Research Article

Cancer Epidemiology, Biomarkers & Prevention

Shift Work, Chronotype, and Melatonin Patterns among Female Hospital Employees on Day and Night Shifts

Michael Leung[1,2], Joan Tranmer[1,3], Eleanor Hung[1], Jill Korsiak[1,2], Andrew G. Day[1,4], and Kristan J. Aronson[1,2]

Abstract

Background: Shift work-related carcinogenesis is hypothesized to be mediated by melatonin; however, few studies have considered the potential effect modification of this underlying pathway by chronotype or specific aspects of shift work such as the number of consecutive nights in a rotation. In this study, we examined melatonin patterns in relation to shift status, stratified by chronotype and number of consecutive night shifts, and cumulative lifetime exposure to shift work.

Methods: Melatonin patterns of 261 female personnel (147 fixed-day and 114 on rotations, including nights) at Kingston General Hospital were analyzed using cosinor analysis. Urine samples were collected from all voids over a 48-hour specimen collection period for measurement of 6-sulfatoxymelatonin concentrations using the Buhlmann ELISA Kit. Chronotypes were assessed using mid-sleep time (MSF) derived from the Munich Chronotype Questionnaire (MCTQ). Sociodemographic, health, and occupational information were collected by questionnaire.

Results: Rotational shift nurses working nights had a lower mesor and an earlier time of peak melatonin production compared to day-only workers. More pronounced differences in mesor and acrophase were seen among later chronotypes, and shift workers working $\geq$3 consecutive nights. Among nurses, cumulative shift work was associated with a reduction in mesor.

Conclusion: These results suggest that evening-types and/or shift workers working $\geq$3 consecutive nights are more susceptible to adverse light-at-night effects, whereas long-term shift work may also chronically reduce melatonin levels.

Impact: Cumulative and current exposure to shift work, including nights, affects level and timing of melatonin production, which may be related to carcinogenesis and cancer risk. *Cancer Epidemiol Biomarkers Prev; 25(5); 830–8. ©2016 AACR.*

Introduction

In 2007, shift work involving circadian disruption was classified as a probable (Group 2A) carcinogen by the International Agency for Research on Cancer (IARC) on the basis of sufficient evidence in experimental models, and limited evidence in humans (1). Epidemiologic studies have suggested that working nights and rotating day/night shifts are associated with increased cancer risk at multiple sites, including breast, colon, and prostate (2–14). Earlier meta-analyses demonstrate that long-term night shift work is associated with an increased risk of 40% to 50% for breast cancer (2, 3); however, due to heterogeneity among studies, a more cautious interpretation of this evidence is warranted. One limitation is the current lack of consensus on the definition of "shift work," which leads to heterogeneity in exposure assessment (15, 16), where most studies have used either current shift work status or duration of shift work without considering other aspects that may be relevant such as the number of consecutive night shifts worked (17). More recent meta-analyses have emphasized the heterogeneity observed between studies, and concluded that night shift work is weakly to moderately associated with increased breast cancer risk (18, 19).

The potential carcinogenic effect of light at night and shift work exposure is hypothesized to be mediated by melatonin (20), a hormone with cancer-protective properties that has been associated with decreased cancer risk (21–27). Although there is a growing body of evidence to support this mechanism, other potential carcinogenic mechanisms may operate in parallel (28). To date, most epidemiologic studies of shift work and melatonin patterns are limited in scope and design, where most assess only cross-sectional urinary 6-sulfatoxymelatonin (aMT6) concentrations, without consideration of timing of peak production. The general pattern observed in these studies is increased exposure to shift work and light at night and decreased melatonin production (29, 30). However, as a result of cross-sectional examination, one methodological issue that arises is the potential for confounding by circadian rhythm since pineal secretion of melatonin is inherently circadian: comparisons using functional times do not account for differences in internal biologic time among individuals. Very few studies have examined melatonin patterns by using and/or modeling time–series data, which takes into account an individual's circadian rhythm: these studies have

[1]Department of Public Health Sciences, Queen's University, Kingston, Ontario, Canada. [2]Division of Cancer Care and Epidemiology, Cancer Research Institute, Queen's University, Kingston, Ontario, Canada. [3]School of Nursing, Queen's University, Kingston, Ontario, Canada. [4]Kingston General Hospital, Kingston, Ontario, Canada.

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

Corresponding Author: Kristan Aronson, Queen's Cancer Research Institute, 10 Stuart Street Second Floor, Queen's University, Kingston, ON, K7L 3N6 Canada. Phone: 613-533-6000, ext. 78522; Fax: 613-533-6473; E-mail: aronson@queensu.ca

doi: 10.1158/1055-9965.EPI-15-1178

AACR

demonstrated that working nights is associated with not only a decrease in melatonin production, but also a delay in peak time of production (31, 32).

Heterogeneity among shift work studies can also be attributed to the lack of consideration of an individual's chronotype (15), a hypothesized effect modifier (33, 34), which may help explain why among night shift workers some develop cancer and others not. Chronotype, or diurnal preference, has been shown to impact an individual's adaptability and tolerance to working at night (35), and subsequent melatonin patterns (31, 33, 36, 37). However, to date, evidence has been discordant regarding which chronotypes are more vulnerable to the adverse effects of night time exposure to light. Two studies have shown that workers with morning preference might be more susceptible to light-at-night effects (31, 37), whereas another study showed the opposite effect, where morning-type night shift workers showed melatonin levels that were closer to levels in day shift workers than did evening-type shift workers (36). Possible reasons for a lack of agreement are: (i) imprecision of various methods for chronotype evaluation [i.e., single item in a questionnaire (ref. 37) vs. Composite Scale of Morningness (ref. 36)]; and, (ii) differences in study population, in terms of work schedules (permanent night shift vs. rotational schedules), geography [nature of chronotype differs by latitude (ref. 38)], and cohort-specific causal and confounding structures. Thus, additional studies with validated, more reliable assessments of chronotype are required to elucidate the interaction between chronotype, shift work, and melatonin patterns.

Few studies have evaluated the effect of shift work on melatonin patterns with precise exposure and outcome assessments, and even fewer studies have looked at potential effect modification by chronotype. Thus, the specific objectives of this study are: (i) to evaluate the associations between shift work exposure metrics (i.e., current status, past history of shift work, and number of consecutive nights) and melatonin patterns defined by cosinor mesor, amplitude and acrophase; and, (ii) to evaluate effect modification by chronotype on melatonin patterns among rotational shift workers. Although there is a consistently expanding body of evidence to support the role of melatonin in cancer, more research is still required to elucidate which aspects of shift work are most relevant to carcinogenesis.

Materials and Methods

Study population

The target population was female personnel at Kingston General Hospital in Kingston, Ontario. Participants were recruited from inpatient units, diagnostic and support services through advertisements on bulletin boards that described the study and asked potential participants to contact the project coordinator, and through direct email communication to all nurses as well as notices in both the local intranet communication. All participants worked either fixed-day schedules or rotating shift schedules. Participants were full-time or part-time employees with a history of the same regular employment for 1 year before the study, and they were asked to self-exclude if they were pregnant or had given birth in the last year. This study was approved by the Health Sciences Research Ethics Board (REB) at Queen's University, and all participants provided written informed consent.

Data collection

Specimen collection was conducted over a 48-hour period, which included two regular workdays for fixed-day workers, and one day and one night shift for rotational shift workers. Before the initiation of the data collection period, the coordinator administered a questionnaire, including information on health and occupational history, including occupational title, status, and length of employment; whereas chronotype, for all participants, was assessed using the original Munich Chronotype Questionnaire (MCTQ; ref. 39) to characterize people based on their mid-sleep time (MSF). The three exposures of shift work were current shift work (as a proxy for light at night exposure), past shift work history, and number of consecutive night rotations. Current shift work status was determined through self-report, where a shift worker was defined as an individual who works in a rotational pattern that includes nights. Cumulative shift duration was determined through interview, where number of years an individual worked in a rotation was estimated using full-time years + 0.5 × part-time years, where the burden of part-time shift work is approximated to be half of full-time shift work. Number of consecutive nights worked in the current rotational schedule was self-reported, and was assumed to be relatively stable over the last year. In the literature, a pattern of six consecutive night shifts has been associated with increased breast cancer risk (40); however, because most participants in our cohort worked 2 or 3 consecutive nights, 3 nights were chosen as the cutoff to dichotomize the variables into the following groups: <3 consecutive night shifts and ≥3 consecutive nights shifts.

Melatonin

During the 48-hour specimen collection period, all urine voids were collected in separate containers. Each urine sample was time-stamped in diaries, aliquoted and stored at −70°C. Concentrations of the primary urinary metabolite of melatonin, 6-sulfatoxymelatonin, were assessed using the Buhlmann ELISA Kit, a competitive immunoassay that uses the capture antibody technique. For the analytic performance of assays, internal and external quality controls were conducted. The reproducibility of standard curve parameters and control values were checked against the established performance characteristics of the assay for melatonin and creatinine; and measurements were also compared with the reference interval of standard kits. To control for urine output, melatonin concentrations were creatinine normalized.

Exclusion criteria

Of the 328 participants initially recruited, 261 were included in the analysis. Participants were first excluded ($n = 44$) if they had an ineligible chronotype measurement. The assessment of chronotype using the MCTQ is based on the assumption that sleep-wake behavior on free days reflects an individual's internal biologic clock. If individuals wake up with an alarm, then this sleep–wake pattern reflects their social/work constraints instead of their internal clock. Thus, individuals who wake up with an alarm cannot be "chronotyped" reliably. Their MSF values are considered to be contaminated by social influences (41), and will be considered ineligible for the analysis, especially because chronotype is a factor of primary interest in this study. Then, participants with incomplete biologic data on both specimen collection days were excluded ($n = 7$); and finally, melatonin values that were considered outliers and/or influential observations were excluded ($n = 16$) by removing participants in the top 5 percentile of each exposure group.

Statistical analysis

Comparisons of work and health characteristics were made between groups using the Wilcoxon Rank Sum test for continuous variables, and χ^2 and Fischer Exact tests for categorical variables. Individual melatonin profiles for each group (day-only workers, shift workers working day, and shift workers working nights) were examined by cosinor analysis where the parameters mesor (24-hour mean concentration), amplitude (difference between peak concentration and mesor), and acrophase (peak time of production) were obtained. Each rotational shift worker had two melatonin patterns, one for their day rotation and one for their night rotation, whereas day workers had one pattern derived from the average over their two specimen collection work days. Because mesor was highly positively correlated with amplitude in all three groups (Supplementary Table S1: Spearman's rank correlation coefficient of >0.92; $P < 0.001$), analysis results are only presented for mesor.

Geometric means and 95% confidence intervals (which represent the inter-individual variation within the group) were calculated for mesor and acrophase. To analyze the effect of light at night or time of day exposure, pairwise comparisons using a Wilcoxon Sign Rank test of mesor and acrophase were conducted between day and night rotations among shift workers only. Because comparisons are matched (i.e., within participant), not only is there no confounding, but it also accounts for the correlated data structure for day and night shift assessments within one person, given the high inter-individual variability in patterns and timing of melatonin secretion (42). Furthermore, a second analysis for time of day exposure was conducted where comparisons are made between day-only workers and shift workers on both their day and night rotations. To see effect modification by chronotype and number of consecutive nights, time of day analyses were stratified by chronotype (tertiles: early, middle, and late) and number of consecutive nights (<3 consecutive night shifts and ≥3 consecutive nights shifts). The associations between cumulative shift work and melatonin patterns were done within each group (day-only, shift workers working days, and shift workers working nights). Multivariable least-squares regression analyses controlling for confounders were conducted to evaluate the between-person associations of shift work and melatonin patterns (where mesor was log-transformed for normality). *A priori* confounders such as age, parity, age at first birth, and education were retained in all models in accordance to our hypothesized causal model and a previously published directed acyclic graph (31). Use of sleep aids was also controlled for in all models, as melatonin supplement usage was not included in the initial exclusion criteria. All statistical analyses were performed using SAS Version 9.4 and STATA Version 13.0.

Results

Two hundred and sixty one women were considered in the analysis, 147 working regular day schedules and 114 working rotating schedules, including nights. As described in Table 1, women working days were older, had a slightly higher number of full-term births, and were more likely to smoke than women working rotating shifts. Furthermore, there were far more nurses among those working rotation shifts (86% vs. 47%) than among day-only workers; and, rotating shift workers also had a longer past shift work history (13 vs. 8 years) compared with women working days only. Both groups were similar in pre/post-menopausal status, body mass index, and reported use of oral contraceptives, sleep aid, and hormone replacement therapy.

Within-participant pairwise comparisons of melatonin mesor and acrophase between day and night rotations among shift workers can be found in Table 2. Rotational shift workers on their night shift had a lower mesor (−1.6 ng/mg) and an earlier acrophase (−0.8 hours) compared with their day shifts. Stratifying the analysis by chronotype, these differences were only observed for middle (−3.4 ng/mg and −0.6 hours) and late (−1.5 ng/mg and −1 hour) chronotype groups.

In Table 3, between-participant comparisons also show an overall time of day effect (Fig. 1)—shift workers working nights had a lower mesor, in both crude (−1.8 ng/mg) and adjusted (−25%) comparisons, and an earlier time of peak production in only the adjusted comparison (−0.5 hours) compared with day-only workers. This was further supported by no observed difference between shift workers working days and day-only workers in both crude and adjusted comparisons. Stratifying the comparisons by chronotype (Fig. 2), the magnitude of the adjusted difference in mesor between night shift workers and day-only workers was more pronounced for later chronotypes (early: −22.1%; middle: −24.7%; late: −30.6%), whereas an adjusted difference in acrophase was only observed in the late chronotype group (−0.9 hours). Stratifying the comparisons by the number of consecutive nights (Fig. 2), differences were more pronounced for shift workers working ≥3 consecutive nights, in both crude (<3 consecutive nights: no difference; ≥3 consecutive nights: -1.7 ng/mg) and adjusted (<3 consecutive nights: −19.7%; ≥3 consecutive nights: −32.8%) comparisons; however, differences were similar for acrophase across the two strata (−0.6 hours for both). In contrast, melatonin mesor and acrophase were not associated with duration of past shift work in all three exposure groups (Table 4).

Two sensitivity analyses were also conducted. First, analyses were repeated among nurses only, because there were considerable fewer nurses in the day-only group, where the differences observed might be attributed to occupational-related stressors that may influence hormonal production. The results of this sensitivity analysis (Supplementary Tables S2–S4) not only support the associations seen in the primary analysis, but also show that melatonin mesor was associated with duration of past shift work among shift workers on day rotations: specifically, melatonin decreased (−1.3%) with every additional year of rotational shift work. Second, analyses were repeated among all recruited participants, where those with ineligible chronotypes were re-included into the analysis (exclusion criteria of incomplete biological data and influential observations still applied) to increase the power and precision of chronotype-independent analyses. The results of this sensitivity analysis (Supplementary Tables S5–S7) were similar to those of the main analysis.

Discussion

Exposure to night shift work, as a proxy for light at night exposure, was associated with a lower mesor when comparing within-person to day rotations (−1.6 ng/mg), and between-person to day-only workers (crude: −1.8 ng/mg; adjusted: −25%). This was further supported by no observed difference between shift workers working days and day-only workers in both crude and adjusted comparisons. These results are in accordance with most, but not all recent studies using time–series data. One

Table 1. Description of the study population

Variable	Day workers ($N = 147$) *n* (%)	Shift workers ($N = 114$) *n* (%)	*P*[a]
Age, y	45.1 ± 9.6	40.6 ± 11.9	<0.01
Number of full-term births	1.6 ± 1.2	1.2 ± 1.2	<0.01
Menopausal status			
Pre	92 (62.6)	73 (64.0)	
Post	55 (37.4)	41 (36.0)	0.8
Age at first birth			
Nulliparous	38 (25.9)	49 (43.0)	
≤29	57 (38.8)	32 (28.1)	
≥29	52 (35.4)	33 (29.0)	0.01
Chronotype (MSF[b])	3.29 ± 0.9	3.6 ± 1.3	—
Body mass index	27.0 ± 6.0	28.5 ± 6.6	0.06
Current smoking status			
Smoker	19 (12.9)	6 (5.3)	
Non-smoker	128 (87.1)	108 (94.7)	0.04
Current oral contraceptive use			
Yes	18 (87.8)	21 (18.6)	
No	129 (12.2)	92 (81.4)	0.2
Current sleep aid use			
Yes	19 (12.9)	20 (17.5)	
No	128 (87.1)	94 (82.5)	0.3
Hormone replacement therapy			
Yes	14 (9.6)	5 (4.5)	
No	132 (90.4)	107 (95.5)	0.1
Employment category			
Nursing	69 (46.9)	98 (86.0)	
Other	78 (53.1)	16 (14.0)	<0.01
Duration of cumulative shift			
work (years)	7.9 ± 8.5	12.8 ± 10.2	<0.01
0 Years	36 (24.5)	0 (0.0)	
<9	62 (42.2)	52 (45.6)	
≥9	49 (33.3)	62 (54.4)	<0.01
Number of consecutive night			
shifts	—	2.7 ± 0.9	—
<3	—	68 (59.6)	
≥3	—	46 (40.4)	—

[a]The *P* value for differences between day-only and rotating shift workers using the Wilcoxon rank-sum test for continuous variables and χ^2 or Fischer's Exact tests for categorical variables.
[b]Mid-sleep time using the Munich Chronotype Questionnaire.

previous study conducted by our research group observed no difference in mesor between day and night shift among female nurses working on a rapid rotating schedule (43); however, the study may have been limited by (i) inadequate power due to a smaller sample size, (ii) selection of confounders using data-driven methods, and (iii) inability to model melatonin pattern by cosinor analysis due to fewer bio-samples. In contrast, the Nurse's Health Study reported a reduction in melatonin concentration of 69% during night shifts (44), whereas two separate studies from Spain observed a 34% reduction of mesor among permanent night workers compared with day workers, (31) and a −38 ng/mg decrease of mesor among rotating shift workers compared with day workers (32). Thus, our study provides further evidence that melatonin is acutely suppressed by nighttime work exposure.

Night shift work was also associated with an earlier time of peak among within-person (−0.8 hours) and between-person (−0.5 hours) comparisons. In contrast, a study from Spain found that rotating shift workers had a later acrophase (+1.3 hours) compared with day workers (32); however, the investigators did not adjust for any confounder or statistically assess the difference in timing of peaks. In another study, a 3-hour delay in peak time was reported among night workers compared to day workers (31); however, all participants were engaged in permanent fixed night shifts, the most extreme group in the shift work exposure

Table 2. Pairwise comparisons of 6-sulfatoxymelatonin mesor and acrophase between day and night rotations among shift workers, stratified by chronotype

		Cosinor parameters (95% CI)			
		Geometric mean mesor (ng/mg)		Geometric mean acrophase (hh:mm)	
	N	Day rotation	Night rotation	Day rotation	Night rotation
Shift workers	114	15.2 (13.7, 16.7)	13.6 (11.3, 15.9)[a]	4:31 (4:17, 4:45)	3:43 (3:20, 4:06)[a]
Chronotype					
Early	38	14.3 (12.0, 16.6)	14.4 (9.9, 18.9)	4:47 (2:06, 5:58)	4:01 (21:44, 5:58)
Middle	38	15.5 (12.5, 18.5)	12.1 (9.7, 14.4)[a]	4:31 (0:03, 5:57)	3:54 (22:03, 5:51)[a]
Late	38	15.9 (13.3, 18.6)	14.4 (9.5, 19.2)[a]	4:14 (1:09, 5:55)	3:14 (20:53, 5:59)[a]

[a]$P < 0.05$ using the Wilcoxon signed-rank test for matched data.

Table 3. Associations between 6-sulfatoxymelatonin mesor and acrophase between day-only workers and shift workers on day and night shifts, stratified by chronotype and number of consecutive night shifts

		Cosinor parameters			
		Mesor (95% CI)		Acrophase (95% CI)	
	N	Crude geometric mean (ng/mg)	Adjusted mean % change[a]	Crude geometric mean (hh:mm)	Adjusted mean difference (h)[a]
Day only, shift work night					
Day (only)[b]	147	15.4 (14.1, 16.7)	Ref	4:23 (4:11, 4:36)	Ref
Night (rotation)	114	13.6 (11.3, 15.9)[c]	−25.0 (−37.7, −9.8)[d]	3:43 (3:20, 4:06)	−0.5 (−1.0, −0.05)[d]
Chronotype (MSF)					
Early	38	14.4 (9.9, 18.9)	−22.1 (−39.3, −0.6)[d]	4:01 (21:44, 5:58)	−0.2 (−0.8, 0.3)
Middle	38	12.1 (9.7, 14.4)	−24.7 (−40.6, −4.7)[d]	3:54 (22:03, 5:51)	−0.5 (−1.0, 0.09)
Late	38	14.4 (9.5, 19.2)	−30.6 (−46.8, −9.2)[d]	3:14 (20:53, 5:59)	−0.9 (−1.5, −0.2)[d]
Number of consecutive nights					
<3	68	14.3 (11.5, 17.1)	−19.7 (−34.3, −1.7)[d]	3:46 (3:17, 4:16)	−0.6 (−1.1, −0.07)[d]
≥3	46	12.6 (8.6, 16.5)[c]	−32.8 (−47.6, −13.8)[d]	3:38 (3:01, 4:16)	−0.6 (−1.2, −0.002)[d]
Day only, shift work day					
Day (only)[b]	147	15.4 (14.1, 16.7)	Ref	4:23 (4:11, 4:36)	Ref
Day (rotation)	114	15.2 (13.7, 16.7)	−7.0 (−21.4, 0.74)	4:31 (4:17, 4:45)	0.2 (−0.2, 0.5)
Chronotype (MSF)					
Early	38	14.3 (12.0, 16.6)	−9.8 (−28.0, 12.9)	4:47 (2:06, 5:58)	0.5 (−0.006, 0.9)
Middle	38	15.5 (12.5, 18.5)	−4.5 (−24.4, 20.9)	4:31 (0:03, 5:57)	0.2 (−0.3, 0.7)
Late	38	15.9 (13.3, 18.6)	−9.2 (−29.3, 16.9)	4:14 (1:09, 5:55)	−0.1 (−0.7, 0.4)
Number of consecutive nights					
<3	68	16.7 (14.6, 18.7)	0.6 (−16.8, 21.7)	4:30 (4:13, 4:47)	0.09 (−0.3, 0.5)
≥3	46	13.1 (11.1, 15.1)	−19.7 (−36.0, 0.7)	4:32 (4:07, 4:57)	0.3 (−0.2, 0.8)

[a]Adjusted models controlled for cumulative shift work, age, education, parity, age of first birth, and use of sleep aid.
[b]Reference group for all comparisons.
[c]$P < 0.05$ using the Wilcoxon rank-sum test.
[d]$P < 0.05$ using the Wald test.

spectrum. Even though this study differs with regard to the direction of the phase shift, the presence of a phase shift itself may signal a form of circadian adaptation, where it has been suggested that time of peak production may change to better align circadian rhythms to a new sleep–wake pattern (45, 46). Thus, the results of our study add to the growing body of literature to show that nighttime light exposure affects timing of peak production. Future studies should try to examine melatonin rhythms in relation to other physiologic rhythms to characterize circadian misalignment, as it has been suggested that the adverse effects of shift work stem not from phase shifting itself, but from the misalignment of multiple circadian rhythms (i.e., melatonin and body temperature; refs. 47, 48).

In this study, shift workers with an earlier chronotype appear to be better protected from the adverse effects of shift work and light at night exposure. Differences in mesor and acrophase were only observed for the middle and late chronotypes among within-person comparisons. Furthermore, for stratified between-person comparisons adjusting for *a priori* confounders, a larger reduction in mesor was observed in shift workers with late chronotypes (−32.5%) relative to early chronotypes (−22.1%) when comparing with day-only workers; whereas an earlier timing of peak

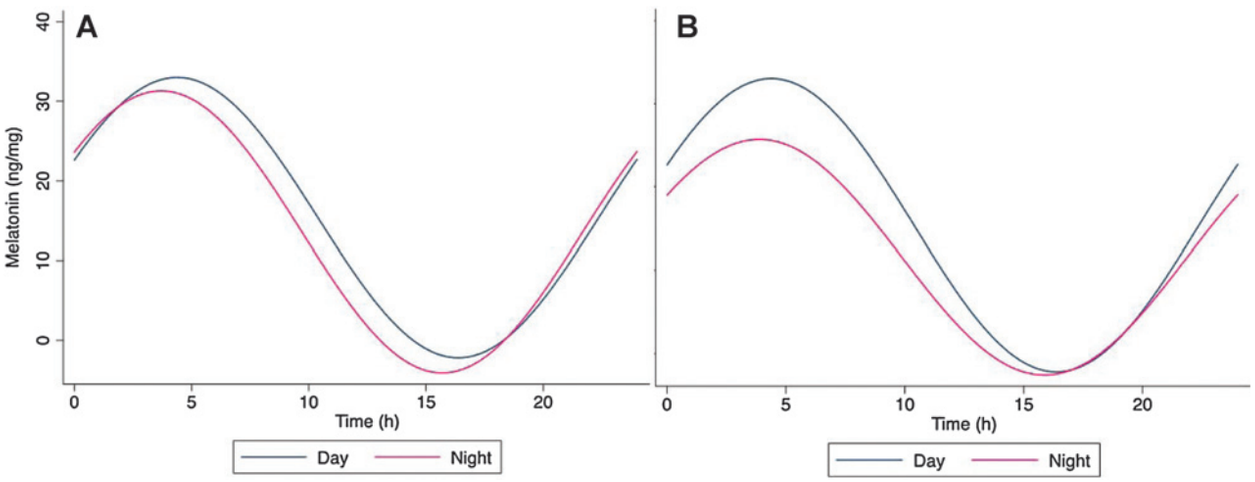

Figure 1.
Crude (A) and adjusted (B) melatonin patterns of night shift workers compared with day-only workers.

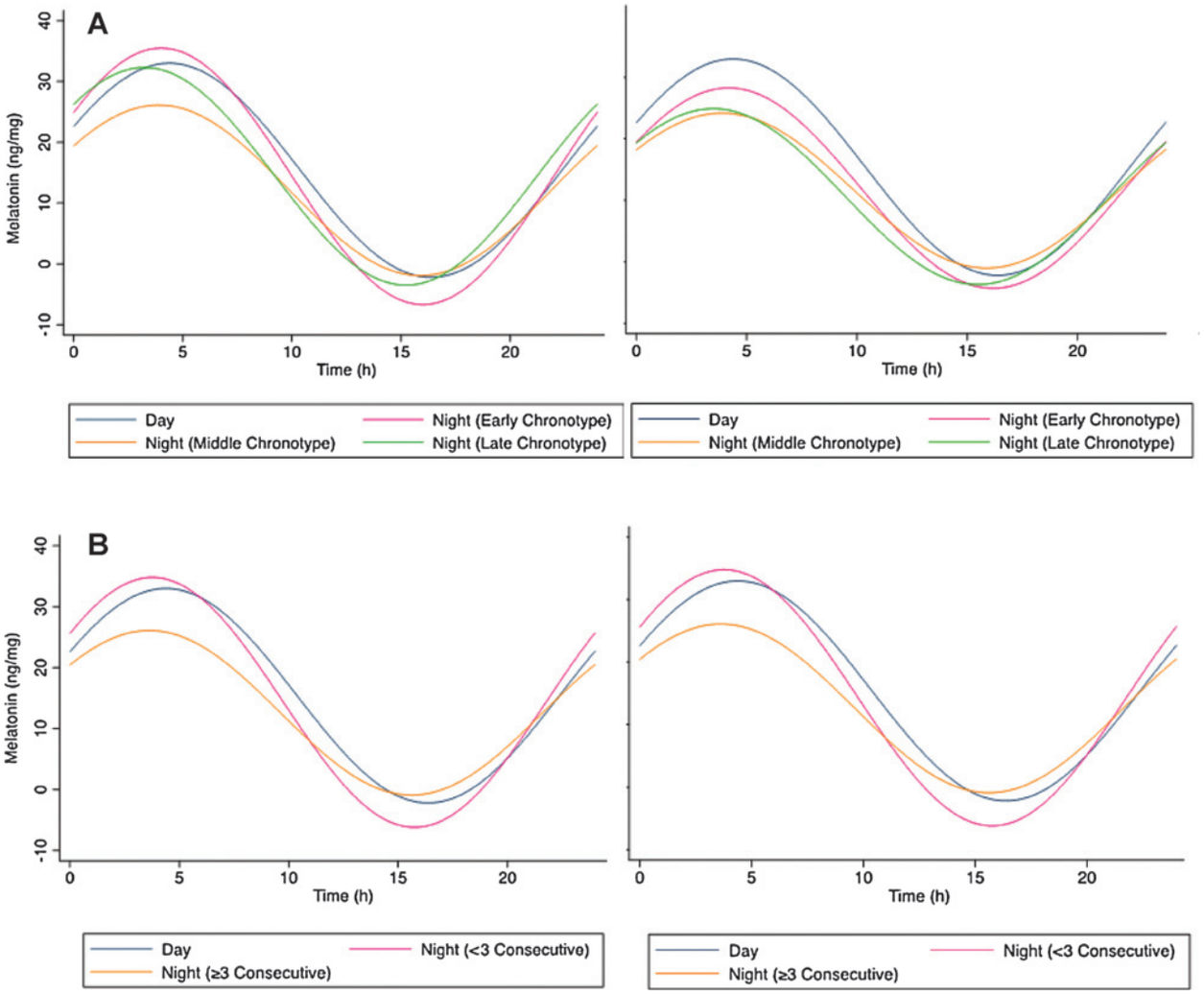

Figure 2.
The effect on Fig. 1 of stratifying in turn for chronotype (A) and number (B) of consecutive nights.

production (−0.9) was observed in shift workers with late chronotypes compared with day-only workers. Recent evidence has suggested that an individual's chronobiologic propensity for sleep and rest may play a significant role in altering the relationship not only between night work and melatonin patterns (33), but also cancer risk (37). However, to date, evidence for effect modification by chronotype has been discordant. One study showed that participants with morning preference might be more susceptible to light-at-night effects (31, 37), whereas another study showed the opposite, where morning-type night shift workers showed melatonin levels that were closer to levels in day shift workers than did evening-type night shift workers (36). Even though it has been hypothesized that individuals who work outside their preferred time window of activity (i.e., early chronotypes working at night) are more susceptible to stress/strain, two studies (this study included) now show that shift workers with early chronotypes are less vulnerable to effects of shift work and light at night exposure (36). This signals that there may be parallel pathways

Table 4. Associations between cumulative shift work and 6-sulfatoxymelatonin mesor and acrophase, stratified by shift work status and time of day

		Cosinor parameters			
		Mesor (95% CI)		Acrophase (95% CI)	
	N	Mean change per year (%)[a]	Adjusted mean change per year (%)[a,b]	Mean difference per year (h)[a]	Adjusted mean difference per year (h)[a,b]
Day (only)	147	−0.5 (−1.6, 0.7)	0.3 (−1.0, 1.5)	−0.01 (−0.04, 0.01)	−0.01 (−0.04, 0.01)
Day (rotation)	114	−1.0 (−2.0, 0.09)	0.4 (−1.4, 2.2)	−0.001 (−0.02, 0.02)	0.02 (−0.02, 0.06)
Night (rotation)	114	−1.1 (−2.3, 0.2)	−0.09 (−2.4, 2.2)	0.01 (−0.02, 0.05)	0.004 (−0.06, 0.07)

[a]All estimates were not significant ($P > 0.05$).
[b]Adjusted models controlled for age, education, parity, age of first birth, and use of sleep aid.

other than the mediation of melatonin that are of greater importance in conferring morning-type susceptibility against the adverse effects of shift work, such as reduced sleep quality and its impact on immune functions (28, 49). It is still an understudied hypothesis, and given the discordant evidence, the impact of chronotype on melatonin pattern and cancer risk among shift workers warrants further investigation, especially as it may help explain why among workers who share the same shift work exposures, some may develop cancers and others not.

Mesor was also differentially affected among shift workers by the number of consecutive night shifts worked. A larger reduction in mesor was observed in those working $\geq$3 consecutive nights relative to those working <3 consecutive nights compared with day-only workers. To our knowledge, this is the first time this has been reported and it supports the evidence in the existing breast cancer literature by demonstrating at a biomarker level the mechanism suggested by experimental research at the population level: Breast cancer risk increases by only 3% for every 5-years of shift work, whereas the increase in risk elevates to 80% for every 5 years with $\geq$6 consecutive nights in a rotation (40). Therefore, we can identify this as an aspect of current rotational shift work that alters circadian melatonin pattern. Because this is the first study to show an association between the number of consecutive night shifts and depressed melatonin rhythms, future studies should try examine and replicate this association, especially because melatonin has been shown to possess cancer-protective properties (21–25).

In terms of past shift work history, cumulative exposure to rotational shift work was not associated with either mesor or acrophase among both shift workers and day-only workers. There are many possible reasons for the lack of association. First, long-term rotational schedules, especially with rapid rotation between day and night shifts, may be insufficient to chronically disrupt melatonin patterns. Second, a number of day-only workers in this cohort, mainly non-nurses, had no past shift work history or close to zero years of shift work. By including these individuals, there will be lots of variability in melatonin mesor and acrophase at or close to zero years of past shift work. In a regression framework, this translates to very little precision at the lower extreme of the distribution, and given that estimates are leveraged by the extremes, it is not surprising that no association was observed. In our sensitivity analysis where non-nurses were excluded, cumulative shift work was associated with a -1.3% reduction in mesor for every additional year of rotational shift work in shift workers on day rotations. Even though most of the evidence in the literature demonstrates acute effects of working at night on melatonin, these results are suggestive of a chronic accumulation of effects, which supports the current breast cancer literature that long-term shift work may increase the risk of breast cancer (4). Given that other studies have not shown associations of cumulative exposure with melatonin patterns, and that our study did not have enough variability in cumulative exposure (i.e., large proportion of sample had zero or close to zero years of past shift work), the association and biologic mechanism for a chronic effect of shift work on melatonin requires further study.

One of the strengths of this study is the collection of urine samples over two 24-hour working days that enabled the characterization of circadian melatonin patterns by modeling time–series data using cosinor analyses. Because we can describe the patterns of melatonin, we can see where the peaks occur regardless of shift schedule, and we can avoid making comparisons that are confounded by an individual's internal biologic time. A second key strength of this study is its observational nature, where circadian melatonin patterns can be captured during working hours of both day and night shifts to demonstrate at the population level a potential mechanism suggested from experimental research. Another strength is the rigorous examination of several exposure metrics of shift work, as well as effect modification by chronotype, to elucidate which aspects of shift work may be related to adaptation, and possible internal desynchrony, which may be related to cancer risk. Furthermore, *a priori* confounders were selected carefully with a hypothesized causal model that coincides with other causal models/DAGs among existing studies.

One limitation of this study is the use of MSF as a proxy for chronotype. At the time of this project's inception (2011), the Munich Chronotype Questionnaire for Shift Workers (MCTQshift; ref. 41) had not yet been published or validated. Instead, chronotypes for both shift workers and day workers were assessed using the original Munich Chronotype Questionnaire (MCTQ; ref. 39). We chose not to correct for sleep-debt (MSFsc) because the algorithm would introduce error into the characterization of chronotype among shift workers as the general MCTQ was intended for individuals workings standard hours (calculating an average sleep duration, which is needed to correct for sleep debt would not be appropriate for shift workers given the differences in sleep–wake behavior for day and night rotations). This correction would subsequently introduce bias, as the sleep-debt adjusted chronotypes would only be correct in day only and not in rotational shift workers. Thus, MSF on free days for both day-only and rotational workers (especially due to long periods of free days in a rotational schedule) was deemed the best parameter to use as a proxy for an individual's chronotype. Future studies should use the MCTQshift for shift workers, as MSFsc is a more reliable indicator of chronotype. Another limitation of this study is the potential lack of generalizability because our study population was restricted to female health care workers who worked either rotational or day-only schedules, and that chronotype was examined by cohort-specific tertiles. Furthermore, even though individual light exposure measures were not included in this study, our previous studies in these hospitals have shown that night lighting is quite dim at 40 to 50 lux (43, 50), which is much lower than experimental conditions. Because intensity and timing of light exposures can impact melatonin patterns (51–53), individual light assessment should be incorporated in future studies. Finally, the modeling of time–series data by cosinor analysis did not take into account serial correlation of observations within participants, which may violate the assumption of homoscedasticity of error terms. A mixed-effects model with a first-order autoregressive structure was attempted to model the autocorrelation of errors, but complications with convergence arose due to unbalanced data (variable number and timing of urine samples across individuals). Thus, future studies should try to extend the usual cosinor analysis by using an autoregressive covariance structure to model the serial correlation often seen in biorhythm data (54).

In conclusion, this study evaluated the association of various exposure metrics of shift work with melatonin patterns, and found that (i) night shift work with $\geq$3 consecutive nights is associated with a more pronounced reduction in melatonin mesor; and, (ii) night shift work is modified by chronotype, where evening chronotypes show more pronounced effects of nighttime light exposure. To our knowledge, this is the first study to assess the number of consecutive nights in relation to melatonin patterns, and one of the very few studies examining effect

modification by chronotype. Although there is a growing body of evidence to support the role of melatonin in cancer, more research is needed to elucidate which specific aspect of melatonin production (24-hour mean concentration or timing of peak production) is most relevant to carcinogenesis. Future mechanistically oriented studies need to be more comprehensive by analyzing the role of melatonin in conjunction with other parallel pathways that may operate between shift work and cancer (28) to guide intervention strategies and healthy workplace policies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Leung, J. Tranmer, K.J. Aronson
Development of methodology: M. Leung, J. Tranmer, J. Korsiak, K.J. Aronson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Tranmer, K.J. Aronson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Leung, J. Tranmer, E. Hung, A.G. Day, K.J. Aronson
Writing, review, and/or revision of the manuscript: M. Leung, J. Tranmer, E. Hung, A.G. Day, K.J. Aronson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Leung, J. Tranmer
Study supervision: J. Tranmer, K.J. Aronson

Acknowledgments

The Canadian Institutes for Health Research and the Workers Safety and Insurance Board (Ontario) supported the main study from which this project originates. The investigative team (L. McGillis-Hall, I. Janssen, and C. Collier); research manager (R. Corbin); and statistical support (X. Sun) and Kingston General Hospital.

Grant Support

This study was funded by the Workplace Safety and Insurance Board of Ontario. This work is part of a Master's thesis by M. Leung, who is supported by the Canadian Institutes of Health Research Canada Graduate Scholarship Master's Award.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 16, 2015; revised February 19, 2016; accepted February 22, 2016; published OnlineFirst March 3, 2016.

References

1. International Agency for Research on Cancer. Painting, firefighting, and shiftwork. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. France; 2010;98:9–764.
2. Megdal SP, Kroenke CH, Laden F, Pukkala E, Schernhammer ES. Night work and breast cancer risk: a systematic review and meta-analysis. Eur J Cancer 2005;41:2023–32.
3. Kolstad HA. Nightshift work and risk of breast cancer and other cancers—a critical review of the epidemiologic evidence. Scand J Work Environ Health 2008;34:5–22.
4. Grundy A, Richardson H, Burstyn I, Lohrisch C, SenGupta SK, Lai AS, et al. Increased risk of breast cancer associated with long-term shift work in Canada. Occup Environ Med 2013;70:831–8.
5. Wang F, Yeung KL, Chan WC, Kwok CCH, Leung SL, Wu C, et al. A meta-analysis on dose–response relationship between night shift work and the risk of breast cancer. Ann Oncol 2013;24:2724–32.
6. Conlon M, Lightfoot N, Kreiger N. Rotating shift work and risk of prostate cancer. Epidemiology 2007;18:182–3.
7. Gapstur SM, Diver WR, Stevens VL, Carter BD, Teras LR, Jacobs EJ. Work schedule, sleep duration, insomnia, and risk of fatal prostate cancer. Am J Prev Med 2014;46:S26–33.
8. Kubo T, Ozasa K, Mikami K, Wakai K, Fujino Y, Watanabe Y, et al. Prospective cohort study of the risk of prostate cancer among rotating-shift workers: findings from the Japan collaborative cohort study. Am J Epidemiol 2006;164:549–55.
9. Kubo T, Oyama I, Nakamura T, Kunimoto M, Kadowaki K, Otomo H, et al. Industry-based retrospective cohort study of the risk of prostate cancer among rotating-shift workers. Int J Urol 2011;18:206–11.
10. Parent M-É, El-Zein M, Rousseau M-C, Pintos J, Siemiatycki J. Night work and the risk of cancer among men. Am J Epidemiol 2012;176:751–9.
11. Papantoniou K, Castaño-Vinyals G, Espinosa A, Aragonés N, Pérez-Gómez B, Burgos J, et al. Night shift work, chronotype and prostate cancer risk in the MCC-Spain case-control study. Int J Cancer 2014;137:1147–57.
12. Schwartzbaum J, Ahlbom A, Feychting M. Cohort study of cancer risk among male and female shift workers. Scand J Work Environ Health 2007; 33:336–43.
13. Tynes T, Hannevik M, Andersen A, Vistnes AI, Haldorsen T. Incidence of breast cancer in Norwegian female radio and telegraph operators. Cancer Causes Control 1996;7:197–204.
14. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, et al. Night-shift work and risk of colorectal cancer in the nurses' health study. J Natl Cancer Inst 2003;95:825–8.
15. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, et al. Considerations of circadian impact for defining "shift work" in cancer studies: IARC Working Group Report. Occup Environ Med 2010;68: 154–62.
16. Papantoniou K, Kogevinas M. Shift work and breast cancer: do we need more evidence and what should this be?Occup Environ Med 2013;70: 825–6.
17. Härmä M, Ropponen A, Hakola T. Developing register-based measures for assessment of working time patterns for epidemiologic studies. Scand J Work Environ Heal 2015;41:268–79.
18. Wang X-S, Armstrong MEG, Cairns BJ, Key TJ, Travis RC. Shift work and chronic disease: the epidemiological evidence. Occup Med 2011;61:78–89.
19. Kamdar B, Tergas A, Mateen F. Night-shift work and risk of breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat 2013;138: 291–301.
20. Stevens RG. Electric power use and breast cancer: a hypothesis. Am J Epidemiol 1987;125:556–61.
21. Bartsch C, Bartsch H, Jain AK, Laumas KR, Wetterberg L. Urinary melatonin levels in human breast cancer patients. J Neural Transm 1981;52:281–94.
22. Bartsch C, Bartsch H, Karenovics A, Franz H, Peiker G, Mecke D. Nocturnal urinary 6sulphatoxymelatonin excretion is decreased in primary breast cancer patients compared to agematched controls and shows negative correlation with tumorsize. J Pineal Res 1997;23:53–8.
23. Khoory R, Stemme D. Plasma melatonin levels in patients suffering from colorectal carcinoma. J Pineal Res 1988;5:251–8.
24. Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. J Natl Cancer Inst 2005;97:1084–7.
25. Travis RC, Allen DS, Fentiman IS, Key TJ. Melatonin and breast cancer: a prospective study. J Natl Cancer Inst 2004;96:475–82.
26. Schernhammer ES, Berrino F, Krogh V, Secreto G, Micheli A, Venturelli E, et al. Urinary 6-sulfatoxymelatonin levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2008;100:898–905.
27. Schernhammer ES, Hankinson SE. Urinary melatonin levels and postmenopausal breast cancer risk in the Nurses' Health Study cohort. Cancer Epidemiol Biomarkers Prev 2009;18:74–9.
28. Fritschi L, Glass DC, Heyworth JS, Aronson K, Girschik J, Boyle T, et al. Hypotheses for mechanisms linking shiftwork and cancer. Med Hypotheses 2011;77:430–6.
29. Marie Hansen A, Helene Garde A, Hansen J. Diurnal urinary 6-sulfatoxymelatonin levels among healthy Danish nurses during work and leisure time. Chronobiol Int 2006;23:1203–15.
30. Schernhammer ES, Rosner B, Willett WC, Laden F, Colditz GA, Hankinson SE. Epidemiology of urinary melatonin in women and its relation to other hormones and night work. Cancer Epidemiol Biomarkers Prev 2004;13: 936–43.

31. Papantoniou K, Pozo OJ, Espinosa A, Marcos J, Castano-Vinyals G, Basagana X, et al. Circadian variation of melatonin, light exposure, and diurnal preference in day and night shift workers of both sexes. Cancer Epidemiol Biomarkers Prev 2014;23:1176–86.
32. Gómez-Acebo I. Association between exposure to rotating night shift versus day shift using levels of 6-sulfatoxymelatonin and cortisol and other sex hormones in women. Chronobiol Int 2014;32:128–35.
33. Erren TC. Shift work and cancer research: can chronotype predict susceptibility in night-shift and rotating-shift workers? Occup Environ Med 2013;70:283–4.
34. Erren TC, Pape HG, Reiter RJ, Piekarski C. Chronodisruption and cancer. Naturwissenschaften 2008;95:367–82.
35. Saksvik IB, Bjorvatn B, Hetland H, Sandal GM, Pallesen S. Individual differences in tolerance to shift work—a systematic review. Sleep Med Rev 2011;15:221–35.
36. Bhatti P, Mirick DK, Davis S. The impact of chronotype on melatonin levels among shift workers. Occup Environ Med 2014;71:195–200.
37. Hansen J, Lassen CF. Nested case-control study of night shift work and breast cancer risk among women in the Danish military. Occup Environ Med 2012;69:551–6.
38. Miguel M, de Oliveira VC, Pereira D, Pedrazzoli M. Detecting chronotype differences associated to latitude: a comparison between Horne–Östberg and Munich Chronotype questionnaires. Ann Hum Biol 2014; 41:105–8.
39. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms 2003;18: 80–90.
40. Lie J-AS, Kjuus H, Zienolddiny S, Haugen A, Stevens RG, Kjærheim K. Night work and breast cancer risk among Norwegian nurses: assessment by different exposure metrics. Am J Epidemiol 2011;173:1272–9.
41. Juda M, Vetter C, Roenneberg T. The Munich ChronoType Questionnaire for Shift-Workers (MCTQShift). J Biol Rhythms 2013;28:130–40.
42. Arendt J. Jet-lag and shift work: (2). Therapeutic use of melatonin. J R Soc Med 1999;92:402–5.
43. Grundy A, Tranmer J, Richardson H, Graham CH, Aronson KJ. The influence of light at night exposure on melatonin levels among Canadian rotating shift nurses. Cancer Epidemiol Biomarkers Prev 2011;20: 2404–12.
44. Davis S, Mirick DK, Chen C, Stanczyk FZ. Night shift work and hormone levels in women. Cancer Epidemiol Biomarkers Prev 2012;21:609–18.
45. Gibbs M, Hampton S, Morgan L, Arendt J. Adaptation of the circadian rhythm of 6-sulphatoxymelatonin to a shift schedule of seven nights followed by seven days in offshore oil installation workers. Neurosci Lett 2002;325:91–4.
46. Barnes R, Deacon S, Forbes M, Arendt J. Adaptation of the 6-sulphatoxymelatonin rhythm in shiftworkers on offshore oil installations during a 2-week 12-h night shift. Neurosci Lett 1998;241:9–12.
47. Lowden A, Åkerstedt T, Wibom R. Suppression of sleepiness and melatonin by bright light exposure during breaks in night work. J Sleep Res 2004; 13:37–43.
48. Lamond N, Dorrian J. The impact of a week of simulated night work on sleep, circadian phase, and performance. Occup Environ Med 2003;60:e13.
49. Haus EL, Smolensky MH. Shift work and cancer risk: potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. Sleep Med Rev [Internet] 2013;17:273–84.
50. Grundy A, Sanchez M, Richardson H, Tranmer J, Borugian M, Graham CH, et al. Light intensity exposure, sleep duration, physical activity, and biomarkers of melatonin among rotating shift nurses. Chronobiol Int 2009;26:1443–61.
51. Zeitzer J, Dijk D. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol 2000;526:695–702.
52. Lewy A. Clinical implications of the melatonin phase response curve. J Clin Endocrinol Metab 2010;95:3158.
53. Paul M, Miller J. Timing light treatment for eastward and westward travel preparation. Chronobiol Int 2009;26:867–90.
54. Greenhouse J, Kass R, Tsay R. Fitting nonlinear models with ARMA errors to biological rhythm data. Stat Med 1987;6:167–83.