Research Article

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Circadian Variation of Melatonin, Light Exposure, and Diurnal Preference in Day and Night Shift Workers of Both Sexes

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Abstract

Background: Light-at-night has been shown in experimental studies to disrupt melatonin production but this has only partly been confirmed in studies of night shift workers. In this cross-sectional study, we examined the circadian variation of melatonin in relation to shift status, individual levels of light-at-night exposure, and diurnal preference, an attribute reflecting personal preference for activity in the morning or evening.

Methods: One hundred and seventeen workers (75 night and 42 day) of both sexes, ages 22 to 64 years, were recruited from four companies. Participants collected urine samples from all voids over 24 hours and wore a data logger continuously recording their light exposure. Sociodemographic, occupational, lifestyle, and diurnal preference information were collected by interview. Concentrations of urinary 6-sulfatoxymelatonin (aMT6s), the main melatonin metabolite, were measured.

Results: Mean aMT6s levels were lower in night [10.9 ng/mg creatinine/hour; 95% confidence interval (CI), 9.5–12.6] compared with day workers (15.4; 95% CI, 12.3–19.3). The lowest aMT6s levels were observed in night workers with morning preference (6.4; 95% CI, 3.0–13.6). Peak time of aMT6s production occurred 3 hours later in night (08:42 hour, 95% CI, 07:48–09:42) compared with day workers (05:36 hour, 95% CI, 05:06–06:12). Phase delay was stronger among subjects with higher light-at-night exposure and number of nights worked.

Conclusions: Night shift workers had lower levels and a delay in peak time of aMT6s production over a 24-hour period. Differences were modified by diurnal preference and intensity of light-at-night exposure.

Impact: Night shift work affects levels and timing of melatonin production and both parameters may relate to future cancer risk. *Cancer Epidemiol Biomarkers Prev; 23(7); 1176–86. ©2014 AACR.*

Introduction

Shift work that involves circadian disruption was classified as a probable carcinogen for humans by the International Agency for Research on Cancer, based on sufficient evidence from animal studies and limited evidence in humans (1). Circadian disruption and abnormal melatonin production are potential underlying biologic mechanisms for the increased risk of cancer among night shift workers (1–5). Pineal melatonin, a key hormone of the circadian system, has oncostatic properties exhibiting antioxidant, antimitotic, antiangiogenic, and immunomodulatory activity (3, 6). Melatonin production typically occurs during the biologic night (peak time 2:00–4:00 hour) and is sensitive to light, the main environmental cue that synchronizes circadian rhythms to the 24-hour day (7). In human experimental studies, exposure to light-at-night suppresses melatonin production in a dose-dependent manner (8–10).

Urinary levels of 6-sulfatoxymelatonin (aMT6s), the major melatonin metabolite, is widely used as a biomarker of circadian phase and disruption. Night shift workers are occupationally exposed to light-at-night and experience lower aMT6s levels as described in some observational studies, performed mostly among nurses (11–19). To date, only a few of these studies have been able to address this issue using objective light exposure measurements (12, 15, 16, 18, 19). Furthermore, after several nights worked, some night shift workers can adapt to their shift schedule and melatonin peak time shifts to coincide with the daytime sleep episode (20–22). In most studies, only absolute aMT6s levels were assessed and not peak time of production. Comparisons of aMT6s levels based on a single void or limited sampling can be confounded by circadian phase differences among individuals (commonly

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found in shift workers). Individual diurnal preference or chronotype is a human attribute with genetic basis that reflects the time of the day functions are active (23). Diurnal preference is suggested to affect shift work adaptation (24). Evening types (subjects with a later melatonin peak) may adapt faster to night shift work by phase delaying their circadian rhythms (21, 25–27), but this is still an understudied hypothesis.

In the present cross-sectional study, we evaluated whether permanent night shift workers produce less aMT6s compared with day workers over a 24-hour working period. We also examined changes in the peak time of urinary aMT6s related with working at night. In both comparisons, we took into account individual diurnal preference and light at night exposure.

Materials and Methods

Study population

Health and Safety department personnel of four companies (two public hospitals, a car manufacturer, and a railway company) in Barcelona, Spain contacted workers and offered them participation in the study. A leaflet with study information and selection criteria was handed to all workers doing fixed day and night shifts in each company. Subjects were not eligible for participation if they had cancer history, were taking oral contraceptives or hormonal therapy, or had been pregnant 6 months before the study. For every two night workers, one day worker from the same company was enrolled as a control. Seventy-five night and 42 day workers of both sexes, ages 22 to 64 years were recruited in the study. Among female participants, all but two were hospital shift nurses or assistants. Among males, all but four were workers in the car industry or the train company. Night shift nurses worked 10 hour fixed shifts (21:00–07:00 hour) 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 hour) 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 hour depending on the working sector. The study was approved by the local ethics committee and all participants signed an informed consent.

Data collection

Subjects were interviewed and information on occupation (working patterns, night shift history), sociodemographics, and lifestyle (physical activity, alcohol consumption, smoking, sleep habits, medication, etc.) was collected. Questions were asked for both habitual patterns as well as for the 24 hours previous to the study. Diurnal preference was assessed using the self-administered Morningness-Eveningness Questionnaire (28). Participants collected samples from all natural urine voids over an approximate 24-hour period on a working day. A total of 1,030 urine samples were collected in 50 mL plastic tubes, which were labeled with the time and date of each collection. Participants were advised to keep the urine samples in a refrigerator immediately after collection and sent them to the laboratory by courier mail 1 or 2 days later. Samples were stored at −80°C until analysis. Four subjects with less than three 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. A mean of 7.8 (SD 2.1) urine samples were collected per participant (7.7; SD 2.1 and 8.0; SD 2.1 among night and day workers, respectively).

6-sulfatoxymelatonin assessment

Urinary aMT6s concentrations were measured at the Chronobiology Group, University of Surrey (Guildford, United Kingdom), using a radioimmunoassay (Stockgrand, Ltd.). Urine samples were analyzed in duplicates in 28 assays and all samples of the same subject were included in the same assay. 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. Interassay 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.) to account for dilution variability and duration between consecutive samples. Limit of detection of the assay was 25.1 mg/dL and interassay variability was 7.6% at 87.4 mg/dL and 9.9% at 198.3 mg/dL. All creatinine concentrations were adjusted to give mg/mL to allow aMT6s values to be quoted as ng/mg creatinine thus giving creatinine-standardized values.

Light exposure assessment

Participants wore a light intensity data logger (HOBOware, Onset Computer Corporation) that continuously (every 12 or 15 seconds) recorded their ambient light exposure over an approximate 24-hour period, simultaneously with the urine collections. The logger was relatively small in size (5.8 × 3.3 × 2.3 cm) and light in weight (18 g) and was carried at the shoulder level. This position was used to obtain measurements that would approximate the amount of light reaching the retina. During sleep, participants placed the logger on a bedside table with the sensor facing upwards and while showering left it nearby in the bathroom. The loggers recorded relative light intensity within the range of 0 to 320,000 lux and were designed for indoor and outdoor settings. Recruitment of participants and therefore light measurements took place from March 15 until June 12 in Barcelona, Spain (latitude 41° 23′N, longitude 2° 10′E); therefore, there was variation in the day length (11.8–15.1 hours of sunlight). Mean light-at night exposure was calculated over different periods, including the period from 24:00 to 05:00 hour (expected time of peak of melatonin), from 22:00 to 07:00 hour

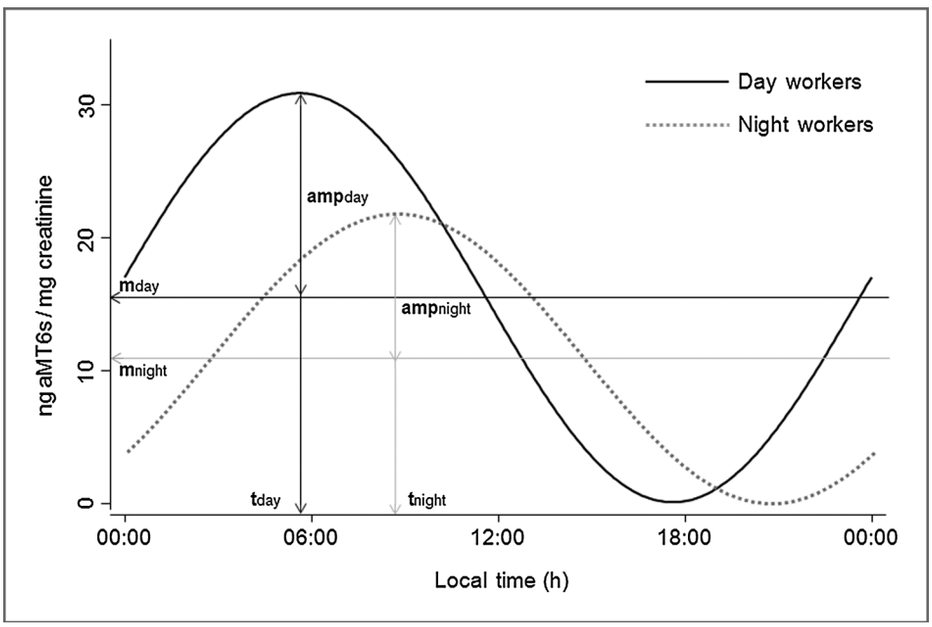

Figure 1. Graphic illustration of the cosinor curves and the derived parameters: mesor (m = circadian mean), amplitude (amp = distance between the maximum and mesor), and acrophase (t = peak time) in day and night workers.

(approximate darkness window during the study period), and during the working period (using time schedules). Morning light exposure immediately after shift work to initiation of sleep was additionally evaluated among night shift workers. Zero values (12% of total) were replaced by half (5.4 lux) of the lowest value recorded by the sensor (10.8 lux) during the night shift to account for intensities below the sensor's detection limit or for the possibility that the sensor was covered by hair or clothes.

Statistical analysis

Cosinor analysis was used to evaluate the aMT6s rhythm (29). Cosinor analysis is a curve fitting procedure that is usually used in circadian and other rhythms with a cyclic nature and an approximate 24-hour period. Our analysis followed a two-step approach. In the first step, cosinor analysis was used to obtain, for each subject, the derived parameters: mesor (circadian mean), amplitude (distance between the maximum and mesor), and acrophase (peak time of production). The mesor from the cosinor analysis is proportional to the AUC (AUC = mesor*24). Two "goodness of fit" measures were used to determine the validity of the cosinor-derived parameters: the percentage variability accounted for the cosine curve and the *P* value for the null hypothesis of no cosine rhythm in the data. In the second step, we calculated geometric means and 95% confidence intervals (95% CI) for each aMT6s cosinor parameter (mesor, amplitude, and acrophase) by shift work status in the study population and also by sex. Linear regression analysis was used to evaluate the association between log-transformed aMT6s cosinor parameters and shift work status. Log transformation was applied to achieve a normal distribution. For aMT6s levels, regression estimates were back-transformed and presented as the geometric mean percentage change. Because mesor was highly positively correlated with amplitude (Spearman rank correlation coefficient 0.95; $P < 0.001$), regression analysis results were only presented for mesor. Phase delay was estimated as the adjusted geometric mean difference of the predicted acrophases in night workers compared with day workers. Light-at-night exposure, short-term (nights worked over the previous 2 weeks before urine collection), and long-term exposure (number of years worked in night shift) to night shift work were categorized using tertiles of exposure among night workers. Standard cutoff points for morningness-eveningness (M-E) score were used to obtain a categorical variable for diurnal preference: evening (M-E score range: 26–41), neither (M-E score range: 42–58), and morning type (M-E score range: 59–67). Associations between aMT6s parameters and shift work were also tested stratifying by night shift workers' diurnal preference, by tertiles of short-term, long-term, and light-at-night exposure. Confounders were assessed using a Directed Acyclic Graph (DAG), shown in online Supplemental Materials (Fig. 1). On the basis of the DAG, all models were adjusted for potential confounders: age, diurnal preference (neither, evening, morning), education (primary school, secondary, university), sex (male, female), menopausal status (pre, post), parity (nulliparous, 1–2 children, 3–4 children), and age of first birth (nulliparous, <30 years, ≥30 years). Analyses were repeated using the criteria of 10% change in the coefficients for confounders' selection. Estimates were slightly different from the simpler models, but differences did not follow a common pattern. Results of this analysis are presented in the online supplements (Table 1 and Table 2). A sensitivity analysis was performed by removing four subjects for whom the aMT6s cosinor fit was not significant ($P > 0.05$) or the percentage of rhythm was low (<50%) and two

Table 1. Characteristics of the study population ($N = 113$)

	Day workers ($N = 41$)	Night workers ($N = 72$)
Age, y; mean (SD)	41.6 (9.5)	44.3 (10.4)
BMI (kg/m^2); mean (SD)	25.2 (4.1)	25.6 (4.2)
Participating companies (%)		
Hospital A	22.0	29.2
Hospital B	24.4	20.8
Car industry	29.3	25.0
Railway company	24.4	25.0
Female sex (%)	48.8	45.8
Premenopausal (%)[a]	80.0	48.5[b]
Nulliparous (%)[a]	20.0	9.7
Age first child >30 y (%)[a]	20.0	30.3
Highest education completed (%)		
Primary school	7.3	16.7
High school	51.2	43.0
University	41.5	40.3
Diurnal preference (%)		
Evening type	12.2	22.2
Neither type	68.3	68.1
Morning type	19.5	9.7[b]
Current smokers (%)	36.6	31.9
Chronic illness (%)	26.4	50.0[b]
Drug use (antidepressants, NSAIDS, β-blockers and calcium channel blockers; %)	9.8	23.6[b]
Sleep problems (%)	90.2	94.4
Sleep duration on a working day (h); mean (SD)	6.4 (0.9)	5.9 (1.4)[b]
Sleep duration on a day off (h); mean (SD)	8.1 (0.8)	8.2 (1.5)
Habits over the last 24 h; mean (SD)		
Alcohol consumption (g)	1.3 (3.9)	2.8 (7.3)
Total caffeinated beverages (*n*)	2.9 (2.1)	2.5 (1.9)
Physical exercise (METS*h)	18.3 (10.4)	14.9 (9.6)

[a]Percentages are calculated among female day ($n = 20$) and night ($n = 33$) shift workers.
[b]$P < 0.05$ (χ^2 for categorical and *t* test for continuous variables).

subjects with extreme aMT6s acrophases and results remained unchanged. All analyses were performed using statistical package Stata version 12.1 (StataCorp LP).

Results

Basic sociodemographic and lifestyle characteristics of day and night shift workers are shown in Table 1. Night shift workers were older [mean (SD); 44.3 (10.4) vs. 41.6 (9.5) years], less educated (83.3 of high school or higher vs. 92.7%), more frequently evening types (22.2 vs. 12.2%), and slept less on working days (5.9 vs. 6.4 hours) compared with day workers. No differences in body mass index (BMI), physical activity, alcohol, or caffeine consumption over the last 24 hours were found between the two groups. Night workers reported more frequently the use (23.6 vs. 9.8%) of medications that can affect melatonin (antidepressants, NSAIDS, beta blockers, calcium channel blockers) or a chronic illness (50 vs. 26.4%), including high cholesterol, hypertension, thyroid disease, allergy, asthma, and depression. A lower percentage of female night workers were premenopausal (48.5 vs. 80%), nulliparous (9.7 vs. 20%), and had their first full-term birth before 30 years of age (70 vs. 80%) compared with day workers. Night shift workers at the time of their participation had worked on average 2.4 (SD 1.1) consecutive nights (2.1 in the hospitals and industry, and 3.7 in the train company), 6.1 nights (SD 2.2) over the past 2 weeks, and had been engaged in a total of 12.9 years (SD 10) of night shift work. Table 2 shows the description of light exposure over different periods in day and night shift workers. From 24:00 to 05:00, night shift workers were exposed to a median light intensity of 38 lux [interquartile range (IQR) 26] which was mostly generated from overhead fluorescent lamps. Mean light exposures over the night shift ranged from 15 to 246 lux but intensities were similar across the three working sectors.

Figure 1 shows the mean aMT6s cosinor curves and Table 3 shows the description of the cosinor-derived

Table 2. Average light exposure in day and night workers

	Day workers ($N = 41$)		Night workers ($N = 72$)	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
24-h light exposure (lux)	1,061 (881)	834 (675)	996 (1,735)	441 (1,222)
Light-at-night exposure (lux)				
from 24:00 to 05:00 h	10 (19)	1 (10)	51 (49)	38 (26)
from 22:00 to 07:00 h	57 (64)	30 (65)	192 (297)	43 (43)
Light exposure at work (lux)[a]				
All sectors	1,250 (1,517)	784 (1,142)	60 (46)	43 (38)
Hospitals	822 (691)	640 (787)	64 (49)	47 (47)
Car industry	1,342 (1,730)	872 (783)	69 (24)	44 (59)
Railway company	1,950 (2,160)	1,544 (1,815)	43 (18)	39 (12)
Morning light after work (lux)[b]	—	—	660 (1,268)	221 (565)

[a]Estimation based on start and end time of work for each participant.
[b]Estimation based on end time of night shift and time of sleep onset on working days for each participant.

parameters in day and night workers. The aMT6s mesor was lower among night workers (10.9 ng/mg creatinine) compared with day workers (15.4 ng/mg creatinine) over a 24-hour working day. On average, the aMT6s peak time occurred 3 hours later in night workers (08:42 hour, 95% CI, 07:48–09:42) compared with day workers (05:36 hour, 95% CI, 05:06–06:12). Peak time was more delayed in male night shift workers (9:54 hour; 95% CI, 8:42–11:30) and particularly among workers in the railway company (10:17 hour; 95% CI, 9:02–11:42). We found a small positive correlation between aMT6s levels and acrophase among night shift workers (Pearson correlation coefficient 0.23, $P < 0.05$) over 24 hours.

The associations between aMT6s cosinor parameters and night shift work, night shift workers' diurnal preference, nights worked over the past 2 weeks, and years worked night shifts among night shift workers are shown in Table 4. After adjusting for potential confounders, night shift workers had 33.8% (95% CI, −48.4 to −15.1) lower aMT6s levels compared with day workers. The corresponding decreases in the hospitals, car industry, and railway company workers were −38.3% (−55.2 to −15.2), −36.3% (−56.5 to −6.7), and −17.1% (−44.3 to 23.5), respectively. In night shift workers, the aMT6s peak occurred significantly later (3 hours, $P < 0.001$) than in day workers. The corresponding delay in the hospital, car

Table 3. Description of aMT6s cosinor-derived parameters: mesor (circadian mean), amplitude (difference between maximum and mesor), and acrophase (time of peak) in the study population

		Crude geometric mean (95% CI)		
	N	Mesor[a]	Amplitude[b]	Acrophase[c]
All subjects				
Day workers	41	15.4 (12.3–19.3)	15.4 (11.2–21.0)	5:36 (5:06–6:12)
Night workers	72	10.9 (9.5–12.6)[d]	10.9 (9.0–13.4)[d]	8:42 (7:48–9:42)[e]
Hospitals	36	11.6 (9.4–14.3)[d]	11.4 (8.4–15.3)	7:42 (6:30–9:12)[d]
Car industry	18	9.2 (7.0–12.0)[d]	10.0 (6.7–14.9)[d]	9:31 (7:03–12:18)[e]
Railway company	18	11.6 (8.2–16.4)	11.2 (7.5–16.9)	10:17 (9:02–11:42)[e]
Females				
Day workers	20	17.1 (11.7–25.1)	16.1 (9.1–28.5)	5:54 (4:48–7:12)
Night workers	33	11.4 (9.2–14.2)[d]	10.9 (8.0–15.0)[d]	7:24 (6:12–8:48)[e]
Males				
Day workers	21	14.0 (10.6–18.3)	14.7 (10.4–20.6)	5:24 (4:54–5:48)
Night workers	39	10.5 (8.6–12.9)[d]	10.9 (8.5–14.3)[d]	9:54 (8:42–11:30)[e]

[a]Expressed in ng/mg creatinine/hour.
[b]Expressed in ng/mg creatinine.
[c]Expressed as local time.
[d]$P < 0.05$ using the Wilcoxon rank-sum test.
[e]$P < 0.001$ using the Wilcoxon rank-sum test.

Table 4. Estimated aMT6s mesor (circadian mean) and acrophase (peak time) by night shift work (shift status, night shift workers' diurnal preference, nights worked over the past 2 weeks, and total years worked in night shift work)

		Mesor			Acrophase		
	N	Crude geometric mean (95% CI)[a]	Adjusted mean % change (95% CI)[b]	*P*[c]	Crude geometric mean (95% CI)[d]	Adjusted mean difference (95% CI)[b]	*P* value[c]
Day workers	41	15.4 (12.3–19.3)	Ref.		5:36 (5:06–6:12)	Ref.	
Night workers	72	10.9 (9.5–12.6)	−33.8 (−48.4 to −15.1)	<0.001	8:42 (7:48–9:42)	3.0 (1.8–4.2)	<0.001
Night shift workers's diurnal preference							
Evening	16	11.8 (8.7–15.8)	−23.0 (−46.8 to 11.5)	<0.001	8:30 (5:48–12:24)	3.0 (1.0–5.1)	0.002
Neither	49	11.5 (9.7–13.6)	−26.9 (−44.3 to −4.1)	<0.001	8:48 (7:48–9:54)	3.1 (1.7–4.4)	<0.001
Morning	7	6.4 (3.0–13.6)	−53.7 (−72.2 to −22.9)	<0.001	8:30 (6:30–11:18)	3.3 (0.3–6.4)	0.012
Nights worked over the past 2 weeks							
≤4 nights	27	10.5 (8.6–12.9)	−40.6 (−56.4 to −19.1)	0.001	8:48 (7:42–10:00)	3.3 (1.6–5.1)	<0.001
5–8 nights	35	11.1 (8.8–13.9)	−30.1 (−48.1 to −5.8)	0.019	7:18 (5:30–9:48)	2.5 (1.0–4.0)	0.001
≥9 nights	10	11.6 (6.9–19.5)	−22.9 (−50.8 to 21.0)	0.255	10:06 (7:36–11:54)	3.7 (0.9–6.5)	0.003
Total years worked in night shift work							
≤9 y	36	11.7 (9.6–14.4)	−34.6 (−50.8 to −12.9)	0.004	9:12 (7:30–11:12)	3.2 (1.7–4.8)	<0.001
10–19 y	16	10.6 (7.2–15.8)	−24.6 (−47.7 to 8.6)	0.127	9:00 (7:42–10:30)	3.6 (1.4–5.8)	<0.001
≥20 y	20	9.8 (7.6–12.7)	−43.9 (−62.4 to −16.3)	0.005	7:42 (6:24–9:06)	1.7 (−0.3 to 3.7)	0.079

[a]Expressed in ng/mg creatinine/hour.

[b]Adjusted for age, chronotype, educational level, sex, menopausal status, parity, and age at first full-term birth.

[c]Wald test.

[d]Expressed as local time.

industry, and railway company workers was 2.0 (0.4–3.6), 3.8 (1.5–6.1), and 4.6 hours (2.0–7.2), respectively. Night shift workers with morning preference had 53.7% lower aMT6s levels ($P < 0.001$), whereas this reduction was 23.0% and 26.9% for night shift workers with evening and neither preference ($P < 0.001$), respectively, compared with day workers. Diurnal preference did not modify the association between night shift work and aMT6s acrophase and all night shift workers had approximately 3 hours phase delay compared with day workers across the three groups of diurnal preference ($P < 0.05$). In contrast, in day workers, aMT6s peak time was earlier in morning types (5:12 hour; 4:36–5:48) compared with neither (5:36 hour; 4:54–6:30) and evening types (6:42 hour; 6:00–7:36). As with night workers, aMT6s levels differed by diurnal preference among day workers. Mesor was 22.4 ng/mg creatinine/hour (95% CI, 13.9–50.3) for day workers with evening preference, 15.5 (95% CI, 11.7–20.6) with neither, and 10.5 (95% CI, 7.4–14.7) with morning preference.

When evaluating aMT6s levels by the number of nights worked over the previous 2 weeks, subjects with $\leq$4 nights had a 40.6% (95% CI, −56.4 to −19.1) lower aMT6s levels compared with day workers, whereas subjects with $\geq$9 nights had a decrease of 22.9% (95% CI, −50.8–21.0), suggesting a phase shift among subjects with more nights worked, compared with day workers (Table 4). Indeed the higher the number of night shifts performed over the previous 2 weeks, the later the time of aMT6s peak phase delay; 3.3 hours (95% CI, 1.6–5.1) among those with $\leq$4 nights and 3.7 hours (95% CI, 0.9–6.5) among those with $\geq$9 nights worked. Long-term exposure to night shift work was associated with lower aMT6s levels with the highest exposed group (>20 years) showing a 43.9% decrease in aMT6s levels (95% CI, −62.4 to −16.3).

As shown in Table 5, aMT6s levels were 30%, 36%, and 35% lower in night workers in the first, second, and third tertile of 24:00 to 05:00 hour light exposure, respectively, compared with day workers ($P < 0.05$). Night shift workers in the second and third tertile of 24:00 to 05:00 hour light exposure had a 3.7-hour delay ($P < 0.001$), whereas those in the first tertile had a 1.7-hour delay ($P = 0.008$) compared with the day workers; however, differences between groups of light exposure were not statistically significant. Night shift workers in the top tertile of light exposure at work had a 37.7% decrease ($P = 0.005$) and 3.2 hours of a delay ($P < 0.001$), whereas those in the lowest tertile had a 27.3% decrease ($P = 0.060$) and 2.5 hours delay ($P = 0.003$) in their acrophase, compared with day workers. Similar results were observed when the aMT6s parameters were compared with 22:00 to 07:00 hour light exposure and morning light exposure (results not shown).

We adjusted all light-at-night models for morning light to account for sunlight exposure immediately after the night shift and results remained unchanged. Furthermore all models in Table 4 were adjusted for light-at-night exposure in an additional analysis and effects became stronger. All analyses were repeated excluding subjects with reported chronic diseases. Results (not shown) were similar to those of the main analysis.

Discussion

Circadian aMT6s variation was evaluated in night and day shift workers over a 24-hour period on a working day. Lower aMT6s levels and later peak times were found among permanent night workers compared with day workers. Differences in aMT6s levels were more pronounced in night shift workers with morning preference, whereas a delayed aMT6s peak time was associated with a higher light-at-night exposure and number of nights worked.

These results are in agreement with some (11–17) but not all (18, 19, 30–32) previous studies, showing a reduction of aMT6s levels related to night shift work. Grundy and colleagues (19) found nonsignificant aMT6s differences between the day and night shift among female nurses working on a rapid rotating shift schedule. Authors suggested that only 2 consecutive nights may not be enough for a disruption to be observed. Davis and colleagues (11) reported a 69% decrease in aMT6s levels during the night working period and a 63% decrease during the daytime sleep period among female health care night shift workers performing more than 2 consecutive nights per week. We found lower aMT6s levels among subjects that worked in schedules with less consecutive nights (hospitals and car industry). However, all participants in our study were engaged in fixed night shifts and might represent a group with extreme exposure among the existing shift schedules and thus more likely to experience detectable biologic changes.

Previous studies have lacked melatonin acrophase assessment, therefore small phase shifts would not have been detected. In the present study, a 3-hour phase delay in aMT6s peak time was observed among night shift workers suggesting an overall partial shift adaptation, which was larger among males. Sex differences in the current study largely represent differences in the working sectors and the respective night shift schedules (start and stop times, hours of work, night shift work intensity, number of consecutive nights, and number of days off) and related habits (e.g., sleep, light exposure, timing of meals, and social activities). Adaptation was more pronounced among night workers with more nights worked (railway company and industry), with aMT6s peaking during their reported sleep (average onset 07:00–08:00 hour). Most hospital night workers exhibited a aMT6s peak before their average reported sleep onset (08:00–09:00 hour) and around the end of their night shift. It has been suggested that some of the adverse effects of night shift work (sleepiness and work-related accidents) may be reduced if circadian adaptation occurred, such that a better alignment of circadian timing with the new sleep-wake pattern is achieved (33, 34). However, even among permanent night shift workers, several

Table 5. Estimated aMT6s mesor (circadian mean) and acrophase (peak time) by light exposure among night shift workers (tertiles of mean light exposure from 24:00 to 05:00 hour and over the night shift)

		Mesor			Acrophase		
	N	**Crude geometric mean (95% CI)[a]**	**Adjusted mean % change (95% CI)[b]**	***P*[c]**	**Crude geometric mean (95% CI)[d]**	**Adjusted mean difference (95% CI)[b]**	***P*[c]**
Day workers	41	15.4 (12.3–19.3)	Ref.		5:36 (5:06–6:12)	Ref.	
Night workers	72	10.9 (9.5–12.6)	−33.8 (−48.4 to −15.1)	<0.001	8:42 (7:48–9:42)	3.1 (1.8–4.4)	<0.001
Mean light-at-night exposure from 24:00 to 05:00 hour (lux)							
≤14	24	11.4 (8.8–14.8)	−30.3 (−49.9 to −3.1)	0.032	7:12 (5:36–9:00)	1.7 (0.1–3.3)	0.008
15–40	24	10.7 (8.4–13.5)	−36.3 (−54.6 to −10.7)	0.009	9:18 (8:00–10:48)	3.7 (1.8–5.6)	<0.001
41–315	24	10.7 (8.0–14.4)	−35.0 (−53.0 to −10.0)	0.010	9:48 (7:48–12:06)	3.7 (1.8–5.5)	<0.001
Mean light-at-night exposure over the night shift (lux)							
<38	24	11.3 (8.7–14.7)	−27.3 (−47.8 to 1.3)	0.060	8:06 (6:18–10:24)	2.5 (0.8–4.3)	0.003
38–55	24	10.3 (8.2–13.0)	−35.3 (−53.1 to −10.8)	0.008	10:18 (8:18–13:00)	3.2 (1.4–5.0)	<0.001
55–246	24	11.2 (8.3–15.0)	−37.7 (−55.1 to −13.6)	0.005	9:12 (7:18–11:30)	3.2 (1.4–5.0)	<0.001

[a]Expressed in ng/mg creatinine/hour.

[b]Adjusted for age, chronotype, educational level, sex, menopausal status, parity, and age at first full-term birth.

[c]Wald test.

[d]Expressed as local time.

consecutive nights or weeks of night shift work seem to be required for full adaptation (aMT6s phase delay) to occur (21, 35). In the present study, we found a smaller decrease in aMT6s levels among workers with a higher number of night shifts that may suggest a rhythm phase delay due to partial adaptation. We also found a positive association between aMT6s mesor and acrophase among night workers. It is possible that the effect of light at night on melatonin levels is stronger among less adapted night workers or subjects with an earlier aMT6s peak time.

Long-term exposure to night shift work was also associated with lower levels of melatonin in our study. Chronic melatonin suppression has been linked to both initiation and promotion of cancer because melatonin is a hormone with oncostatic properties (4, 6, 36, 37). However, the anticancer effects of melatonin depend on the circadian stage, as suggested by animal studies (reviewed in ref. 38). Therefore, nocturnal timing of melatonin rhythm might be as important as absolute levels produced on a daily basis for its oncostatic role to be expressed and maintained. It is still unknown whether partial phase shifts (3 hours) as described here can increase cancer risk in humans and future studies should consider possible rhythm changes when assessing night shift work-related circadian disruption.

In this study, subjects with morning preference had lower melatonin levels, compared with those with evening preference. To date, evidence is mixed for the association between diurnal preference and aMT6s levels in shift workers (19, 39) as well as nonshift workers (40, 41). Morning preference has been associated with an earlier melatonin peak time (26, 27, 39, 42). Individual circadian phase assessed by melatonin timing (21, 25) and chronotype has been associated with workers' adaptability to shift-work schedules (24). Evening types report better tolerance for night work and quicker adaptation (shift of the circadian rhythms) than morning types (20). We did not find an association between diurnal preference and aMT6s acrophase in night shift workers, as we had expected, perhaps because we lacked subjects with extreme morning or evening preferences, or due to low numbers. Very little is known about the association between chronotype and cancer risk (43). Chronotype has been suggested as a possible effect modifier for the night shift work-cancer association (43, 44). Our findings provide evidence that subjects with morning preference might be a group more susceptible to light-at-night effects and night shift work-related circadian disruption.

Lower aMT6s levels and later acrophases were found among night shift workers compared with day workers despite low light exposure at work (<38 lux). In experimental studies, light-at-night suppresses melatonin in a dose–response manner; however, even low intensities of light exposure (100 lux) are able to both suppress and phase shift the human melatonin rhythm (8). Not only intensity, but also timing of light exposure is important for the direction of the aMT6s phase shift, as described by the phase–response curve (45, 46). We not only examined the phase shifting abilities of light exposures occurring during the night shift, but also tested the time period immediately after the shift and up to initiation of sleep and results were similar when morning light was taken into account. A few observational studies have included light measurements in their assessments. Only one study evaluated 24-hour melatonin production and monitored personal light exposure simultaneously including 13 rotating night workers (18). Light-at-night exposure at work (median 73 lux) did not have an effect on nighttime melatonin production although it reduced total 24-hour melatonin output. Our results are in agreement with this study; we additionally found a phase delay among night workers that was stronger with higher light exposures. The light loggers we used, however, may provide lower estimates compared with calibrated powermeters, particularly for low intensities of light (Peter Morgan, University of Surrey 2011).

One of the strengths of the present study is the use of repeated aMT6s measurements over a 24-hour period that enabled the assessment of the peak time of melatonin production. Comparisons are not likely to have been confounded by interindividual variation in circadian phase, a main limitation in existing shift work studies. Furthermore, participants of both sexes and from three different occupational settings were recruited, thus increasing the external validity of the results. Potential confounders were carefully selected in this analysis and the effect of night shift work on melatonin did not change substantially after adjustment. In this study, however, due to its cross-sectional design, we cannot rule out the possibility that subjects who engaged in night shift work had lower baseline levels of aMT6s compared with day workers for other nonnight shift work-related reasons. An additional strength of this study is the assessment of individual light exposure using objective light measurements. The light monitors we used measured light intensity but not wavelength (e.g., blue light), an aspect of light that might be important for melatonin suppression and circadian resetting (47, 48). The characterization of diurnal preference is a final strength of this study. We used the Horne–Östberg questionnaire, a validated instrument but designed for the general population. Future studies could consider the use of the Munich Chronotype Questionnaire (MCTQ) and its extended version (MCTQShift), to assess chronotype in night and rotating shift workers (49).

In conclusion, this study evaluated the association of night shift work and individual characteristics with melatonin production and timing, the main mechanism proposed to underlie the association between night shift work and cancer risk. Our findings show that exposure to permanent night shift work is associated with lower aMT6s levels and a phase delay in aMT6s peak time compared with day workers, even under low intensities of light at night. This study also provides

new evidence indicating that night shift work might have a greater impact on melatonin production among morning types.

Disclosure of Potential Conflicts of Interest

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

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