	250.5 (2.5)
Pregnanetriol	334.0 (4.0)	417.5 (1.5)	581.4 (1.9)	966.5 (2.4)	378.9 (1.6)	462.3 (2.6)
16-Androstenol	486.5 (3.0)	469.3 (2.8)	276.5 (3.6)	665.7 (2.9)[b]	384.6 (1.5)	285.6 (3.2)
Total progestagens	954.5 (3.4)	1,114.1 (1.7)	1,457.9 (2.1)	3,289.2 (2.2)[b]	1,009.5 (1.6)	1,099.9 (2.4)
Androgens						
Testosterone	13.0 (3.0)	14.5 (2.5)	4.0 (2.6)	8.0 (2.0)[b]	3.5 (3.5)	5.5 (2.3)
Epitestosterone	16.2 (3.9)	15.6 (2.0)	9.5 (1.8)	13.6 (2.5)	2.3 (2.5)	5.0 (1.8)[b]
DHEA	16.7 (2.3)	20.2 (1.7)	25.4 (2.1)	38.7 (2.2)	23.8 (1.6)	22.0 (2.1)
Androsterone	796.4 (4.5)	1,117.8 (1.5)	684.7 (1.7)	881.1 (2.1)	489.8 (1.3)	412.6 (1.9)
Etiocholanolone	795.8 (4.3)	936.1 (1.6)	1,089.2 (1.6)	1,195.4 (1.8)	679.8 (1.3)	764.9 (2.0)
11β-OH-androsterone	227.1 (4.5)	307.1 (1.6)	397.7 (2.1)	555.7 (2.3)	501.6 (1.8)	556.7 (2.3)
4-Androstenedione	1.1 (1.6)	1.2 (1.6)	2.0 (2.0)	2.8 (2.1)	1.1 (2.9)	1.2 (4.3)
6α-OH-androstenedione	0.5 (2.0)	0.6 (1.5)	1.1 (2.3)	1.8 (2.4)	0.9 (2.9)	3.3 (2.4)
3α,5α-Androstanediol	29.5 (3.7)	39.7 (1.6)	21.3 (1.9)	36.2 (2.2)[b]	23.6 (1.6)	4.3 (2.2)
3α,5β-Androstanediol	52.0 (4.0)	60.1 (2.2)	35.7 (2.9)	62.2 (3.3)	50.6 (3.3)	250.5 (2.5)
Total androgens	2,070.6 (3.8)	2,613.3 (1.4)	2,498.7 (1.4)	3,082.1 (1.8)	1,870.8 (1.3)	1,989.9 (1.9)

Abbreviation: GM, geometric mean; GSD, geometric standard deviation.

[a]Levels of metabolites are expressed in ng/mg creatinine/h.

[b]$P_{difference} < 0.05$ for two-sided t test using the log-transformed variable.

Morningness-Eveningness scores (48.4 vs. 55.2 in men and 46.7 vs. 50.4 in women) indicating more evening preference, compared with day shift workers. Male night shift workers reported less current smoking (33.3 vs. 42.9%), higher alcohol consumption (33.3 vs. 14.3% consumed any alcohol) and shorter sleep duration (5.6 vs. 6.4 hours) over the previous 24 hours, compared with male day workers. Male night workers were less likely to have university education compared with female night workers (17.9 vs. 66.7%); however, no differences in the educational level was found between shifts for both sexes. Women had worked less nights over the previous 2 weeks compared with men (5.2 vs. 6.8 nights) but had on average been engaged in night shift work for more years (15.4 years) compared with male night workers (10.8 years). Men working at night reported earlier sleep onset on a working day (06:00–07:00 hours) than women (07:00–08:00 hours). Amongst females, night shift workers were more frequently postmenopausal (51.5 vs. 20%), parous (90.9 vs. 80%), and likely to report menstrual irregularities (30 vs. 19.1%) and sleep problems (90.9 vs. 85%), compared with day workers. There were no differences in the distribution of womens' menstrual cycle phases or menstrual cycle length between shift types.

Table 2 shows the 24-hour mean aMT6s and sex hormone levels in night and day workers among men, premenopausal, and postmenopausal women. Levels of androgens and their metabolites (testosterone, DHEA, androsterone, 11β-OH-androsterone, 6α-OH-androstenedione) were higher among male night workers compared with day workers, although differences were not statistically significant. Amongst premenopausal women, testosterone (8.0 vs. 4.0 ng/mg creatinine/hour) and 3α,5α-androstanediol (36.2 vs. 21.3 ng/mg creatinine/hour) levels were significantly higher in women working at night compared with day workers. Total progestagens were significantly higher among premenopausal night workers (3289 vs. 1458 ng/mg creatinine/hour), compared with day workers. Total estrogens were also higher in premenopausal night workers (26.3 vs. 21.3 ng/mg creatinine/hour), though differences were not statistically significant. No differences were found between postmenopausal night and day shift female workers (Table 2). Comparisons in postmenopausal women were limited because the control day worker group only compromised four subjects and most of the samples with nondetectable estrogens belonged to this group.

In the full study population (Table 3), night shift work was associated with significantly increased total progestagen levels (GMR 1.65; 95% CI, 1.17–2.32), after adjustment for a wide range of potential confounders. Individual progestagen metabolites were higher among night shift workers (pregnanediol: GMR 1.74; 95% CI, 1.15–2.64, pregnanetriol: 1.46; 1.06–2.01, 16-androstenol: 1.32; 0.84–2.01), compared with day workers. Total androgen production was also higher among night shift workers (GMR: 1.44; 95% CI, 1.03–2.00). All androgens and their metabolites were higher in night shift workers compared with day workers. Differences were statistically significant or of borderline significance for testosterone (GMR 1.43; 95% CI, 0.95–2.14), DHEA (1.38; 1.05, 1.82), androsterone (1.40; 0.97–2.02), 11β-OH-androsterone (1.42; 0.99–2.04), 4-androstenedione (1.36; 1.05–1.78), 6α-OH-androstenedione (1.41; 1.09–1.84), and 3α5α-androstanediol (1.38; 0.99–1.91). Both estradiol (GMR 1.20; 95% CI, 0.83–1.74) and estrone (1.17; 0.86, 1.59) were higher in night shift workers but differences were not statistically significant. Levels of 6-sulfatoxymelatonin were lower in night compared with day workers (GMR, 0.67; 0.51–0.89). Differences in estrogen, progestagen, and androgen levels between night and day workers persisted and if anything were more

Table 3. Estimated geometric mean ratio (GMR) and 95% CIs of 24-hour mean metabolite levels (mesor) in night workers compared with day workers in the full study population ($N = 113$)

	Mean levels (mesor)		
Metabolite	**GMR (95% CI)[a]**	**GMR (95% CI)[b]**	**GMR (95% CI)[c] (+aMT6s levels)**
Melatonin			
6-Sulfatoxymelatonin	0.72 (0.55–0.93)	0.67 (0.51–0.89)	—
Estrogens			
Estradiol	1.08 (0.74–1.57)	1.20 (0.83–1.74)	1.36 (0.93–1.99)
Estrone	1.09 (0.81–1.47)	1.17 (0.86–1.59)	1.28 (0.94–1.75)
Estriol	1.11 (0.76–1.63)	1.05 (0.68–1.62)	1.19 (0.76–1.85)
Total estrogens	1.08 (0.80–1.46)	1.19 (0.86–1.64)	1.21 (0.87–1.67)
Progestagens			
Pregnanediol	1.61 (1.11–2.34)	1.74 (1.15–2.64)	1.88 (1.22–2.89)
Pregnanetriol	1.40 (1.00–1.96)	1.46 (1.06–2.01)	1.55 (1.11–2.17)
16-Androstenol	1.28 (0.83–1.98)	1.32 (0.84–2.08)	1.41 (0.88–2.27)
Total progestagens	1.44 (1.05–1.97)	1.65 (1.17–2.32)	1.67 (1.18–2.35)
Androgens			
Testosterone	1.42 (0.98–2.06)	1.43 (0.95–2.14)	1.57 (1.03–2.38)
Epitestosterone	1.20 (0.85–1.71)	1.16 (0.80–1.69)	1.27 (0.86–1.88)
DHEA	1.31 (1.01–1.72)	1.38 (1.05–1.82)	1.44 (1.08–1.92)
Androsterone	1.31 (0.95–1.82)	1.40 (0.97–2.02)	1.52 (1.04–2.22)
Etiocholanolone	1.15 (0.84–1.58)	1.23 (0.86–1.76)	1.37 (0.94–1.98)
11β-OH-androsterone	1.33 (0.93–1.90)	1.42 (0.99–2.04)	1.54 (1.06–2.25)
4-Androstenedione	1.29 (0.99–1.69)	1.36 (1.05–1.78)	1.43 (1.08–1.89)
6α-OH-androstenedione	1.40 (1.04–1.89)	1.41 (1.09–1.84)	1.48 (1.13–1.95)
3α,5α-Androstanediol	1.41 (1.03–1.94)	1.38 (0.99–1.91)	1.47 (1.05–2.07)
3α,5β-Androstanediol	1.27 (0.80–2.00)	1.32 (0.87–2.02)	1.49 (0.96–2.30)
Total androgens	1.24 (0.93–1.65)	1.44 (1.03–2.00)	1.45 (1.04–2.02)

[a]Adjusted for age, sex, BMI, menopausal status (premenopausal, postmenopausal), and menstrual cycle phase (follicular, mid-cycle, luteal).
[b]Additionally adjusted for education (primary school, high school, university), smoking status (current smoker, former, never), physical activity last 24-h (No, ≤14 METS-hours, >14 METS-hours), alcohol consumption last 24-h (yes, no), number of caffeine beverages last 24-h, sleep duration last 24-h diurnal preference (M-E score), parity (nulliparous, 1–2, 3 or more children) and age at first full-term birth, chronic symptoms (yes, no), drug use (yes, no), and hours of sunlight last 24 hours.
[c]Additionally adjusted for aMT6s levels.

pronounced after additional adjustment for 6-sulfatoxymelatonin (Table 3). Figure 1 shows the estimates of the individual metabolites in night workers compared with day workers in the two largest study groups: men and premenopausal women, adjusted for age, body mass index (BMI), and menstrual cycle phase (premenopausal women). Night shift work was associated with a 20% to 60% increase in individual androgens and their metabolites in both sexes. Testosterone was 2-fold (GMR 2.03; 1.06–3.88) and pregnanediol almost 3-fold (GMR 2.75; 95% CI, 1.28–5.94) higher in premenopausal female night workers compared with day workers. Among premenopausal women, night shift workers showed higher progestagen and androgen levels across the three menstrual cycle phases but estimates became less precise as comparisons were based on smaller subject numbers (Supplementary Table S2).

As shown in Table 4, peak time of testosterone (12:14 vs. 08:35 hours), epitestosterone (13:35 vs. 09:11 hours), DHEA (13:43 vs. 10:24 hours), and etiocholanolone (12:40 vs. 09:46 hours) occurred significantly later in night workers compared with day workers. After adjusting for potential confounders, all androgen metabolites showed a later peak time in night shift workers compared with day workers, statistically significant for epitestosterone (5.8 hours later; 95% CI, 2.5–9.2), DHEA (3.4 hours; 0.6–6.2), etiocholanolone (2.8 hours; 0.1–5.6), 6α-OH-androstenedione (3.1; 0.1–6.0), and borderline significant for testosterone (3.2; −0.6–6.9) and 3α5β-androstanediol (3.1; −0.1–6.3). Estriol peak time was also later in night workers (08:58 hours; 07:38–10:34) compared with day workers (06:12 hours; 04:15–09:02), although not statistically significant after adjustment for confounders. Peak time of aMT6s occurred 3 hours later in night workers (08:42 hours; 95% CI, 07:48–09:42) compared with day workers (05:36 hours; 95% CI, 05:06–06:12). The effects of night shift work on the peak time of sex hormone production were attenuated after adjusting for aMT6s acrophase (Table 4). Effects of shift status on peak time were found in both sexes, but were stronger among men (Fig. 2 and Supplementary Table S3).

We found no correlation between 24-hour urinary production of aMT6s and estrogens, progestagens, and androgens (Supplementary Fig. S2 and Supplementary Table S4). The percentage of cosinor fits was lower in sex hormones (40%–45% for estrogens, 44%–47% for progestagens, and 44%–58% for androgens) compared with aMT6s (83%), considered the best marker of circadian phase. The peak time of aMT6s was positively correlated ($P < 0.05$) with the peak time of testosterone (correlation coefficient $r = 0.41$), epitestosterone ($r = 0.59$), etiocholanolone ($r = 0.35$), and 6α-OH-androstenedione ($r = 0.26$) in men and with estrone ($r = 0.30$) in women. Similar results were obtained in analyses stratified by shift status (Supplementary Table S5) and in multivariate analysis adjusting for confounders (results not shown).

Discussion

We have evaluated the association of 16 sex hormones and their metabolites with night shift work using repeated samples over a 24-hour period. Significantly increased levels of androgens and progestagens were observed among night workers compared with day workers in both sexes. Smaller differences, statistically non-significant, were observed for estrogens. Night shift work was also associated with a later peak time for androgens in both sexes and estriol in women. The effect of night shift work on sex hormone

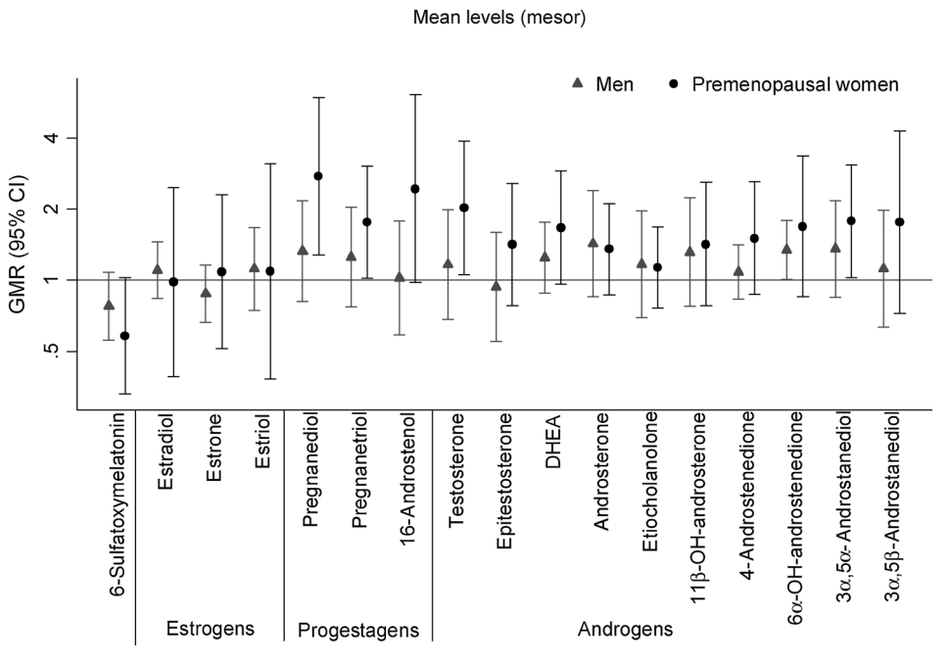

Figure 1.
Estimated GMR and 95% CIs of individual 24-hour mean metabolite levels (mesor) in night workers compared with day workers in men and premenopausal women, adjusted for age, BMI, and menstrual cycle phase (follicular, mid-cycle, luteal).

levels was independent of melatonin production, while the observed delayed peak time of their production was mirrored by a phase delay in melatonin production, considered a reliable marker of circadian phase.

The increase in estrogens, androgens, and progestagens among night workers may be a biologically plausible mechanism for the link between night shift work and hormone-dependent cancers. Higher levels of estrogens have been associated with breast cancer risk in women, as well as combined exposure to increased estrogens and progestagens (22, 24, 29, 30). Androgens also increase female breast cancer risk, either directly by increasing growth and proliferation of cancer cells, or indirectly via conversion to estrogen (31–33). In men, the role of androgens in prostate carcinogenesis has long been discussed but results are inconsistent (34). It has been hypothesized, at least for estrogens, that hormone metabolism may also play a role in cancer etiology (35). The present study has shown an increase of sex hormones and their metabolites as well as changes in their peak time of production in night shift workers. It is not yet known whether abnormally timed rhythms of sex hormone production may affect breast and prostate cancer risk in addition to higher levels.

The increase in 24-hour production of androgen and progestagen metabolites among night workers is a novel finding. A few studies, all in women, have evaluated sex hormones in spot-samples of urine in relation to night shift work (12, 15, 16, 20, 36). Similar to our results, two studies showed higher levels of progesterone and DHEA after recent night shift work and increased estrogens in women with long-term and any night shift work history (12, 15). However, two nights of work in a rotating night shift schedule did not alter sex hormone levels (20, 36). Interestingly, higher testosterone was found among pregnant women with higher light at night exposure (37).There is some evidence for a possible direct effect of bright light on LH and FSH and subsequent stimulation of sex hormone production (38) and higher gonadotropin levels (LH and FSH) have been reported among permanent night workers.

Findings from the present study suggest a direct effect of night shift work on steroid sex hormone production that is independent of melatonin. We have previously reported from the same study that exposure to permanent night shift work was associated with lower aMT6s levels compared with day workers (39). It has been suggested that higher levels of steroid hormones might be related to light-induced melatonin suppression that occurs with night working (11). Melatonin may modulate sex hormone production, downregulating the hypothalamus-pituitary-gonadal axis and possibly inhibiting aromatase, an enzyme that transforms androgens into estrogens (17, 40). At high levels exogenous melatonin reduces estrogen, androgen, and gonadotropins, as demonstrated by some, but not all, clinical trials (41–45). We found no association between endogenous 24-hour urinary levels of aMT6s and a range of steroid hormones and metabolites. Other studies evaluating endogenous melatonin have shown negative (46–48), null (15, 20, 21, 49), and positive associations (12) with plasma sex hormones, therefore the interrelation between melatonin and sex hormone levels is not yet confirmed.

Another novel finding of the study is the later peak time of androgen production observed among night shift workers, particularly among men. Previous studies have only compared levels of steroid hormones based on a single morning blood sample and therefore lacked assessment of steroid hormone rhythmicity and peak time of production (12, 15, 16, 20, 21, 36). Sex hormones and gonadotropins show some daily variation in plasma (50–53), although steroid metabolite rhythms have not been well studied in urine (54). Night shift workers are exposed to irregular light/dark cycles but also disturbed sleep/wake patterns that disrupt the worker's circadian timing organization (55). In the present study, 6-sulfatoxymelatonin acrophase, a reliable marker of circadian phase, was positively correlated with some of the androgens' peak times, suggesting that these rhythms are partly driven by the

Table 4. Peak time (acrophase) of metabolite production [geometric mean (GM) and 95% CIs] in day and night workers and estimated hours of difference in metabolites' peak time [geometric mean difference (GMD) and 95% CI] between night and day workers

	Peak time (acrophase)[a]			
Metabolite	Day workers ($N = 41$) GM (95% CI)	Night workers ($N = 72$) GM (95% CI)	GMD (95% CI)[b]	GMD (95% CI)[c] (+aMT6s peak time)
Melatonin				
6-Sulfatoxymelatonin	05:36 (05:06–06:12)	08:42 (07:48–09:42)	2.9 (1.5–4.3)	—
Estrogens				
Estradiol	09:14 (07:05–12:03)	08:39 (06:53–10:52)	−0.6 (−4.1–2.9)	−0.6 (−4.5–3.3)
Estrone	08:28 (06:43–10:38)	09:10 (07:30–11:11)	0.1 (−2.9–3.2)	−0.8 (−4.0–2.5)
Estriol	06:12 (04:15–09:02)	08:58 (07:38–10:34)[d]	2.2 (−0.9–5.2)	1.7 (−1.6–5.0)
Progestagens				
Pregnanediol	08:46 (06:49–11:16)	09:20 (07:37–11:25)	−0.3 (−3.6–3.0)	−1.1 (−4.7–2.5)
Pregnanetriol	10:01 (07:55–12:40)	10:35 (08:36–13:02)	0.4 (−3.4–4.1)	0.2 (−3.9–4.3)
16-Androstenol	12:20 (10:08–15:03)	12:18 (10:40–14:11)	−1.0 (−4.4–2.3)	−1.5 (−5.2–2.1)
Androgens				
Testosterone	08:35 (06:52–10:46)	12:14 (10:06–14:48)[d]	3.2 (−0.6–6.9)	1.3 (−2.6–5.2)
Epitestosterone	09:11 (08:03–10:28)	13:35 (11:26–16:07)[d]	5.8 (2.5–9.2)	4.6 (1.1–8.2)
DHEA	10:24 (08:03–10:28)	13:43 (12:18–15:17)[d]	3.4 (0.6–6.2)	2.8 (−0.3–5.8)
Androsterone	11:33 (09:54–13:29)	13:04 (11:34–14:47)	0.7 (−2.0–3.5)	0.7 (−2.3–3.7)
Etiocholanolone	09:46 (07:56–11:59)	12:40 (11:20–14:09)[d]	2.8 (0.1–5.6)	2.2 (−0.8–5.3)
11β-OH-androsterone	11:59 (10:27–13:44)	12:38 (11:08–14:22)	0.3 (−2.4–3.0)	0.0 (−2.9–2.9)
4-Androstenedione	08:28 (06:25–11:11)	10:55 (08:52–13:26)	2.8 (−1.2–6.8)	2.5 (−1.9–6.8)
6α-OH-androstenedione	11:37 (10:20–13:03)	12:52 (11:10–14:52)	3.1 (0.1–6.0)	2.8 (−0.4–6.0)
3α,5α-Androstanediol	08:37 (06:31–11:23)	11:28 (09:15–14:13)	2.3 (−1.7–6.3)	2.0 (−2.4–6.3)
3α,5β-Androstanediol	08:40 (06:50–10:58)	11:13 (09:33–13:12)	3.1 (−0.1–6.3)	2.2 (−1.3–5.6)

[a]Peak time is expressed in local time.
[b]Adjusted for age, diurnal preference (M-E score), education (primary school, high school, university), sex, menopausal status (premenopausal, postmenopausal), parity (nulliparous, 1–2, 3 or more children), age at first full-term birth, BMI, physical activity last 24-h (No, ≤14 METS-hours, >14 METS-hours), number of caffeinated beverages last 24-h, sleep duration last 24-h, sleep problems (yes/no), chronic symptoms (yes/no), drug use (yes, no), and hours of sunlight last 24 hours.
[c]Additionally adjusted for aMT6s peak time.
[d]$P_{difference} < 0.05$ for the two-sided t test using log-transformed variable.

central SCN clock. Some sex hormones such as testosterone can also be strongly influenced by sleep, particularly its timing and duration (56, 57). Controlling for self-reported sleep duration in analysis of the current data did not affect the results. However, because night shift work is closely linked to daytime sleep, it is possible that the observed effect on the peak time of androgen production is also due in part to acute sleep restriction. In the current study, the observed differences between men and women are probably not due to sex, but likely are related to differences in the shift schedules that the men and women experienced

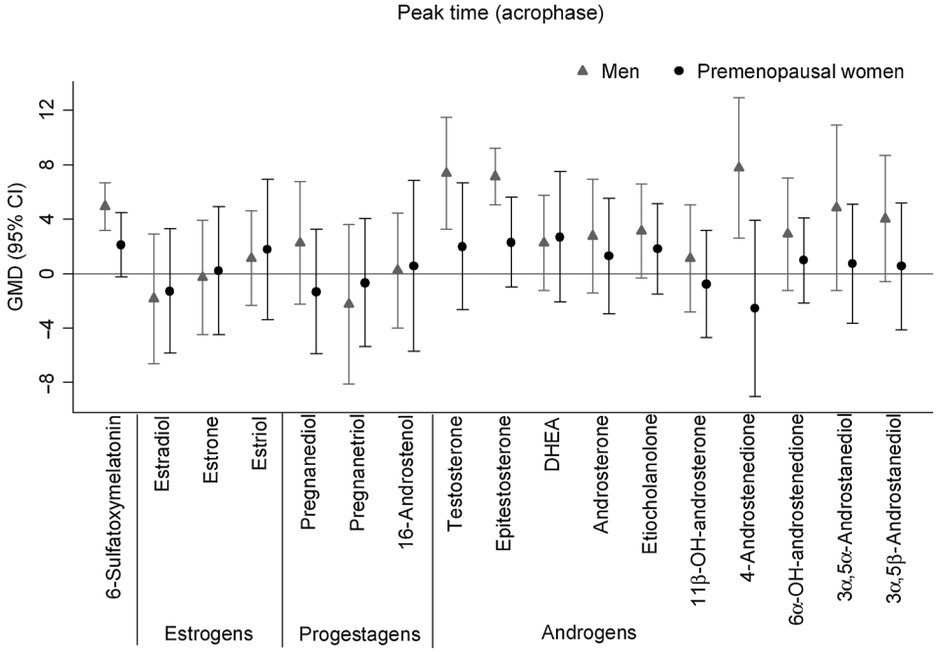

Figure 2.
Estimated hours of difference (GMD and 95% CI) in individual metabolites' peak time (acrophase) between night and day workers in men and premenopausal women, adjusted for age, BMI, menstrual cycle phase (follicular, mid-cycle, luteal), and diurnal preference.

including the intensity of the shift schedules (5 vs. 3 consecutive nights), the shift length (10 vs. 8 hours), the end time of the shift (06:00 vs. 07:00 hours), and the light intensity in the work place, all of which would have led to different daily sleeping, eating, and light exposure patterns.

The extensive evaluation of hormone production in night shift workers by measuring a large number of metabolites together with multiple urine sample collections over a complete 24-hour period is the strength of the current study. The use of individual cosinor analysis enabled us to determine the daily variation of aMT6s and sex steroid hormones, and overcome the limitation of comparing samples that are collected at different time-points. An earlier study (58) proposed a metric for night shift work based on the use of post sleep/post work ratio of urinary 6-hydroxymelatonin sulfate melatonin to help identify workers at increased risk for accidents or injuries. The use of this metric and its extension to include other hormones goes beyond the objectives of the current analysis. Although the timing of the urine collections across the 24-hour period differed between individuals, being natural voids, this would not have affected the cosine-derived parameters (mesor, acrophase). The goodness of fit of the cosine curve for melatonin was high, as expected for this SCN-driven endogenous rhythm. The goodness of fits for the sex hormones were lower indicating a less marked, though present, daily rhythm. An additional asset of the study is the inclusion of a sample of workers including both sexes and different occupational settings that facilitates extrapolation of the results to larger populations of permanent night shift workers. However, in subgroup analysis (by menopausal status or menstrual cycle phase), numbers were small and thus accuracy was reduced. Another limitation of this study is that the menstrual cycle phase calculation was based on the date of the last menstruation before and not following participation/urine collection and that premenopausal women were not able to be studied during the same menstrual cycle phase. Results, however, were very similar between women in the different menstrual cycle phases. All the measured urinary biomarkers are primary liver and kidney metabolites of the hormones, thus, the associations described might also reflect potential effects of night shift work on these organs and peripheral metabolism rather than changes solely in the organs of primary hormone synthesis (pineal, gonads, adrenals). Although day and night workers might differ with respect to lifestyle and reproductive characteristics, we carefully controlled analysis for potential confounders. Predictors may differ between sex hormones and metabolites; however, for simplicity, we adjusted all sex hormone models for the same confounders although the sets of confounders for peak time and levels were selected separately.

In summary, this study shows an association between permanent night shift work and increased urinary levels of androgens and progestagens in both sexes, independent of melatonin suppression. In addition, like melatonin, peak androgen production was delayed among night shift workers. The higher sex hormone levels and mistimed hormone production may reflect a more generalized hormonal disruption that goes beyond melatonin suppression and estrogen increase described by the classical "melatonin hypothesis" for the link between night shift and hormone-dependent cancers.

Disclosure of Potential Conflicts of Interest

B. Middleton and D.J. Skene are the directors of Stockgrand Ltd. No potential conflicts of interest were disclosed by the other authors.

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