Research Article

Cancer Epidemiology, Biomarkers & Prevention

The Influence of Light at Night Exposure on Melatonin Levels among Canadian Rotating Shift Nurses

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Abstract

Background: Shift work has been identified as a risk factor for several cancer sites in recent years, with melatonin as a potential intermediate on the proposed causal pathway. This study examined the influence of nighttime light exposure on melatonin levels among 123 rotating shift nurses.

Methods: Nurses working a rotating shift schedule (two 12-hour days, two 12-hour nights, and five days off) were recruited and participated on a day and night shift in both the summer and winter seasons. Over each 48-hour study period, nurses wore a light data logger and provided two urine and four saliva samples.

Results: Saliva measurements showed that the pattern of melatonin production did not differ between day and night shifts. Mean light exposure was significantly higher ($P < 0.0001$) when nurses were working at night, although peak melatonin levels ($P = 0.65$) and the daily change in melatonin levels ($P = 0.80$) were similar across day/night shifts. Multivariate analysis did not show an association between light exposure and melatonin levels when data from both shifts was combined; however, when data from the night shift was considered alone, a statistically significant inverse relationship between light and change in melatonin was observed ($P = 0.04$).

Conclusion: These results show that light exposure does not seem to be strongly related to reduced melatonin production among nurses on this rapidly rotating shift schedule.

Impact: Future research considering more extreme shift patterns or brighter lighting conditions could further clarify the relationship between light exposure and melatonin production in observational settings.
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Introduction

Shift work has been recognized as a risk factor for several cancer sites (1–12) and, in 2007, was classified as a probable carcinogen by the International Agency for Research on Cancer (IARC; ref. 13). The majority of epidemiologic studies of shift work and cancer have focused on relationships with breast cancer, in which results from meta-analyses show a 40% to 50% increase in risk with long-term shift work (14, 15).

Chronodisruption (altered circadian rhythms) associated with shift work is thought to be responsible for observed increases in cancer risk (15, 16). Although the exact biological pathway is unknown, melatonin, a hormone produced according to circadian rhythms with peak levels seen at night (17), has been suggested as a potential intermediate. It is hypothesized that light exposure during night work could be responsible for an increased risk of cancer through a reduction in nighttime melatonin production (18, 19). Several anticarcinogenic properties of melatonin have been suggested, including inhibition of tumor development and reduction in levels of reproductive hormones, thought to play a role in cancer etiology (20–25). Although results are not consistent, prospective epidemiologic studies have shown an inverse association between melatonin and breast cancer risk (26–30).

Experimental studies have shown a dose–response relationship between light exposure and melatonin levels, in which increased light is associated with decreased melatonin production (31–35). Epidemiologic studies, primarily among shift workers, have used multiple methods to assess exposure (36–41) and results generally show an inverse relationship between light and melatonin (36–41). However, several existing studies have been limited by issues related to the timing of melatonin assessment, in which functional time points (e.g., after sleep), as opposed to chronologic (clock) times, have been compared between shift groups (37, 39, 40). This melatonin assessment strategy assumes that the timing of melatonin production among individuals working nonday shifts will be altered.

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However, if this assumption is not met, comparisons of nighttime light exposure and melatonin levels across shift groups may be confounded by circadian rhythm, when comparing melatonin levels from samples obtained at different times of day (40). Therefore, observational studies with comparisons of chronologic time points between shift groups are needed to determine whether the relationship between light and melatonin in experimental work (31–35) is also seen in the context of an observational study. Recent work also indicates a need to incorporate other factors related to melatonin production, such as chronotype, when investigating these relationships (42).

Because shift work is necessary for many occupations, understanding the mechanism linking it with cancer risk is important to develop healthy workplace policy. However, the IARC classification of shift work as a "probable carcinogen" was based on limited evidence from studies in humans, due to inconsistencies in definitions of shift work in existing literature (1–13, 43). Furthermore, although the influence of light on melatonin is often cited as the mechanism linking shift work and cancer, evidence from observational biomarker studies supporting this relationship is limited (37, 39, 40). Epidemiologic research with objective measurements of light exposure that avoids confounding of melatonin measures by circadian rhythm through careful consideration of the timing of melatonin assessment is needed. Therefore, a longitudinal study among full-time nurses was conducted, the objectives of which were to investigate the influence of nocturnal light exposure and shift work history on melatonin levels among a group of rotating shift nurses. It was hypothesized that both light exposure and history of long-term shift work would be associated with decreased melatonin production.

Methods

Study population

Female full-time registered nurses working the two 12-hour days, two 12-hour nights, 5 days off (DDNN) shift pattern at Kingston General Hospital were offered the opportunity to participate in this study. Nurses were not eligible if they had been pregnant or lactating in the previous 6 months or if they were taking melatonin supplements. Study participation, including these exclusion criteria, was advertised through posters and pamphlets sent to all full-time nursing staff, as well as through presentations to specific hospital units, with those who were ineligible asked to self-exclude. There are approximately 700 full-time nurses at the hospital; however, the number not working the DDNN shift schedule or ineligible for other reasons was unavailable, meaning response rates could not be calculated. Data collection occurred from May 2008 to August 2009 and was approved by the Queen's University Health Sciences Research Ethics Board.

The study included 4 data collection periods, in which nurses were asked to participate in the study twice (day and night shift), in both the summer and winter seasons. For logistical reasons, participants were recruited in 2 cohorts: one in 2008 starting in the summer and one in 2009 starting in the winter. A total of 123 nurses were enrolled in the study, with 118 completing both shifts in the first season and 103 completing the day shift and 96 completing the night shift in the second season. Reasons for loss to follow-up included changes in work schedule, pregnancy, illness/injury, and being too busy to complete the study protocol.

Procedures

Each participation session took place over a 48-hour time period covering either a day or a night shift. During each session, participants were asked to wear a Stow-Away light data logger (Hoskin Scientific Ltd.) and provide 4 saliva and 2 urine samples over a 24-hour period (Fig. 1). The light loggers, which measured ambient light intensity in lumens/m^2, began recording at the beginning of the first day or first night shift in the rotating shift pattern and took readings every minute for the duration of the study period. Participants wore the loggers around their neck for the entire 48 hours of study participation and placed the logger on the bedside table while sleeping.

Participants completed a study questionnaire prior to the first data collection period and a study diary during all 4 data collection periods. The questionnaire collected personal information and a history of health, lifetime

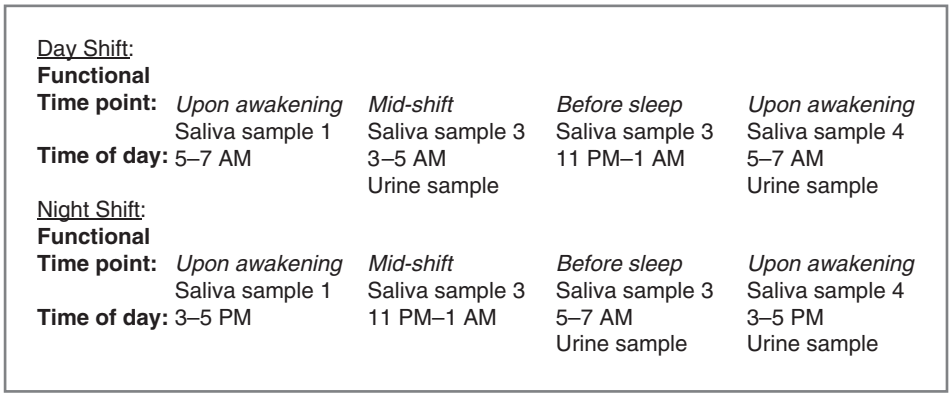

Figure 1. Urine and saliva sample collection time line. Sample collection occurred during the first day shift and second night shift of the rotating shift pattern.

employment, and lifestyle characteristics, including lifetime smoking and alcohol consumption patterns. The diary collected information about physical activity, lighting conditions, smoking, alcohol and caffeine consumption, use of medications, and sleep duration and timing during the 24 hours of melatonin collection. Participants' height and weight were measured by trained study personnel when nurses enrolled in the study. After study participation had begun, the Horne–Ostberg Questionnaire (44) about chronotype was added. Participants were sent a copy of the questionnaire and asked to return it by intrahospital mail.

Melatonin laboratory analysis

Levels of the primary urinary melatonin metabolite, 6-sulfatoxymelatonin, were assessed from urine samples using the Bühlmann 6-sulfatoxymelatonin ELISA Kit (ALPCO Diagnostics). Urinary 6-sulfatoxymelatonin levels in the first morning void represent approximately 70% of blood levels and are considered a good measure of absolute overnight melatonin production (45). To account for differences in urine volume, creatinine levels were assessed using the Parameter Creatinine assay (R&D Systems, Inc.) and melatonin levels adjusted for creatinine. Salivary melatonin levels were directly assessed from saliva samples using the Bühlmann Saliva Melatonin EIA Kit (ALPCO Diagnostics). Salivary melatonin represents approximately 30% of blood levels and is not a good measure of absolute melatonin levels; however, saliva is considered a good marker of melatonin variability within an individual over a 24-hour period (46). Saliva levels were used to characterize production patterns over each 24-hour study period to facilitate comparisons between day and night shifts. Samples for all 3 assays were run in duplicate and median coefficients of variation were 9.7% for urinary melatonin, 12.3% for salivary melatonin, and 9.2% for creatinine measures. According to the manufacturer's instructions, all melatonin assays were run with 6 standards, a blank and a high and low control of known concentration, and creatinine assays were run with 7 standards and a blank.

Statistical analysis

For characteristics measured through the study questionnaire, means and SDs were calculated for continuous variables and percentages for categorical variables. For characteristics specific to each data collection period measured in the study diary, least squares means and SEs in a mixed model with a random subject effect were calculated for continuous variables and percentages for categorical variables. Differences between characteristics on day and night shifts were compared within each season using difference in least squares means estimates for continuous variables and McNemar's test for categorical variables.

Light exposure was characterized as the average light intensity from 12 AM to 5 AM, the expected time of peak melatonin production for both day and night shifts. Peak melatonin levels were those from urine samples collected during the early morning for both day and night shifts, and the change in melatonin levels over the 24-hour period was characterized as the difference in 6-sulfatoxymelatonin levels measured from the 2 urine samples (Fig. 1). For both melatonin characterizations, geometric least squares means (back transformed means of log-transformed variables) were calculated, as neither untransformed melatonin measure was normally distributed.

To characterize melatonin secretion patterns on day and night shifts, mean salivary melatonin levels from samples taken at similar times of day were compared between the day and night shifts within each of the 2 seasons using difference in least squares means estimates. These means were then graphed (Fig. 2) to compare timing of melatonin secretion across shift types.

Multivariate associations between light exposure from 12 AM to 5 AM and both peak and change in 6-sulfatoxymelatonin levels were assessed using mixed multiple linear regression in a random effects model to account for the repeated measures within individuals. Models were built using an all-possible-models backwards selection procedure (47), in which potential confounders that were associated with the outcome at $P < 0.25$ were included in the modeling process, and only variables changing the parameter estimate by more than 10% were included in the final model. Variables considered as potential confounders were as follows: age, body mass index (BMI), total years of shift work, general lifestyle characteristics including smoking status, pack-years smoking, and lifetime alcohol consumption patterns; reproductive characteristics including age at menarche, ever having been pregnant and number of pregnancies, number of days since previous menstrual period, and menopausal status; season; regular use of antidepressants, beta-blockers, hormone replacement therapy, or migraine medication; use of nonsteroidal anti-inflammatory drug (NSAID), sedatives, or oral contraceptives during the 24 hours of melatonin assessment; the number of alcoholic and caffeinated beverages consumed and smoking behavior

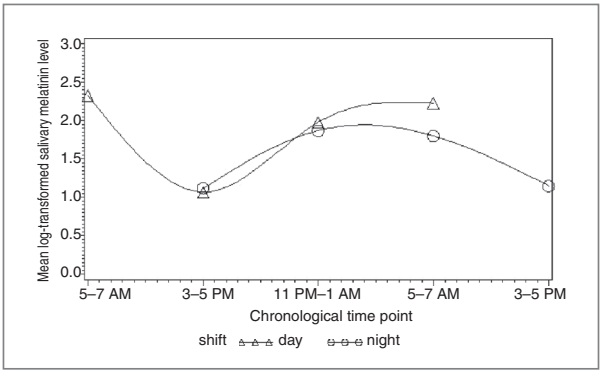

Figure 2. Salivary melatonin levels comparing chronologic time points in season 1. For comparisons between day and night workers: 3PM–5PM: $P = 0.99$; 11PM–1AM: $P = 0.84$; 5AM–7AM: $P = 0.04$.

during the 24 hours of melatonin assessment; and sleep duration and wearing a mask for daytime sleep. Analyses stratified by shift type (day/night), season (summer/winter), and among premenopausal women only were also conducted using the same model building strategies.

The relationship between shift work history (total years of work in jobs in which at least 50% of shifts were night shifts to capture both rotating and permanent night shift patterns) and melatonin production on the night shift was investigated using multiple linear regression. Because lifetime history of shift work was assessed only once in the study questionnaire, melatonin levels measured during the first season of data collection were used as the outcome measure to maximize sample size. Shift work history was characterized both as a continuous variable and as a categorical variable (>20 vs. $\leq$20 years shift work; ref. 5). Multivariate models were built using the same process described above.

Chronotype was categorized using the Horne–Ostberg questionnaire (44) into one of 5 categories (Table 1). Because chronotype was assessed only once, geometric means and 95% CIs for both peak and change in melatonin levels were calculated for each chronotype category using melatonin measures from the first season to maximize sample size. All statistical analyses were conducted using SAS, Version 9.2.

Results

Characteristics of the study population and comparisons between day and night shifts in the first season are described in Table 1. Comparisons across shift types were also conducted for the second season and results were similar (data not shown). Participants reported significantly longer sleep duration and consuming a greater number of alcoholic beverages during the 24 hours of melatonin collection when working their day shift. There were also differences in the number of days since the beginning of the previous menstrual period between day and night shifts in both seasons. However, because these were in opposite directions across seasons, they were likely due to factors related to scheduling of study participation sessions.

Mean light intensity levels from 12 AM to 5 AM were significantly higher when nurses were working their night shift (Table 1), although actual light exposure on the night shift was much lower (maximum 37.2 lux) than the approximately 200 lux found to decrease melatonin production in experimental research (34, 35). However, there were no significant differences in either peak urinary melatonin or in the change in melatonin levels when nurses were working the day and night shifts (Table 1).

Mean salivary melatonin levels from saliva samples taken at similar times of day (Fig. 1) were compared for nurses working their day and night shifts. Figure 2 (comparing day and night shifts in the first season) confirms that peak melatonin production occurred at night when nurses were working both day and night shifts (40), suggesting the absence of a major phase shift in melatonin production with night work, a pattern also seen in the second season (data not shown). Thus, in our study population, early morning urine samples from both the day and night shifts are the best to capture peak melatonin production, as found in our previous study (40).

Mixed multiple linear regression was used to examine the relationship between light exposure and both melatonin measures for data from both seasons combined. Only observations with complete data for both light exposure and melatonin were included in this analysis. Of 435 eligible observations, 27 were missing light data, 20 were missing morning urinary melatonin levels and 35 were missing values for the change variable. In the full study population, neither peak melatonin nor change in melatonin were associated with light exposure (Table 2). In the model examining change in melatonin levels, 5 overly influential individuals were removed from the analysis to improve model fit, although neither model (full or reduced) showed a significant relationship between light exposure and melatonin production.

An analysis stratified by shift was conducted to account for the observed wide range of interindividual variability in melatonin levels. On the day shift, there was little variation in light exposure from 12 AM to 5 AM, but large interindividual variability in melatonin levels, such that lack of exposure variability in this group could be hiding a potential relationship between light and melatonin, when data from both shift types was examined together. On the night shift, in which an effect of light exposure on melatonin would be expected, there was more variability in both light exposure and melatonin values. When working the night shift, a slight inverse relationship between light exposure and peak melatonin levels was suggested (Table 2), and a small inverse association between light exposure and change in melatonin levels was observed (Fig. 3).

In bivariate analysis, the number of days since previous period was associated with melatonin levels. Therefore, a restricted analysis among premenopausal women (both shift types) was conducted to allow this potential confounder to be considered; however, no relationship between light and either melatonin measure was detected (Table 2). An analysis stratified by season was also conducted to investigate potential differences across seasons; however, no relationship with either melatonin variable was observed in summer (peak $= 0.018$, $P = 0.79$; change $= 0.03$, $P = 0.68$) or winter (peak $= -0.07$, $P = 0.37$; change $= -0.12$, $P = 0.14$).

The relationship between the number of years of shift work, defined as the number of years spent working a job that included 50% or more nights, and melatonin levels was also examined. When a continuous representation of years of shift work was used with melatonin levels following the night shift, a statistically significant positive relationship was observed, in which shift work history was associated with an increase in peak melatonin levels

Table 1. Characteristics of study population

Variable	Day shift[a] Mean (SE)/*N* (%)	Night shift Mean (SE)/*N* (%)	*P*[b]
Age	40.5 (1.02)	—	—
BMI (kg/m^2)	28.4 (0.82)	—	—
# Years of shift work	14.0 (1.01)	—	—
Ethnicity			
White	113 (95.76%)	—	—
Other	5 (4.24%)	—	—
Reproductive characteristics			
Age at menarche	12.55 (0.13)	—	—
Number of pregnancies	1.58 (0.18)	—	—
Ever been pregnant			
Yes	69 (58.47%)	—	—
No	49 (51.53%)	—	—
Menopausal status			
Premenopausal	89 (75.42%)	—	—
Postmenopausal	29 (24.58%)	—	—
Number of days since previous period	13.83 (1.73)	19.58 (1.71)	0.009
Sleep characteristics			
Sleep duration	6.91 (0.15)	5.23 (0.15)	<0.0001
Sleep interrupted	15 (12.71%)	19 (16.10%)	0.45
Lights on for more than 1 hour if interrupted	3 (2.54%)	8 (6.78%)	0.09
Experience sleep problems	69 (58.47%)	—	—
Diagnosed with sleep disorder	4 (3.49%)	—	—
Medication use			
Antidepressants	12 (10.17%)	—	—
Beta-blockers	2 (1.69%)	—	—
Hormone replacement therapy	7 (5.93%)	—	—
Migraine medication	6 (5.08%)	—	—
Pain medication (NSAIDs)	29 (24.58%)	28 (23.73%)	0.86
Sedatives or muscle relaxants	6 (5.08%)	7 (5.93%)	0.74
Oral contraceptives	19 (16.10%)	15 (12.70%)	0.25
Lifestyle characteristics			
Pack-years smoking	2.77 (0.57)	—	—
Smoked during 24 hours of melatonin collection	10 (8.47%)	11 (9.32%)	0.56
Caffeine consumption (# drinks during 24 hours melatonin collection)	2.61 (0.22)	2.97 (0.22)	0.10
Alcohol consumption (# drinks during 24 hours melatonin collection)	0.33 (0.07)	0.06 (0.07)	0.008
Lifetime alcohol consumption (# drinks/wk)			
Teen	2.52 (0.33)	—	—
20s	4.30 (0.38)	—	—
30s	2.83 (0.34; $n = 95$)	—	—
40s	2.71 (0.36; $n = 66$)	—	—
50s	2.75 (0.74; $n = 26$)	—	—
Chronotype			
Definite morning type	3 (3.57%)	—	—
Moderate morning type	18 (21.43%)	—	—
Neither type	55 (65.48%)	—	—
Moderate evening type	8 (9.52%)	—	—
Definite evening type	0	—	—
Light exposure			
Log-transformed mean light intensity (log lumens/m^2)	−2.14 (0.06)	−0.06 (0.06)	<0.0001
Urinary 6-sulfatoxymealtonin[c]			
Morning 6-sulfatoxymelatonin (ng/mg creatinine)	27.25 (1.11)	25.49 (1.11)	0.65
Change in 6-sulfatoxymealtonin (ng/mg creatinine)	23.48 (1.14)	24.53 (1.14)	0.80

[a]Characteristics assessed in the study questionnaire (administered once) are shown in the "Day shift" column.
[b]Differences between day and night shifts are compared using difference of least squares means estimates in a mixed model with a random subject effect for continuous variables and using McNemar's test for categorical variables.
[c]Geometric means (calculated by back-transforming log-transformed variables) are presented here.

Table 2. Association between light and urinary melatonin

Model	Regression coefficient	*P*
Light exposure		
Full population		
Peak urinary melatonin[a]	−0.03301	0.49
Change in urinary melatonin[b]	−0.03128	0.58
Night shift only		
Peak urinary melatonin[c]	−0.04037	0.07
Change in urinary melatonin[d]	−0.05494	0.04
Day shift only		
Peak urinary melatonin[e]	−0.08371	0.47
Change in urinary melatonin[f]	0.04392	0.75
Premenopausal women only		
Peak urinary melatonin[g]	0.04535	0.50
Change in urinary melatonin[h]	−0.07648	0.27

[a]Adjusted for use of antidepressant medication and the number of caffeinated beverages consumed during the 24 hours of melatonin assessment.
[b]Adjusted for use of antidepressants, oral contraceptives, and the numbers of caffeinated beverages consumed during the 24 hours of melatonin assessment.
[c]Adjusted for total number of years of shift work and use of antidepressant medication.
[d]Adjusted for use of antidepressant medication.
[e]Adjusted for menopausal status and the number of caffeinated beverages consumed during the 24 hours of melatonin collection.
[f]Adjusted for menopausal status, antidepressant and migraine medication use, the number of caffeinated beverages consumed during the 24 hours of melatonin assessment and total number of years of shift work.
[g]Adjusted for total number of years of shift work, number of days since previous menstrual period, smoking, and both number of alcoholic beverages and number of caffeinated beverages consumed during the 24 hours of melatonin assessment. Four overly influential individuals removed to improve model fit.
[h]No variables changed the parameter estimate by more than 10%, therefore no confounders included in model.

and a borderline positive relationship with the change in melatonin levels was also observed (Table 3). Furthermore, studies of shift work history and breast cancer suggest that it is long-term shift work that increases cancer risk (14, 15); therefore, an analysis to investigate the effects of long-term shift work on melatonin levels was also conducted using a cut point of 20 years (5). A positive relationship (although not significant) in which long-term shift work was associated with increased melatonin levels was also observed (Table 3).

Finally, relationships between melatonin and chronotype were explored. Eighty-four nurses (71%) returned

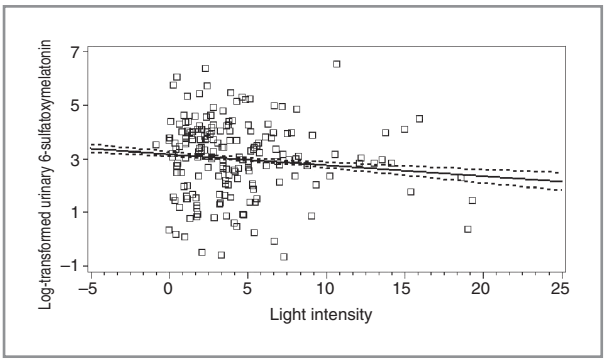

Figure 3. Association between light intensity (lumens/m^2) and log-transformed change in urinary melatonin (log ng/mg creatinine). Change in melatonin is calculated as the difference in melatonin values from the 2 urine samples. Parameter estimate = −0.05494 (P = 0.04). Model adjusted for use of antidepressants.

the chronotype questionnaire. Three were classified as extreme morning types, 18 as moderate morning type, 8 as moderate evening type, and 55 as neither type. No relationship between mean melatonin values on the day or night shift from the first season and chronotype was observed (data not shown). Furthermore, the influence of chronotype as a confounder of the shift work–melatonin relationship was explored. Whereas the distribution of chronotypes differed by long-term shift work status (Table 3), chronotype had little effect on the shift work—melatonin relationship and thus was not included in the final statistical models.

Discussion

These results show that the pattern of melatonin production was similar when rotating shift nurses were working days or nights. Furthermore, light exposure was not strongly related to reduced melatonin production, although a slight inverse relationship with change in melatonin (but not peak) was seen when nurses were working at night. If replicated, these findings may imply that the rapidly rotating shift schedule examined here (or exposure to low light levels while working at night) is not sufficiently disruptive to produce changes in melatonin production. Alternatively, these results may point to a role for other biological pathways, in addition to melatonin, in the relationship between shift work and cancer risk (48).

Main strengths of this study are its objective measures of both light intensity and melatonin while investigating a commonly worked shift schedule. Although light exposure and melatonin are often cited as intermediates between shift work and cancer, few studies with objective measures of light intensity examining relationships with melatonin in an observational setting have been published (37, 39, 40). In epidemiologic studies, associations between shift work history, a proxy for light exposure, and melatonin may be confounded by other behaviors associated with this

Table 3. Influence of shift work history on melatonin

Model	Regression coefficient		*P*
Number of years shift work (continuous)			
Peak urinary melatonin[a]	0.03251		0.02
Change in urinary melatonin[b]	0.03373		0.05
>20 vs. ≤20 y shift work			
Peak urinary melatonin[b]	0.55187		0.07
Change in urinary melatonin[b]	0.63605		0.08
Chronotype[c]	≤20 y shift work	>20 y shift work	
Definite morning type	2 (3.64%)	1 (3.45%)	0.04
Moderate morning type	7 (12.73%)	11 (37.93%)	
Neither type	39 (70.91%)	16 (55.17%)	
Moderate evening type	7 (12.73%)	1 (3.45%)	

[a]Adjusted for age.
[b]Adjusted for age and light exposure between 12 AM and 5 AM during 24 hours of melatonin assessment.
[c]Chronotype frequency by long-term shift work status.

work schedule that are related to melatonin production. Thus, the use of an objective exposure measure in our study allowed the role of nighttime light in melatonin production to be specifically examined in an observational setting.

As well, by comparing melatonin levels from biological samples collected at similar times of day, this study accounts for potential confounding by natural circadian variations in melatonin production when comparing individuals on day and night shifts, a feature that has been absent from most published observational studies of the light–melatonin relationship (37, 39, 40). Specifically, one study found melatonin levels were lower after sleeping and higher after working among permanent night shift workers compared with day workers (39), whereas another found similar results among rotating shift workers (37). Our previous study observed an inverse relationship between light exposure and melatonin levels following sleep; however, this relationship was no longer significant when results were stratified by shift type to account for differences in the timing of urine sampling across shift groups, and salivary melatonin analysis revealed that timing of melatonin production was similar across shift types (40). These results suggest that differences in melatonin levels among individuals sleeping during the day compared with those sleeping at night observed in previous studies (37, 39) could be partially confounded by natural circadian variations in melatonin (40). Thus, uncontrolled confounding in other studies could explain why our findings differ.

Given that previous studies have examined more extreme shift schedules (permanent nights, different rotation patterns; refs. 37–41), and the potential for residual confounding by circadian rhythms in biomarker studies, differences in the results of this study compared with previously published work are understandable. Furthermore, nighttime lighting conditions in the hospital in our study may have been too dark to reach the threshold required to produce a strong effect on melatonin. Although light levels observed here were significantly higher on the night shift, mean light levels were much lower than those used in experiments to produce changes in melatonin production (34, 35). Future studies among individuals with a wider range of light exposure variability will have greater power to detect relationships between light exposure and melatonin in an observational setting. Additional study strengths included the use of urine and saliva samples, which allowed us to measure validated biomarkers of melatonin production (45, 46) and adjustment of urinary melatonin measures for creatinine allowed for differences in urine diluteness to be controlled.

This study also investigated the association between shift work history and melatonin production. When examined as a continuous variable, a small positive relationship between shift work history and melatonin production on the night shift was seen; however, no statistically significant relationships between long-term shift work history and melatonin production were found. Two reports from the Nurses Health Study found decreased melatonin levels among women with recent (previous 2 weeks) shift work history (36, 49), although only one was significant (36). Furthermore, long-term history of shift work was not associated with melatonin levels in the Nurses Health Study (49) and ever having worked the "graveyard" shift was not associated with melatonin among Japanese women (41), consistent with our findings. Although we found that individuals with less than 20 years of shift work experience were more likely to be evening types, this difference is likely age related, as chronotype becomes earlier with age (50). Nurses in the shift work experience group of more than 20 years were substantially older

and the addition of chronotype to models looking at shift work history and melatonin that already included age had little impact on observed relationships. Previous studies (41, 49) have not considered the role of chronotype in the shift work–melatonin relationship, therefore, future studies that include chronotype are needed to confirm our results.

Although the inclusion of nurses working only the DDNN rotating shift pattern allowed for a sample collection scheme that accounted for the influence of circadian melatonin variations, the use of a single shift schedule limits generalizability to other patterns of shift work. More extreme shift schedules, such as more consecutive nights, may be associated with a greater degree of circadian disruption, and there may be a greater effect of light exposure on melatonin. Several previous studies have focused on populations working fixed shifts and differences in shift schedules between these studies and ours could partially explain observed differences in the influence of light exposure on melatonin production (38, 39). Furthermore, although the use of an objective measure of light was a strength of this study, light loggers were worn by nurses around their neck, such that there may have been small differences between logger-measured light intensity and actual intensity perceived by the retina. However, such differences would be quite small and unlikely to substantially impact study results.

We also observed a wide range of interindividual variability in melatonin levels measured on both day and night shifts, which may have limited power to detect small relationships with light exposure, particularly given the low lighting levels when working at night. In addition, whereas first morning urine has been shown to reflect overnight melatonin production during sleep, because urine was not collected over the entire night during the night shift, morning urine samples collected following the night shift may represent an underestimate of melatonin production. However, because peak melatonin levels measured following both shifts were similar, the potential underestimation of melatonin production on the night shift is unlikely to have influenced study findings. Finally, it has been suggested that phase shifts (altered timing) of melatonin production may be important to cancer risk (48, 51); however, given the timing of both urine and saliva sampling (Fig. 1), this study was unable to detect small phase shifts in melatonin production across shift types.

This study observed no difference in the pattern of melatonin production between the day and night shifts, and only a small inverse relationship between light exposure and change in melatonin on the night shift. Future studies of different shift schedules with brighter light exposure are needed to provide context to these results. If future work also fails to find strong relationships between light exposure and melatonin production, this could suggest that other mechanisms (48) are responsible for the observed link between shift work and cancer (1–12, 52). Alternatively, if melatonin is indeed the pathway through which shift work influences cancer risk, our results suggest the prevalent rotating shift pattern of two 12-hour days, two 12-hour nights with relatively low levels of nighttime light exposure followed by 5 days off is minimally disruptive to melatonin production.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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