

## Research Article

## Night Shift Work and Levels of 6-Sulfatoxymelatonin and Cortisol in Men

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## Abstract

**Background:** Night shift work is associated with cancer among men, but the biologic mechanism is unclear. We investigated whether male night shift workers showed changes in levels of melatonin and cortisol, potential biomarkers of cancer risk.

**Methods:** Urine was collected from 185 night shift and 158 day shift-working male healthcare providers, aged 22 to 55 years, throughout work and sleep periods, and assayed for 6-sulfatoxymelatonin and cortisol. Morning serum was collected within 90 minutes of completing the night and assayed for cortisol.

**Results:** Night shift workers had significantly lower 6-sulfatoxymelatonin levels during daytime sleep, nighttime work, and nighttime sleep on off-nights (57%, 62%, and 40% lower, respectively), relative to the day shift workers during nighttime sleep ( $P < 0.0001$ ); urinary cortisol in night shift workers was 16% higher during daytime sleep and 13% lower during nighttime sleep on off-nights ( $P < 0.05$ ). Morning serum cortisol post-work and post-sleep in night shift workers were 24% and 43% lower, respectively, than post-sleep levels among day shift workers ( $P < 0.0001$ ). Within-subject comparisons among the night shift workers revealed significantly lower melatonin levels and significantly higher urinary cortisol levels during daytime sleep and nighttime work, relative to nighttime sleep ( $P < 0.01$ ); morning serum cortisol levels post-work were lower than those post-sleep.

**Conclusions:** Night shift workers have substantially lower 6-sulfatoxymelatonin during night work and daytime sleep, and levels remain low when night shift workers sleep at night. Chronic reduction in melatonin among night shift workers may be an important carcinogenic mechanism. Cortisol secretion patterns may be impacted by night shift work, which could affect cancer risk.

**Impact:** Shift work could be an important risk factor for many types of cancer. *Cancer Epidemiol Biomarkers Prev*; 22(6); 1079–87. ©2013 AACR.

## Introduction

Although the primary focus of epidemiologic studies of shift work and cancer has been on breast cancer risk, several studies have reported an increased risk of cancer among men. Prostate cancer has been associated with jobs that involve some degree of work at night, including airline pilots, police officers, and health practitioners (1–9). There is compelling evidence that a major pathway that underlies the carcinogenicity of shift work lies in the suppressive effects of light-at-night on melatonin levels, a

hormone that is not only a primary circadian pacemaker (10), but also possesses well-established growth inhibitory and oncostatic properties (11–13). In addition to light-at-night exposure, night shift workers maintain schedules that are out of sync with the typical daily light–dark cycle, putting them further at risk for circadian disruption. Cortisol, another hormone important in circadian regulation (14; 15) with implications for cancer risk through its effects on immune function (16; 17), could be affected not only by the exposures mentioned above, but also by sleep disruption experienced by night shift workers. Thus, through the impact on melatonin and cortisol levels, the carcinogenicity of shift work may be broadly applicable to cancer at many sites among both men and women.

Previously, we reported significantly lower levels of urinary 6-sulfatoxymelatonin, the primary metabolite of melatonin, among female night shift workers during nighttime work, daytime sleep, and nighttime sleep periods on off-nights, relative to day shift workers (18). Here, we report results from a similar study conducted in men. This is the first study to evaluate melatonin and cortisol levels in men at multiple critical time points throughout

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the course of a shift worker's typical "work day" and subsequent sleep.

## Materials and Methods

### Study participants

Participants were men aged 22 to 55 years employed as healthcare workers in the Seattle metropolitan area. Eligibility was based on the following criteria: body mass index (BMI) between 18 and 30 kg/m<sup>2</sup>, no use of hormones or other medications or supplements typically taken to treat benign prostate conditions at least 30 days prior, no personal history of prostate cancer or chemotherapy, no use of supplements containing melatonin, and not having undergone general anesthesia or major surgery at least 8 weeks prior. These criteria were intended to ensure that participants were not under the influence of known factors that may alter levels of the hormones under study. Night shift workers were required to work the graveyard shift (i.e., stop work no earlier than 6 am and work at least 8 hours per shift) at least 24 hours per week and to sleep at night during off-days. Day shift workers were required to work the day shift (i.e., begin work no earlier than 6 am and work at least 8 hours per shift) exclusively at least 24 hours per week and were chosen to have a similar age distribution as the night shift workers. Participants were required to have worked their current shift schedule for at least 30 days before enrollment in the study.

### Data collection

**Overview.** After obtaining informed consent, a structured interview was administered to collect information about physical activity, work shift history, current work and sleep schedules, recent antidepressant use, and current job description. For the night shift workers, urine and blood collections were scheduled to coincide with days when at least 2 consecutive shifts were to be worked, followed by an off-night of sleep.

**Urine and blood sample collection.** Following completion of the first work shift, night shift workers collected 4 separate urine samples as follows: (i) all urine excreted during the subject's daytime sleep period and the first void upon rising, (ii) all urine excreted after the first void upon rising from day sleep, until the start of the second work shift, (iii) all urine excreted during the second night shift and the first void immediately following the nighttime work shift, and (iv) all urine excreted during the following night's sleep (the off-night) and the first void the next morning. In addition, morning blood samples were drawn within 90 minutes of completing the second night shift and within 90 minutes of rising from the off-night sleep.

Day shift workers collected 3 urine samples as follows: (i) all urine excreted during the subject's daytime work period and the first void upon completion of the work shift, (ii) all urine excreted after the first void upon completing the day shift, until the start of the nighttime sleep period, and (iii) all urine excreted during the night-

time sleep period and the first void the next morning. In addition, a morning blood sample was drawn within 90 minutes of rising from the nighttime sleep period.

Before collecting the first urine sample for day shift workers, and urine samples 1 and 4 for night shift workers, the participant was instructed to void his bladder and discard the urine. After each sample collection period, participants completed a form that included questions about problems with sample collection (e.g., spillage or a missed collection). Each sample was collected in an opaque collection bottle, placed in a cooler, and picked up by the interviewer at the time of blood sample collection and immediately delivered to the Fred Hutchinson Cancer Research Center Specimen Processing Shared Resource (Seattle, WA). Study participation entailed multiple tasks in which the precise timing was essential for complete and accurate data collection. Interviewers kept in close contact with participants throughout the data collection protocol; this was extremely useful in helping participants adhere to the stringent data collection procedures.

**Assessment of urinary 6-sulfatoxymelatonin.** Samples were assayed for creatinine concentration based on a kinetic modification of the Jaffe reaction using reagents supplied by Pointe Scientific Inc. on a Roche Cobas Mira Plus chemistry analyzer. Intra- and inter-assay coefficients of variation were 3.9% and 2.2%, and 2.7% and 2.3%, respectively, at 73 and 125 mg/dL. Urinary concentrations of the primary metabolite of melatonin, 6-sulfatoxymelatonin, were determined with a radioimmunoassay kit (Stockgrand Ltd.). The assay was run in duplicate with low, medium, and high kit controls as well as an in-house control using a urine sample from a volunteer. Assay sensitivity was 0.5 ng/mL urine. Intra- and interassay coefficients of variation were 10.7%, 9.7%, and 9.3%, and 19.8%, 11.2%, and 15.4%, respectively, at 4.4, 13.8, and 26.5 ng/mL.

**Assessment of urinary cortisol.** Urinary cortisol was quantitated using a 7890A gas chromatograph in conjunction with a model 7000B triple quadrupole mass spectrometer operating in electron impact mode (Agilent Technologies), as previously described (19;20). Cortisol was extracted from 10 mL of urine with 500 mg bed volume, C-18, solid phase extraction columns (Agilent) followed by elution with methanol. After drying, glucuronides and sulphates were subjected to hydrolysis using crude *b*-glucuronidase (*H. pomatia*, Sigma) and *b*-glucuronidase/arylsulphatase (*H. pomatia*, Roche Molecular Biochemicals) in sodium acetate buffer (pH 4.8). The digest was reextracted, eluted with methanol, dried down, and derivatized to the methyloxime-trimethylsilyl ethers [Sigma; 2% (w/v) methoxyamine hydrochloride in pyridine for 1 hour at 60°C] and trimethylsilylimidazole (Regis Technologies; overnight at 100°C). The products, in cyclohexane, were purified by passing them through a column of hydroxyalkoxy-propyl dextran, Type IX (Sigma). A total of 1  $\mu$ L aliquot was chromatographed (Agilent; VF-1ms column, 30 m) and eluted as described previously (12). Cortisol was quantitated via multiple-

reaction-monitoring with Agilent Mass Hunter software. Cortisol-d4 (CDN Isotopes) served as the internal standard. All batches included blinded quality control urine samples. Results indicated acceptable reproducibility and accuracy. The intra- and interassay coefficients of variation were 5.4% and 7.7%, respectively.

**Assessment of serum cortisol.** Serum cortisol levels were measured by a solid-phase, competitive chemiluminescent enzyme immunoassay on the Immulite 2000 Analyzer (Siemens Healthcare Diagnostics). The intra- and interassay coefficients of variation were 5.0% and 7.2%, respectively.

### Statistical Analysis

Primary analyses used linear regression models (SAS Proc REG, SAS Institute) to evaluate differences in hormone levels between the day and night shift workers, with covariate adjustment for factors known or suspected to influence the hormones under study. These included: participant age; day length (calculated for the Seattle area from US Naval Observatory data); BMI [weight (kg)/height (m)<sup>2</sup>]; number of alcoholic beverages consumed in the previous 24 hours; nicotine/tobacco consumption in the previous 24 hours; and psychotherapeutic, sedative, beta blocker, thyroid preparation, or steroidal medication use in the previous 24 hours. Covariates were specified *a priori* based on results from previous studies showing these factors to be associated with urinary 6-sulfatoxymelatonin levels (21–23).

A secondary objective was to investigate whether 6-sulfatoxymelatonin levels are lower and cortisol levels are higher during daytime sleep relative to nighttime sleep within the night shift workers. SAS Proc MIXED was used to fit linear regression models with correlated error structure, allowing for time-dependent covariate adjustment (24–26). These models were adjusted for the same covariates listed above.

Urinary and serum hormone values were approximately log-normally distributed. Log-transformed hormone levels, normalized to creatinine concentration, were analyzed as continuous response variables. All statistical tests were 2 sided. Parameter estimates from regression models were exponentiated to display results as percent observed difference in hormone level for the comparison of interest. SEs and 95% confidence intervals were constructed using the Delta Method (27).

Several sensitivity analyses were undertaken to investigate the varying effects of age, job duration, and use of corticosteroid medications on the primary results. An interaction term for age and night shift work was evaluated to determine whether younger participants (i.e., ≤35 years) were more or less susceptible to the effects of night shift work. Similarly, we evaluated an interaction term between night shift work and duration of the present shift status using the median value (2.0 years) of reported duration. Finally, 3 participants reported taking steroidal medications during the data collection period; these

resulted in serum and urinary cortisol levels that were approximately double overall mean levels; analyses were conducted to determine whether exclusion of these participants affected the primary analysis results.

### Results

Of the 253 eligible night shift and 190 eligible day shift-working men, 240 night shift (94.8%) and 182 day shift (95.7%) workers agreed to participate, for a total of 422 participants. Of these, 35 became ineligible before data collection and 4 withdrew from the study. An additional 30 night shift and 10 day shift workers were removed from analyses due to significantly compromised data as a result of protocol or sample collection errors, for a final total of 185 night shift (73% of initially eligible) and 158 day shift (83% of initially eligible) workers available for analysis.

Participants were 22 to 55 years old, (mean = 34.7 for night shift workers and 36.5 for day shift workers). Night and day shift participants were remarkably similar with respect to BMI (mean = 25.8 kg/m<sup>2</sup> and 25.3 kg/m<sup>2</sup>, night vs. day shift), although night shift workers were slightly more likely to be in the highest BMI category (Table 1). Night and day shift participants were similar with respect to tobacco use and alcohol consumption during study participation, with the exception that, among drinkers, night shift workers consumed more alcohol in 24 hours ending with the nighttime sleep period (mean = 3.1 drinks and 2.0 drinks, night vs. day shift). Day shift participants were slightly more likely to use psychotherapeutics during study participation, but the use was very similar for both groups in most other medication categories.

Night shift workers had 57% lower urinary 6-sulfatoxymelatonin levels during daytime sleep compared with day shift-working men during nighttime sleep (raw mean = 17.0 vs. 29.7 ng/mg creatinine, night vs. day shift), and the difference was highly statistically significant ( $P < 0.0001$ ; Table 2). Similarly, night shift workers had 6-sulfatoxymelatonin levels that were 62% lower during nighttime work (raw mean = 12.9 ng/mg creatinine) compared with day shift workers during nighttime sleep ( $P < 0.0001$ ). Urinary 6-sulfatoxymelatonin levels were also significantly lower, by 40%, in night shift workers during their off-night of sleep (raw mean = 20.9 ng/mg creatinine), relative to day shift workers during their nighttime sleep ( $P < 0.0001$ ).

Night shift workers had 16% higher urinary cortisol levels during daytime sleep, relative to day shift workers during nighttime sleep (raw mean = 53.7 vs. 47.3 ng/mg creatinine, night vs. day shift;  $P < 0.05$ ; Table 2). There was no evidence of a difference in urinary cortisol levels during night work (raw mean = 46.6 ng/mg creatinine), compared with night sleep in the day shift workers. Night shift workers had 13% lower urinary cortisol levels during nighttime sleep on off-nights (raw mean = 43.7 ng/mg creatinine), relative to day shift workers ( $P < 0.05$ ). Morning serum cortisol levels post-work among night shift workers were approximately 43% lower than post-sleep

**Table 1.** Descriptive characteristics of male shift worker study participants according to work shift status

Covariate	Day shift		Night shift	
	N	% <sup>a</sup>	N	% <sup>a</sup>
Age group, y <sup>b</sup>				
20–25	20	13	25	13
26–30	32	20	55	30
31–35	24	15	25	13
36–40	31	20	37	20
41–45	19	12	13	7
46–50	14	9	15	8
51–55	18	11	15	8
BMI [wt (kg)/ht (m) <sup>2</sup> ]				
<18	1	1	0	0
18–25	65	41	67	36
25–30	91	57	112	61
>30	1	1	6	3
Alcohol consumption <sup>c</sup>				
Any	45	28	73	39
Number of drinks				
0	113	71	114	62
1	17	11	18	10
2	15	9	17	9
3 or more	13	8	36	19
Tobacco use (any) <sup>c</sup>				
Any	13	8	20	11
By type				
Cigarettes	9	6	18	20
Patch	2	1	1	0
Other tobacco	3	2	2	1
Medication use (any) <sup>c</sup>				
Psychotherapeutics	14	9	9	5
Sedatives	1	1	4	2
Beta blockers	0	0	4	2
Thyroid medications	0	0	1	1
Steroids	1	1	1	1

<sup>a</sup>Percentage calculated from 158 day shift participants and 185 night shift participants.

<sup>b</sup>Calculated according to date of first urine collection.

<sup>c</sup>In the 24 hours ending the nighttime sleep period

levels in day shift workers (raw mean = 9.0 µg/dL vs. 14.1 µg/dL, night vs. day shift;  $P < 0.0001$ ; Table 2). Night shift workers had 24% lower morning serum cortisol levels after an off-night of sleep (raw mean = 12.3 µg/dL), relative to day shift workers post-nighttime sleep.

Among night shift workers, daytime sleep was associated with statistically significantly lower 6-sulfatoxymelatonin and higher urinary cortisol levels, relative to nighttime sleep on off-nights (Table 3). Urinary 6-sulfatoxymelatonin levels were approximately 29% lower, and urinary cortisol levels were approximately 29% higher during daytime sleep, relative to nighttime sleep on off-

nights ( $P < 0.01$ ). Nighttime work was also associated with statistically significantly lower 6-sulfatoxymelatonin levels and higher urinary cortisol levels, relative to nighttime sleep on off-nights; urinary 6-sulfatoxymelatonin levels were approximately 37% lower ( $P < 0.0001$ ), urinary cortisol levels were 15% higher ( $P < 0.01$ ). Morning serum cortisol levels were 25% lower after night work than levels following an off-night of sleep ( $P < 0.0001$ ).

In exploratory analyses (described above), results were not affected by younger age, longer duration of night shift work, or steroidal medication use. There was one exception: younger night shift workers seemed to have even greater reductions in 6-sulfatoxymelatonin levels during nighttime work relative to nighttime sleep in the day shift workers (results not tabulated).

## Discussion

Night shift workers experience a variety of physical symptoms and adverse health effects, including headache, fatigue, and gastrointestinal disturbances (reviewed in refs. 29; 31; 32), and ischemic stroke (33; 34). Night shift workers get considerably less sleep, and such sleep is of lower quality and efficiency than that of day shift workers (28;29;31;35;36). Of increasing concern is the mounting evidence of an association between night shift work and cancer risk in both women and men (37), including prostate (1–9), breast (reviewed in ref. 38), endometrial (39), and colon (4;40) cancer; the recently published study by Parent and colleagues also reported increased risk of lung, bladder, rectal, and pancreatic cancers, as well as non-Hodgkin lymphoma, associated with night shift work (4). The present study was undertaken to investigate 2 inter-related mechanisms by which night shift work could increase cancer risk.

It is well established that night shift workers are at high risk for circadian disruption and the resulting effects on hormonal regulation. Melatonin is a primary output signal of the central circadian pacemaker (i.e., suprachiasmatic nucleus) that synchronizes the internal hormonal environment to the light–dark cycle of the external environment; it is primarily produced and secreted by the pineal gland, a neuroendocrine transducer that is stimulated by darkness and suppressed by light as perceived by the retina (41). Melatonin acts as a chemical code for the night: the longer the night, the longer the duration of secretion (42). Hence, during the typical sleep–wake period of the non-night shift worker, circulating melatonin concentrations are low during the day and higher at night, exhibiting a characteristic rise in concentration after darkness and peak near the midpoint of the dark interval (43).

A mechanism whereby night shift work could increase cancer risk lies in the well-described growth-inhibitory and oncostatic properties of melatonin: melatonin both protects cells from DNA damage and promotes repair of DNA damage once it has occurred (11–13). Recently, Blask and colleagues conducted a series of experiments, which

**Table 2.** Results from regression analyses of melatonin and cortisol levels between night shift workers (NSW), relative to day shift workers (DSW), all participants ( $n = 158$  day and 185 night shift workers)

Comparison <sup>a</sup>	% higher (+) or lower (–) NSW hormone levels, relative to DSW levels <sup>b</sup>	95% confidence interval
Day sleep (NSW), relative to night sleep (DSW)		
Urinary 6-Sulfatoxymelatonin (ng/mg creatinine)	–57.5% <sup>c</sup>	(–66.1%, –48.9%)
Urinary cortisol (ng/mg creatinine)	+15.7% <sup>d</sup>	(+1.0%, +30.3%)
Night work (NSW), relative to night sleep (DSW)		
Urinary 6-Sulfatoxymelatonin (ng/mg creatinine)	–62.0% <sup>c</sup>	(–69.0%, –55.0%)
Urinary cortisol (ng/mg creatinine)	+3.3%	(–9.0%, +15.7%)
Serum cortisol, morning (μg/dL) <sup>e</sup>	–42.7% <sup>c</sup>	(–49.5%, –35.8%)
Off-night sleep (NSW), relative to night sleep (DSW)		
Urinary 6-Sulfatoxymelatonin (ng/mg creatinine)	–39.8% <sup>c</sup>	(–50.5%, –29.1%)
Urinary cortisol (ng/mg creatinine)	–12.7% <sup>d</sup>	(–24.4%, –0.9%)
Serum cortisol, morning (μg/dL) <sup>e</sup>	–24.4% <sup>c</sup>	(–32.7%, –16.0%)

<sup>a</sup>Analyzed using the natural log transformation.<sup>b</sup>Adjusted for the effects of age, hours of darkness, BMI, number of alcoholic beverages consumed, nicotine/tobacco consumption, and use of medications specified a priori.<sup>c</sup> $P < 0.0001$ , using 2-sided  $t$  test.<sup>d</sup> $P < 0.05$ , using 2-sided  $t$  test.<sup>e</sup>Single serum sample collected within approximately 90 minutes of rising from night sleep or completing the night shift.

showed directly the inverse relationship that exists between melatonin level and tumor activity, using both steroid receptor-positive and -negative human breast cancer xenografts in rats (44; 45); the same team has since showed similar results with prostate cancer xenografts (46). Other studies have reported a reduction in growth of malignant prostate tumor cells by both pharmacologic and physiologic doses of melatonin (47–55), although

such findings are not always consistent (56, 57). The present study found that night shift work is associated with lower urinary 6-sulfatoxymelatonin levels during night work and daytime sleep, and that levels remain low even during night sleep on off-nights. Within night shift workers, 6-sulfatoxymelatonin levels were significantly lower during both daytime sleep and nighttime work, relative to nighttime sleep on off-nights.

**Table 3.** Results from regression analyses of melatonin and cortisol levels within the night shift workers ( $n = 185$  urinary measurements and 182 serum measurements) during daytime sleep or nighttime work, relative to nighttime sleep

Comparison <sup>a</sup>	% higher (+) or lower (–) hormone levels, relative to nighttime sleep levels <sup>b</sup>	95% confidence interval
Daytime sleep, relative to nighttime sleep		
Urinary 6-Sulfatoxymelatonin (ng/mg creatinine)	–28.7% <sup>c</sup>	(–44.5%, –13.1%)
Urinary cortisol (ng/mg creatinine)	+28.9% <sup>c</sup>	(+11.5%, +46.4%)
Nighttime work, relative to nighttime sleep		
Urinary 6-Sulfatoxymelatonin (ng/mg creatinine)	–37.5% <sup>d</sup>	(–45.7%, –29.2%)
Urinary cortisol (ng/mg creatinine)	+15.3% <sup>c</sup>	(+4.1%, +26.6%)
Serum cortisol, morning (μg/dL) <sup>e</sup>	–24.6% <sup>d</sup>	(–32.7%, –16.6%)

<sup>a</sup>Analyzed using the natural log transformation.<sup>b</sup>Adjusted for the effects of age, hours of darkness, body mass index, number of alcoholic beverages consumed, nicotine/tobacco consumption, and use of medications specified a priori.<sup>c</sup> $P < 0.01$ , using 2-sided  $t$  test.<sup>d</sup> $P < 0.0001$ , using 2-sided  $t$  test.<sup>e</sup>Single serum sample collected within approximately 90 minutes of rising from night sleep or completing the night shift.

This study also found that night shift work is associated with an altered secretory pattern of cortisol, as measured both in urine (an integrated measure over time) and serum (a "spot" moment-in-time measure). In healthy individuals, cortisol levels are typically peak in the morning shortly after rising, followed by a steep morning drop, which lessens as evening approaches; levels reach a nadir shortly after nighttime sleep onset, gradually rising until the peak shortly after awakening (58). This study observed urinary cortisol levels among night shift workers that were higher during daytime sleep and lower during nighttime sleep; these results, coupled with markedly lower morning serum cortisol levels following both night work and night sleep, provide evidence that the normal secretory pattern of cortisol is flattened among night shift workers. Cortisol exerts a regulatory effect related to both immunity and inflammation; cortisol deficiency may result in an unresponsive immune system, whereas too much cortisol suppresses immune responses (59). Atypical cortisol rhythms, such as low morning levels and decreased variability in levels overall, have been associated with metabolic dysfunction (60). Moreover, chronic dysregulation of the circadian cortisol rhythm has been associated with higher levels of inflammation (16), and inflammation plays a critical role in carcinogenesis (17). Sephton and colleagues reported that loss of the normal diurnal variation in cortisol predicts early mortality in metastatic breast cancer (61).

At least 9 studies, predominately of women, have examined melatonin levels in night shift workers; 7 reported significantly decreased measures of melatonin levels (62–68), whereas 2 did not (69; 70). Of note is the study conducted by Burch and colleagues (68), which reported an altered sleep:work urinary melatonin ratio, indicating the lack of a robust diurnal melatonin rhythm typically seen in the non-night shift-working population. With respect to cortisol, several studies of night shift workers report similar results to those presented here (71–74), including lower waking cortisol levels among male officers working short-term night shifts (71), altered cortisol secretory patterns among male and female night shift workers (72), amplitude reduction in cortisol levels among men after light exposure under controlled laboratory conditions (73), and decreased rhythm amplitude in serum cortisol levels among night shift workers (74).

This study has a number of strengths. Unlike previous studies, which focused on women, the present study evaluated 6-sulfatoxymelatonin and cortisol levels simultaneously at multiple critical time points throughout the course of a shift worker's typical "work day" and subsequent sleep. The combination of urine sample collection, which allows for integrated measures of melatonin and cortisol secretion, combined with "spot" measure of morning serum cortisol levels to capture the critical morning rise, allowed for a comprehensive picture of cortisol and melatonin secretion throughout the participant's "day". This combination of urine and serum collections allowed us to observe the flattening of the night shift workers'

cortisol rhythms, relative to the day shift workers. Our comparisons of urinary melatonin levels during nighttime sleep and nighttime work (night shift workers) to nighttime sleep (day shift workers) indicate that night shift workers have constitutively lower levels of melatonin. In addition to comparisons between night and day shift-working groups, the study design allowed us to observe that both nighttime work and daytime sleep resulted in 6-sulfatoxymelatonin levels that were substantially lower than nighttime sleep levels within the same participant. Even if many night shift workers could be self-selected individuals who naturally produce lower levels of melatonin such that observed differences between night and day shift workers might not entirely be attributable to night shift work in of itself, these analyses indicate that 2 necessities of the job, both working at night and sleeping during the day, were each associated with substantially lower melatonin levels compared with levels during nighttime sleep within the same participant. A further strength was the ability to analyze melatonin and cortisol secretion over a continuous 24-hour period. Although we did not observe significant differences in 24-hour urinary melatonin or cortisol levels between the night and day shift workers, indicating that the night shift workers secrete non-negligible amounts of cortisol and melatonin at times other than sleep and nighttime work (data not shown), evidence suggests that the diurnal rhythm of these hormones may be more biologically relevant than simply the cumulative levels attained over a 24-hour period (75). Sensitivity analyses were undertaken to investigate whether results of the primary analyses were affected by younger age, duration of night shift work, or removal of participants who took steroidal medications. In all but one instance (described above), results were essentially unchanged. Finally, with respect to melatonin, the results of this study are in close agreement with results from our companion study in women (18); in that study, we found similar reductions in 6-sulfatoxymelatonin levels throughout the same critical time points of the night shift worker's sleep-work cycle; cortisol levels were not evaluated in that study.

This study is subject to several limitations. Fritschi and colleagues assert that there are at least 5 potential mechanisms by which night shift work may increase cancer risk (76). Although we addressed several key lifestyle disturbances associated with shift work by adjusting for body mass, alcohol consumption, and smoking, we did not evaluate sleep disruption typically experienced by night shift workers. However, sleep disruption activates stress-response mechanisms, apparent through measurement of cortisol levels (77; 78). Indeed, this study observed atypical cortisol levels in night shift workers, relative to day shift workers, at several key time periods. Second, it is well known that there are many factors that contribute to the substantial interindividual variation in melatonin levels; although our data collection instruments were designed to collect as much information as possible on factors believed to contribute to such variability for use in

covariate adjustment (e.g., hours of daylight, BMI, medication use, etc.), it is likely that there are other factors not measured here (namely, measurement of actual light levels at home or work). If any of these unmeasured factors did, in fact, have an effect on any of the hormones under study, then our inability to adjust for such exposures would most likely attenuate the actual effect of night shift work on hormone levels. Third, using a protocol that included urine samples collected over shorter time intervals would have potentially allowed us to observe approximately when peak melatonin or cortisol levels occur and evaluate any phase shift in the secretion of these hormones associated with night shift work. However, the collection of urine during specific, predefined periods of the shift worker's "day" (e.g., night work, night sleep, etc.) did allow for some degree of measurement of phase shift without being disruptive to the participant's schedule, which would have its own effect on the hormones under study (e.g., interruption during sleep to collect more frequent samples).

This study investigated 2 interrelated mechanisms by which night shift work could increase cancer risk, via reductions in melatonin levels and/or altered cortisol secretion. The substantial alterations observed in 6-sulfatoxymelatonin and cortisol levels among night shift workers during both sleep and work periods, relative to day shift workers, may indicate that shift work, via the direct oncogenic properties of melatonin and/or the effects of cortisol on immune regulation, could be an important risk factor in the development of many cancers. Another mechanism whereby night shift work could affect prostate cancer risk in particular is through the potential for circadian disruption to affect the regulation of androgens that are potentially important in prostate cancer development, possibly through melatonin's critical role in regulation of gonadal function (79). Evaluation of urinary and serum androgen levels in this study population are being pursued.

Given the broad applicability of the mechanisms evaluated in this study, future epidemiologic studies of shift work need to expand beyond breast and prostate cancer.

These should include identification of subgroups of night shift workers that are more or less negatively affected by shift work, either through identification and evaluation of key polymorphisms important in the regulation of the circadian clock, or through more in-depth examination of biomarkers, such as melatonin, cortisol, and temperature, of circadian dysfunction. Given that at least some night shift work is unavoidable (e.g., law enforcement, fire fighters, hospital emergency staff), an examination of work schedules and prescribed treatments to mitigate the negative effects of night shift work should be part of the future study agenda.

#### Disclosure of Potential Conflicts of Interest

F.J. Nordt is employed as a president and has ownership interest (including patents) in Rhein Consulting Laboratories. No potential conflicts of interest were disclosed by the other authors.

#### Authors' Contributions

**Conception and design:** D.K. Mirick, F.J. Nordt, S. Davis  
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