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## The Impact of Chronotype on Melatonin Levels Among Shift Workers

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### Abstract

**Objectives**—The association between shift work and cancer, which is thought to be mediated by effects on circulating melatonin levels, may be modified by chronotype (i.e. the inherent preference for activity in the morning or the evening); however, few studies have examined the potential impact of chronotype on the carcinogenic effects of shift work. The authors analyzed the impact of chronotype on previously reported differences in melatonin levels among healthcare workers that exclusively worked night or day shifts.

**Methods**—The cross-sectional study included 664 men and women (310 day shift and 354 night shift workers) from which urine samples were collected throughout work and sleep periods and were assayed for 6-sulfatoxymelatonin. Participants also completed the Composite Scale of Morningness, a questionnaire used to assess chronotype.

**Results**—Among both morning and evening-type night shift workers, 6-sulfatoxymelatonin levels were constitutively lower during daytime sleep, nighttime sleep and night work compared to dayshift workers during nighttime sleep. However, morning-type shift workers consistently showed 6-sulfatoxymelatonin levels that were closer to levels in day shift workers than did evening-type night shift workers. Differences in 6-sulfatoxymelatonin levels between morning-type and evening-type night shift workers relative to day shift workers were statistically significant in every instance ( $p < 0.05$ ).

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#### Contributors

All authors participated substantially in the analysis and interpretation of data and the drafting and revision of the manuscript. All authors approved the manuscript for publication.

#### Competing Interests

None to report.

#### Ethics approval

Institutional Review Board of the Fred Hutchinson Cancer Research Center.

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**Conclusion**—These results suggest that morning-type night shift workers may be better able to maintain a ‘normal’ circadian pattern of melatonin production as compared to evening-type night shift workers. The impact of this chronotype effect on cancer risk among shift workers requires further study.

### Keywords

shift work; chronotype; melatonin; cancer

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### Introduction

Since the International Agency for Research on Cancer’s 2007 classification of shift work as a probable human carcinogen, epidemiologic evidence for an association between shift work and cancer continues to be mixed (1–3). The mixed evidence has been attributed to a variety of factors including crude exposure assessment and the lack of consideration of individual characteristics that may impact adaptability to shift work schedules such as chronotype (4).

Chronotype or diurnal preference has been previously associated with tolerance to shift work. Individuals with a preference for activity during the evening (i.e. evening-types) have reported higher job satisfaction and better work performance than individuals with a preference for activity in the morning (i.e. morning-types) (5). A recent study found that morning-type women who work the night shift had a higher risk of breast cancer than evening-type women when comparing subjects with high cumulative night shift work to those that never worked the night shift (6). While chronotype was assessed based only on a single item on a questionnaire in that study, it suggests that evening-type individuals may have a reduced susceptibility to the carcinogenic effects of night shift work. Thus, additional studies with more reliable assessments of chronotype could be of tremendous value to occupational disease prevention efforts.

The potential carcinogenic effects of shift work are thought to be mediated through melatonin, which has been shown to have direct oncostatic properties and has been associated with decreased risks of breast cancer (7–9). Specifically, post-sleep 6-sulfatoxymelatonin levels (6-sulfatoxymelatonin is an established urinary marker of circulating melatonin levels), were associated with decreased breast cancer risk. However, in a study that evaluated 24-hour urinary 6-sulfatoxymelatonin, no association was observed (10). Taken together, these previous studies seem to indicate that the decreased risks of cancer are not simply conferred by general increases in the levels of circulating melatonin but involves the correct timing of melatonin secretion (i.e. maintaining high levels of circulating melatonin at night and low levels during the day) (11).

Few studies have evaluated the potential effect of chronotype on melatonin levels in a population actually engaged in shift work. Previously, the authors reported significantly reduced urinary 6-sulfatoxymelatonin levels among exclusive night shift workers during nighttime work, daytime sleep and nighttime sleep periods on off-nights, relative to exclusive day shift workers during nighttime sleep in cross-sectional studies of female and male healthcare workers (12,13). To better understand the potential effect of chronotype on melatonin, an analysis of the impact of chronotype, assessed using the Composite

Morningness Questionnaire, was conducted (14) on these previously reported differences in melatonin levels associated with shift work. In light of the previous report of lower breast cancer risk in evening-type shift workers compared to morning-type shift workers, it is hypothesized that evening-type individuals would have less disruption of their melatonin levels associated with night shift work as compared to morning-type individuals.

## Methods

Study methods have been previously described in detail (12,13) and are briefly summarized below.

### Study participants

Participants were women aged 20 to 49 years (recruitment and data collection from November 2003 to August 2007) or men aged 20 to 55 years (recruitment and data collection from October 2007 to May 2011) employed as healthcare workers in the Seattle metropolitan area. Participants were also required to have a body mass index (BMI; weight [kg]/height [m]<sup>2</sup>) between 18 and 30 kg/m<sup>2</sup>, and could not be using hormones or supplements containing melatonin at least 30 days prior to enrollment. Among women, additional eligibility criteria included: regular menstrual periods; no personal history of breast cancer, chemotherapy or tamoxifen therapy; no pregnancy or breast feeding within the past year; no use of supplements containing phytoestrogens or isoflavones and consumption of no more than 5 servings per week of soy-based foods. Male participants could not be using medications or supplements used to treat benign prostate conditions within 30 days of participation, could not have a personal history of prostate cancer or chemotherapy and could not have undergone general anesthesia or major surgery at least 8 weeks prior to enrollment.

Night shift workers were required to work at least 20 hours per week exclusively during the graveyard shift and to sleep at night during off days. In addition, to be eligible for the study, night shift workers were required to stop work no earlier than 6 a.m. and work at least 8 hours per shift to ensure that they were exposed to light during the late evening when melatonin levels typically rise, and that they were well into their shift around the time of typical peak melatonin secretion (1 – 2 am). Day shift workers were required to be employed at least 20 hours per week and work exclusively during the day shift (i.e., begin work no earlier than 6 a.m. and work at least 8 hours per shift) and were chosen to have a similar age distribution as the night shift workers.

### Data Collection

After obtaining informed consent, a structured interview was administered to collect information about physical activity, employment history, current work and sleep schedules, reproductive and menstrual history (for female participants) and current medication use. The interviewer also assessed BMI. Subjects completed a Shift Work Questionnaire that contained a 13-item Composite Scale of Morningness. This scale was developed by Smith et al (1989) to address deficiencies observed in three widely used questionnaires to assess chronotype among shift workers (14). Though Smith et al (1989) originally validated the

Composite Scale of Morningness among a group of undergraduate college students, the scale has subsequently been validated among shift workers, and was shown to be stable over time even when subjects transition between day and night work schedules (15). The scale is used to assign a total chronoscore ranging from 13 to 55 points. Smith et al (1989) classified individuals with total scores of 22 and less as evening-types, 23 to 43 as intermediate-types and 44 and above as morning-types (14). However, these cut-points are considered arbitrary as they are simply based on the 10<sup>th</sup> and 90<sup>th</sup> percentiles of the distribution of chronoscores in their student study population which has a much different age distribution than the current study (15). As such, other cut-points were analyzed including simple dichotomization at the exact midpoint of the chronoscore scale (evening-types 33 or less and morning-types 34 or higher) and classification of individuals that are  $\pm 5$  points from the exact midpoint of the scale as intermediate-types such that evening-types are those with scores of 28 or less, intermediate-types are those with scores from 29–39 and morning-types are those with scores of 40 or higher. Total sleep time and sleep efficiency (ratio of total sleep time to total time in bed) were also determined for participants during daytime sleep (night shift workers) and nighttime sleep (day shift workers) using actigraphy, a method for providing electrophysiological measures of sleep in the home (16).

Urine collections were scheduled for days when at least two consecutive shifts were to be worked, followed by an off-night (for the night shift workers) or at least one day shift worked, followed by a night of sleep (for the day shift workers). Just prior to each urine collection period the participant was instructed to void his/her bladder and discard the urine; all subsequent urine excreted throughout either the work shift or the sleep period was collected, including the first void immediately following the end of the time period. Night shift workers collected all urine excreted during the daytime sleep period (following the first night shift) and the first void upon rising. During the second night shift, the participant collected all urine excreted during the shift and the first void immediately following the shift. During the following night's sleep (the 'off' night), the participant collected all urine excreted and the first void the next morning. Day shift workers collected all urine excreted during the day work shift and the first void immediately following the shift, as well as all urine excreted during the subsequent night of sleep and the first void the next morning. Day shift and night shift workers were asked to report alcohol consumption, tobacco use and medication use during study participation.

### Urinary 6-sulfatoxymelatonin Assay

Each sample was assayed for creatinine concentration based on a kinetic modification of the Jaffe reaction using Diagnostic Chemicals Ltd. reagents supplied by Roche Diagnostic Systems (Nutley, New Jersey) on a Roche Cobas Mira Plus chemistry analyzer. Intra- and inter-assay coefficients of variation were 0.9–1.3% and 1.8–2.3%, respectively. Urinary concentrations of the primary metabolite of melatonin, 6-sulfatoxymelatonin, were determined with a radioimmunoassay kit (Stockgrand Ltd., Guildford, Surrey, UK). 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 inter-assay coefficients of variation were 5.1–12.8% and 11.2–17.4%, respectively.

## Statistical Methods

Urinary 6-sulfatoxymelatonin values were approximately log-normally distributed. Log-transformed 6-sulfatoxymelatonin, normalized to creatinine concentration, was analyzed as a continuous response variable. Linear regression models (SAS Proc REG, SAS Institute, Cary, NC) were employed to evaluate the impact of chronotype on differences in 6-sulfatoxymelatonin levels between the day and night shift workers. Because previous results indicated significant reductions in 6-sulfatoxymelatonin levels among the night shift workers relative to levels in day shift workers, the primary aim of this analysis was to evaluate whether such reductions among night shift workers varied between morning, intermediate and evening-type individuals. Urinary levels of 6-sulfatoxymelatonin during nighttime sleep among the day shift workers were compared to levels in morning, intermediate and evening-type night shift workers at each of the following time points: daytime sleep following a night shift, nighttime sleep on an off-night and nighttime work. In each regression model, an interaction term between night shift status and chronotype was included to determine whether there was a statistically significant difference in the effect of night shift work between the three chronotype categories. Additionally, there were two within-subject comparisons of urinary 6-sulfatoxymelatonin levels among the night shift workers: daytime versus nighttime sleep and night work versus nighttime sleep. These analyses employed SAS Proc MIXED (SAS Institute, Cary, NC) to fit linear regression models with correlated error structure and adjustment for time dependent covariates. All models were adjusted for participant age, gender, hours of darkness (calculated for the Seattle area from US Naval Observatory data), BMI, number of alcoholic beverages consumed the previous 24 hours and psychotherapeutic use in the previous 24 hours. All statistical tests were two-sided and statistical significance was declared at  $p < 0.05$ . Parameter estimates from the regression models were exponentiated to display results as percent increases or decreases in 6-sulfatoxymelatonin levels for the comparisons of interest. Standard errors and 95% confidence intervals were constructed using the Delta Method (17).

## Results

Of the 914 eligible subjects identified, 869 agreed to participate. Forty-five eligible subjects declined to participate before the study commenced because of the necessary time commitment or could not be contacted by study staff. An additional 24 subjects were lost for these reasons during the study. One-hundred and thirty-nine subjects became ineligible during the study because of work schedule changes, use of melatonin supplements, irregular menstrual periods and/or pregnancy. After removing subjects with incomplete data collection, 664 participants remained for the primary analysis. Table 1 displays distributions of the primary covariates used in the analysis by shift status and gender. Male night shift workers tended to be younger (mean age = 34.4 versus 36.5, night versus day shift) and reported consuming more alcohol (mean number of drinks = 1.2 versus 0.6, night versus day shift) than male day shift workers. Female night shift workers tended to have a slightly higher BMI than female day shift workers (mean BMI = 24.4 versus 23.6, night versus day shift). There were no other notable differences in covariates between day shift and night shift workers. In Table 2, the distributions of male and female day and night shift workers by various chronotype classification schemes are provided. The Smith et al, 1989 scheme

resulted in very small numbers of evening-type individuals, so it was not included in any further analyses. Using the dichotomous measure of chronotype, morning types were generally more prevalent overall; a higher proportion of day shift workers were morning types, whereas night shift workers were more evenly divided between morning and evening types. Results of analyses of the impact of chronotype (dichotomized and three-level classification schemes) on melatonin differences between night shift workers and day shift workers, for men and women combined, are provided in Table 3. For dichotomized chronotype, evening-type night shift workers had 53% lower 6-sulfatoxymelatonin levels during daytime sleep relative to day shift workers during nighttime sleep, whereas morning-type night shift workers had 65% lower 6-sulfatoxymelatonin levels relative to day shift workers. When comparing night shift workers during nighttime sleep on their off-nights to day shift workers during nighttime sleep, evening-type participants had 49% lower 6-sulfatoxymelatonin levels, and morning-type participants had 30% lower 6-sulfatoxymelatonin levels. During night work, evening-type night shift workers had 73% lower 6-sulfatoxymelatonin levels compared to day shift workers during nighttime sleep, but morning-type night shift workers had a reduction of 54%. These differences in the effect of chronotype were statistically significant in every instance ( $p$ -interaction  $< 0.01$ ). Results for morning and evening-types were similar for the three-level chronotype analyses (Table 3).

Melatonin differences for intermediate types were consistently between those of morning types and evening types regardless of the comparison under consideration, although the difference between intermediate and evening-types was only statistically significant when comparing night work melatonin levels among night shift workers to night sleep melatonin levels among day shift workers.

As shown in Table 4 for the dichotomized chronotype analysis, within the night shift workers, daytime sleep levels of 6-sulfatoxymelatonin among evening-type participants were 21% lower than levels during nighttime sleep on an off-night, whereas daytime sleep levels among morning-type participants were further reduced (41%) relative to nighttime sleep levels. When comparing 6-sulfatoxymelatonin during night work to nighttime sleep, evening-types had a significantly greater reduction (52%) in levels of 6-sulfatoxymelatonin than morning-types (33%). These differences by chronotype were statistically significant ( $p$ -interaction  $< 0.01$ ). Results for the morning and evening types in the three-level chronotype analyses were similar (Table 4). Once again, melatonin differences for intermediate types fell between those of morning and evening types.

Gender stratified analyses (results not shown) did not produce materially different results except when examining effects during daytime sleep. When comparing daytime sleep levels among nightshift workers to nighttime sleep levels among dayshift workers, female evening-types had 62% (95% CI: -71%, -52%) lower levels of 6-sulfatoxymelatonin while male evening-types had 45% (95% CI: -57%, -31%) lower levels (female and male morning-types were 64 and 65% lower, respectively). When comparing daytime and nighttime sleep levels within nightshift workers, female evening-types had 36% (95% CI: -54%, -18%) lower levels of 6-sulfatoxymelatonin, but males had only 5% (95% CI: -3.0%, 20%) lower levels (female and male morning-types were 39 and 43% lower, respectively).

## Discussion

The results demonstrate that morning, intermediate and evening-type participants exclusively engaged in night shift work suffer from constitutively lower melatonin levels. However, as compared to evening-type night shift workers, morning-type night shift workers were able to maintain melatonin at levels that were more comparable to day shift workers at similar times of the day. Though not always statistically significant, intermediate-types, as compared to evening-types, also better maintained melatonin levels that were comparable to day shift workers at similar times of the day. For instance, during night work, which occurs at a time of day that is typically associated with sleep and high melatonin levels, morning and intermediate-types suffered less of a disruption in melatonin levels as compared to evening-types. In the case of daytime sleep, which occurs at a time of day that is typically associated with wakefulness and low melatonin levels, morning and intermediate-types better suppressed their melatonin levels as compared to evening-types. Gender stratified analyses revealed that the reduced suppression of melatonin among evening-types during daytime sleep was largely restricted to men.

A previous study of light at night exposure and melatonin among 123 rotating shift nurses did not observe an association between chronotype and melatonin levels (18); however, few subjects were classified as morning and evening-types under the classification system that was used. Furthermore, chronotype was not assessed as a potential modifier of the null shift work-melatonin association that was observed. The lack of an association may be attributed to the rapidly rotating shift schedule (two 12-hour days, two 12-hour nights and 5 days off), which the authors speculated may have been insufficient to disrupt melatonin secretion.

Among the female shift workers, we previously reported a significant effect of Asian race on melatonin differences, whereby Asian night shift workers were able to maintain melatonin levels that were closer to their day shift counterparts than White night shift workers during day sleep, night work and night sleep (19). Among female night shift workers, we observed no significant difference in chronoscore by race [mean (SD) chronoscore for White night shift workers = 32 (9); mean (SD) chronoscore for Asian night shift workers = 32 (8)]. In addition, including a variable for race in our regression models did not have a substantial impact on our point estimates of interest (results not shown). This suggests that race and chronotype are having independent effects on shift-related differences in melatonin levels.

The differences in melatonin levels between night shift and day shift workers observed in this study have been associated with tumor growth. Blask et al (2005) demonstrated that 40% decreases in melatonin among premenopausal women when exposed to 90 minutes of bright white light at night were associated with significant increases in tumor growth and activity in breast cancer xenografts (20). In conjunction with these findings, results suggest that relative to evening-types, morning-type night shift workers may be somewhat protected against the carcinogenic effects of melatonin suppression.

Very limited research has been completed on the impact of chronotype on cancer risk among shift workers, but based on a crude analysis of chronotype and breast cancer risk among night shift workers that found evening-types to have a lower shift work related risk of breast

cancer than morning-types (6), one might expect that evening-types would be able to better maintain normal melatonin levels. If evening-types do indeed have a lower risk of breast cancer associated with shift work as compared to morning-types, then the results might suggest that pathways other than those related to melatonin are of greater importance in conferring some degree of protection to evening-types against the carcinogenic effects of shift work. One potential pathway is lifestyle disturbances leading to poor diet, lack of exercise, increased alcohol consumption and increased tobacco use (21). There was no evidence for a difference in BMI between evening-type (mean BMI=25.1 kg/m<sup>2</sup>) and morning-type (mean BMI=25.0 kg/m<sup>2</sup>) nightshift workers in this study; however, BMI may not be a good indicator since those with BMI >30 kg/m<sup>2</sup> were not eligible to participate. There was also no significant difference between evening and morning-type nightshift workers in the mean number of alcoholic beverages consumed in the 24-hours prior to completing their nighttime sleep period (1.1 versus 0.8). The limited tobacco use among study participants precluded evaluation of differences by chronotype. Another potential pathway is sleep disruption leading to stress responses and immune suppression (21). There was a marginal difference in total sleep time between evening-type (466 minutes) and morning-type (456 minutes) nightshift workers. No difference in mean sleep efficiency was observed (79 versus 81% for evening-type and morning-type nightshift workers, respectively).

A previous laboratory-based study that collected hourly blood samples for the assessment of melatonin during sleep observed that chronotype was strongly related to melatonin acrophase (i.e. timing of peak melatonin secretion), but not amplitude (22). Data in the current study did not allow for assessment of acrophase among study subjects. A protocol that included urine samples collected over shorter time intervals would have potentially allowed for the assessment of acrophase; however, the disruption to participants' schedules would have likely impacted melatonin measurements, (e.g., interruption during sleep and light-at-night exposure to collect more frequent samples). Another limitation of this study is that it did not evaluate health effects, specifically cancer, in association with the differing reductions in 6-sulfatoxymelatonin levels. Given the large amount of time that it would take to accrue sufficient cancer cases in a prospective study of melatonin and cancer risk among shift workers, in the interim, studies such as this, involving the evaluation of biomarkers of circadian disruption and potentially cancer risk, can be useful. Despite these limitations, this is the first study to the authors' knowledge to evaluate the impact of chronotype on differences in melatonin levels between fixed day shift and night shift workers. Though the study population consisted of healthcare workers, results should be mostly generalizable to other occupational settings. However, since the study focused on fixed day and night shift workers that were employed at least 20 hours per week, results may not be applicable to rotating shift workers or part-time employees working less than 2 shifts per week. In addition, as a cross-sectional study, there is a possibility that the night shift workers that participated represented a self-selected group that was able to better tolerate the negative effects of working the night shift. Thus, a longitudinal study including new shift workers before they have a chance to drop out may observe even larger differences than those reported here. Detailed data collection including multiple urinary measures of 6-



sulfatoxymelatonin at multiple critical time points for each study subject and the rigorous assessment of chronotype are major strengths of the study.

The results of this study provide evidence that, with respect to disrupted melatonin levels, morning-types may be better protected from the negative effects of shift work relative to evening-types. The impact of chronotype on cancer risk needs to be examined more extensively in future studies of shift workers, with detailed lifestyle and sleep quality data to investigate the contribution of other pathways underlying the association between shift work and cancer. Examination of other biomarkers in addition to 6-sulfatoxymelatonin and consideration of gender-specific effects would also be useful in this endeavor.

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### What this paper adds

- Chronotype may impact the carcinogenic potential of shift work, but it has received limited attention in previous population-based studies.
- We evaluated the impact of chronotype on the association between shift work and melatonin; melatonin disruption has been previously linked to carcinogenesis
- We found that morning-type night shift workers were better able to maintain normal patterns of melatonin secretion as compared to evening-type night shift workers, suggesting that morning-types may be protected against the negative effects of shift work-related melatonin disruption.
- Detailed evaluations of chronotype should be included in future studies of cancer risk as it may identify subgroups that are particularly sensitive to the carcinogenic effects of shift work

**TABLE 1**  
Descriptive characteristics of Shift Worker Study participants according to work shift status and gender

Covariate	Day Shift				Night Shift			
	Female		Male		Female		Male	
	N	%*	N	%***	N	%*	N	%***
Age group (years) †								
20 – 25	20	13	20	13	25	15	25	14
26 – 30	25	16	32	20	31	18	55	30
31 – 35	26	17	24	15	34	20	25	14
36 – 40	39	26	31	20	37	21	36	20
41 – 45	41	27	19	12	42	24	13	7
46 – 50	1	1	14	9	3	2	15	8
51 – 55	0	0	18	11	0	0	13	7
Body mass index (wt (kg)/ht (m) <sup>2</sup> )								
< 18	0	0	1	1	0	0	0	0
18 – 25	97	64	65	41	95	55	67	37
25 – 30	54	35	91	58	75	44	109	60
> 30	1	1	1	1	2	1	6	3
Number of Alcoholic Beverages Consumed <sup>§</sup>								
0	109	72	113	71	125	73	112	61
1	24	16	17	11	17	10	17	9
2	12	8	15	9	10	6	17	9
3 or more	7	5	13	8	20	12	36	20
Tobacco use (any) §								
Any	10	7	13	8	16	9	20	11
By type								
Cigarettes	10	7	9	6	16	9	18	10
Patch	0	0	2	1	0	0	1	1
Medication use (any) <sup>§</sup>								
Psychotherapeutics	20	13	14	9	16	9	8	4
Sedatives	2	1	1	1	1	1	4	2

Covariate	Day Shift				Night Shift			
	Female		Male		Female		Male	
	N	%*	N	%***	N	%*	N	%***
Beta blockers	2	1	0	0	3	2	3	2
Thyroid medications	11	7	0	0	11	6	1	1
Steroids	7	5	1	1	7	4	1	1

\* Percent calculated from 152 female day shift participants and 172 female night shift participants with nighttime sleep urine samples

\*\*\* Percent calculated from 158 male day shift participants and 182 male night shift participants with nighttime sleep urine samples

† Calculated according to date of first urine collection

§ In the 24 hours ending the nighttime sleep period

TABLE 2

Chronotype measures of Shift Worker Study participants according to work shift status and gender

Chronotype Measure	Day Shift				Night Shift			
	Female		Male		Female		Male	
	N	%	N	%	N	%	N	%
Smith et al <sup>†</sup>								
Evening	3	2	5	3	26	15	12	7
Intermediate	125	82	114	73	124	73	155	86
Morning	24	16	38	24	20	12	13	7
Trichotomous <sup>‡</sup>								
Evening	15	10	22	14	62	36	48	26
Intermediate	84	55	76	48	70	41	93	51
Morning	53	35	60	38	40	23	41	23
Dichotomous <sup>§</sup>								
Evening	39	26	55	35	88	51	88	49
Morning	112	74	103	65	83	49	92	51

\* Percent calculated from 152 female day shift participants and 172 female night shift participants with nighttime sleep urine samples

\*\* Percent calculated from 158 male day shift participants and 182 male night shift participants with nighttime sleep urine samples

<sup>†</sup> Based on chronoscore and assigned according to *Smith et al*: Evening: 22 or less; Intermediate: 23–43; Morning: 44 or higher.<sup>‡</sup> Based on chronoscore and assigned as follows: Evening: 28 or less; Intermediate: 29–39; Morning: 40 or higher.<sup>§</sup> Based on chronoscore and assigned as follows: Evening (33 or less) or Morning (34 or higher)

TABLE 3

Results from regression analyses of melatonin levels by chronotype, night shift workers (NSW) relative to all day shift workers (DSW)

Comparison	% difference in NSW 6- sulfatoxymelatonin levels, relative to DSW levels <sup>¶</sup>	95% Confidence Interval
Day sleep (NSW) v. night sleep (DSW)		
Dichotomous <sup>†</sup>		
Evening	-53.2% **	(-61.1%, -45.3%)
Morning <sup>*</sup>	-64.6% **	(-70.5%, -58.7%)
Trichotomous <sup>§</sup>		
Evening	-51.3% **	(-60.8%, -41.7%)
Intermediate	-57.2% **	(-64.6%, -49.9%)
Morning <sup>*</sup>	-71.2% **	(-77.5%, -64.9%)
Night sleep (NSW v. DSW)		
Dichotomous <sup>†</sup>		
Evening	-49.0% **	(-57.0%, -41.0%)
Morning <sup>*</sup>	-30.0% **	(-40.9%, -19.1%)
Trichotomous <sup>§</sup>		
Evening	-49.3% **	(-58.7%, -39.8%)
Intermediate	-38.5% **	(-48.4%, -28.6%)
Morning <sup>*</sup>	-31.0% **	(-45.2%, -16.8%)
Night work (NSW) v. night sleep (DSW)		
Dichotomous <sup>†</sup>		
Evening	-73.2% **	(-77.5%, -69.0%)
Morning <sup>*</sup>	-54.4% **	(-61.5%, -47.2%)
Trichotomous <sup>§</sup>		
Evening	-76.2% **	(-80.5%, -71.8%)
Intermediate <sup>*</sup>	-62.2% **	(-68.3%, -56.1%)
Morning <sup>*</sup>	-51.0% **	(-61.0%, -41.0%)

\* test for difference from Evening Type category:  $p < 0.01$ , using two-sided t-test

\*\*  $p < 0.001$ , two-sided t-test

<sup>†</sup> Evening: chronoscore 33 or less; Morning: chronoscore 34 or higher

<sup>§</sup> Evening: chronoscore 28 or less; Intermediate: chronoscore 29–39; Morning: chronoscore 40 or higher

<sup>¶</sup> Analyzed using the natural log transformation and adjusted for the effects of age, gender, hours of darkness, body mass index, number of alcoholic beverages consumed, and use of psychotherapeutics; referent category is all Day Shift Workers (DSW); e.g. in dichotomous analysis, evening type night shift workers during daytime sleep had 53.2% lower levels of 6-sulfatoxymelatonin than all day shift workers during nighttime sleep

TABLE 4

Results from regression analyses of melatonin levels by chronotype within the night shift workers (NSW)

Comparison	% difference in 6- sulfatoxymelatonin levels <sup>¶</sup>	95% Confidence Interval
Day sleep v. night sleep		
Dichotomous <sup>†</sup>		
Evening	-21.5% **	(-36.7%, -6.3%)
Morning <sup>*</sup>	-40.9% ***	(-52.5%, -29.4%)
Trichotomous <sup>§</sup>		
Evening	-17.7%	(-36.4%, +1.1%)
Intermediate	-27.9% ***	(-42.1%, -13.6%)
Morning <sup>*</sup>	-52.8% ***	(-65.0%, -40.7%)
Night work v. night sleep		
Dichotomous <sup>†</sup>		
Evening	-52.3% ***	(-58.1%, -46.5%)
Morning <sup>*</sup>	-32.5% ***	(-40.6%, -24.4%)
Trichotomous <sup>§</sup>		
Evening	-57.3% ***	(-63.6%, -51.0%)
Intermediate <sup>*</sup>	-39.8% ***	(-47.3%, -32.4%)
Morning <sup>*</sup>	-27.4% ***	(-39.5%, -15.3%)

\* test for difference from Evening Type category:  $p < 0.01$ , using two-sided t-test

\*\*  $p < 0.05$ , two-sided t-test

\*\*\*  $p < 0.001$ , two-sided t-test

<sup>†</sup> Evening: chronoscore 33 or less; Morning: chronoscore 34 or higher

<sup>§</sup> Evening: chronoscore 28 or less; Intermediate: chronoscore 29–39; Morning: chronoscore 40 or higher

<sup>¶</sup> Analyzed using the natural log transformation and adjusted for the effects of age, gender, hours of darkness, body mass index, number of alcoholic beverages consumed, and use of psychotherapeutics; reference category is night sleep; e.g. in dichotomous analysis, evening type night shift workers during daytime sleep had 53.2% lower levels of 6-sulfatoxymelatonin than all night shift workers during nighttime sleep