Original Contribution

Racial Differences in the Association Between Night Shift Work and Melatonin Levels Among Women

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Reduced suppression of melatonin in response to working the night shift among people of Asian ancestry has been suggested as a possible explanation for the null results observed in a recent analysis of shift work and breast cancer risk in a Chinese cohort. The authors analyzed the impact of Asian versus white race on previously reported differences in urinary 6-sulfatoxymelatonin levels in a 2003–2008 study in Seattle, Washington, of female health-care workers that exclusively worked night or day shifts. A total of 225 white and 51 Asian participants were included in the analysis. Although 6-sulfatoxymelatonin levels were affected by night shift work in both racial groups, Asian night shift workers consistently showed 6-sulfatoxymelatonin levels that were closer to levels in day shift workers than did white night shift workers. Furthermore, differences in 6-sulfatoxymelatonin levels between white and Asian night shift workers relative to day shift workers were statistically significant in every instance (P < 0.05). These results suggest that Asians may be better able to maintain a "normal" circadian pattern of melatonin production compared with whites and suggest a biological mechanism by which Asian night shift workers may be at a reduced risk of cancer.

cancer; melatonin; race; shift work

In their review of the experimental and epidemiologic evidence, the International Agency for Research on Cancer classified shift work resulting in circadian disruption as a probable human carcinogen (1). This was based on strong experimental evidence demonstrating associations among light at night, reduced melatonin, and carcinogenesis. Not only has reduced melatonin been associated with increased circulating estrogen, an important risk factor for breast cancer, but melatonin has been shown to have direct oncostatic properties (2), meaning that reduced melatonin may be more broadly related to cancer at many sites. Comparatively, the epidemiologic evidence, mostly from studies of breast cancer, was considered limited. Results from studies of breast cancer have been inconsistent (3–11), which may be attributable to inconsistent definitions of shift work and relatively crude "exposure" assessment across the studies.

In a recent analysis of shift work and breast cancer that was conducted in a large prospective population-based cohort of Chinese women, with relatively detailed shift-work data, no evidence of an association was found (11). A racial difference in the suppression of melatonin among shift workers was proposed as a potential explanation for this null finding (12). Indeed, previous studies have suggested that Asians produce lower levels of melatonin relative to Caucasians and that melatonin suppression due to light exposure is reduced among Asians compared with Caucasians (13–15). However, no studies have evaluated this potential effect of race on melatonin levels in a population actually engaged in night-shift work.

Previously, we reported significantly reduced urinary 6-sulfatoxymelatonin levels among female exclusive night-shift workers during nighttime work, daytime sleep, and nighttime sleep periods on "off nights," relative to exclusive day-shift workers in a cross-sectional study of female health-care workers (16). The study did not observe major differences in reproductive hormone levels. This may suggest that the oncostatic properties of melatonin itself may be important as a mechanism by which night-shift...
work may be carcinogenic in humans. Here, we describe an analysis of the impact of Asian versus white racial category on differences in the reductions of urinary 6-sulfatoxymelatonin levels observed among the night-shift workers.

**MATERIALS AND METHODS**

Study methods have been previously described in detail (16) and are briefly summarized below.

**Study participants**

The study period was from November 1, 2003, to April 30, 2008. Participants were women aged 20–49 years employed as health-care workers in the Seattle, Washington, metropolitan area. 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 our study, night-shift workers were required to stop work no earlier than 6 AM and to 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 in the middle of 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 to work exclusively during the day shift (i.e., begin work no earlier than 6 AM and work at least 8 hours per shift); they were chosen so as to have an age distribution similar to that of the night-shift workers.

**Data collection**

Urine collections were scheduled for days when at least 2 consecutive shifts were to be worked, followed by an off night (for the night-shift workers) or at least 1 day shift worked, followed by a night of sleep (for the day-shift workers). Prior to urine collection, the interviewer met with the participant to collect a detailed employment history, demographic information, reproductive and menstrual history, and information on physical activity and current hormone and medication use. With respect to race, participants were asked, “What race are you?” They could choose one or more of the following options: American Indian/Alaska Native, Asian, black or African American, Native Hawaiian or Other Pacific Islander, and white. The current analysis was restricted to participants identifying themselves as Asian or white. If a participant identified herself as belonging to more than one racial group, she was assigned to a primary group as follows: 1) If a participant identified herself as “white” plus any other racial category, the participant was assigned to the other racial category; 2) if a participant identified herself as “Asian” plus any other racial category, the participant was assigned to the Asian category. Because the current analysis was restricted to participants identifying themselves as Asian or white, other racial categories were not assigned.

Just prior to each urine collection period, the participant was instructed to void 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.

**Urinary 6-sulfatoxymelatonin assay**

Each sample was assayed for creatinine concentration based on a kinetic modification of the Jaffe reaction by using Diagnostic Chemicals, Ltd., reagents supplied by Roche Diagnostic Systems (Nutley, New Jersey) on a Roche Cobas Mira Plus chemistry analyzer. Intra- and interassay 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, United Kingdom). 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 of urine. Intra- and interassay 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 the creatinine concentration, was analyzed as a continuous response variable. Linear regression models (SAS Proc REG; SAS Institute, Inc., Cary, North Carolina) were used to evaluate 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 Asian and white racial categories. Urinary levels of 6-sulfatoxymelatonin during nighttime sleep among the day-shift workers were compared with levels in Asian and white 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. To each regression model, an interaction term between night-shift status and racial category was added to determine whether there was a statistically significant difference in the effect of night-shift work between the 2 racial categories. Additionally, there were 2 within-subject comparisons of urinary 6-sulfatoxymelatonin levels among the night-shift workers: daytime versus nighttime sleep and night work versus nighttime sleep. The analyses used SAS Proc MIXED (SAS Institute, Inc.) to fit linear regression models with correlated error structure and adjustment for
time-dependent covariates. All models were adjusted for participant age, day length (calculated for the Seattle area from US Naval Observatory data), body mass index (weight (kg)/height (m)$^2$), number of pregnancies lasting 6 months or longer, number of alcoholic beverages consumed the previous 24 hours, and psychotherapeutic use in the previous 24 hours. All statistical tests were 2 sided. 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 by using the delta method (17).

RESULTS

A total of 276 participants, 225 white and 51 Asian, were included in the primary analysis. Table 1 displays distributions of the primary covariates used in the analysis, by Asian versus white racial category. Asian and white participants in this study differed with respect to many of these covariates. A greater proportion of Asian participants were night-shift workers compared with white participants. In addition, Asian participants tended to be older and to have a lower body mass index, a greater number of pregnancies, lower alcohol consumption, and reduced psychotherapeutic drug use compared with white participants. Descriptive statistics of urinary 6-sulfatoxymelatonin levels by race and shift type at each of the time points are provided in Table 2. Unadjusted and adjusted results from analyses comparing exclusive night-shift workers with exclusive day-shift workers are provided in Table 3. Adjustment for age, hours of darkness, body mass index, number of pregnancies, number of alcoholic beverages consumed, and use of psychotherapeutic medications had a limited impact on the point estimates of interest; nevertheless, we describe the adjusted analyses below. During daytime sleep, white night-shift workers had 60% lower 6-sulfatoxymelatonin levels relative to day-shift workers during nighttime sleep ($P < 0.0001$), whereas Asian night-shift workers had 72% lower 6-sulfatoxymelatonin levels relative to day-shift workers ($P < 0.0001$). This difference between white and Asian participants was statistically significant ($P = 0.04$). When comparing night-shift workers during nighttime sleep on their off nights with day-shift workers during nighttime sleep, white participants had 47% lower 6-sulfatoxymelatonin levels ($P < 0.0001$). Asian participants, however, had only 18% lower 6-sulfatoxymelatonin levels ($P = 0.24$). The difference between white and Asian participants was statistically significant ($P = 0.01$). During night work, white night-shift workers had 72% lower 6-sulfatoxymelatonin levels compared with day-shift workers during nighttime sleep ($P < 0.0001$), but Asian night-shift workers had a significantly lower reduction of 60% ($P < 0.0001$); the difference between whites and Asians was statistically significant ($P = 0.04$). As indicated in Table 4, within the night-shift workers, daytime sleep levels of 6-sulfatoxymelatonin among white participants were 32% lower than levels during nighttime sleep on an off night, whereas daytime sleep levels among Asian participants were further reduced (36%) relative to nighttime sleep levels. The difference between the groups was statistically significant ($P = 0.05$). Although reductions in levels of 6-sulfatoxymelatonin during night work relative to nighttime sleep were statistically significant (white: 50%; Asian: 46%) ($P < 0.0001$ for each), they did not differ between white and Asian participants ($P = 0.44$). Adjustment for covariates had a limited impact on the point estimates of interest for the within-subject analyses of the night-shift workers.

DISCUSSION

The results show that both Asian and white participants engaged exclusively in night-shift work suffer from
constitutively lower melatonin levels. However, compared with white night-shift workers, Asian night-shift workers were able to maintain melatonin at levels that were more comparable to those of 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, Asians suffered less of a disruption in melatonin levels compared with whites. In addition, when switching back to night sleep on an off night, Asian night-shift workers had melatonin levels that were near to those among day-shift workers during their night sleep. This ability to maintain “normal” levels of melatonin under the influence of night-shift work may explain the null results observed in the recent analysis of shift work and breast cancer in a cohort study of Chinese women (11).


<table>
<thead>
<tr>
<th>Participants by Shift Status and Racial Category</th>
<th>6-Sulfatoxymelatonin Level, ng/mg of Creatinine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sleep (Nighttime)</td>
<td>Work (Daytime)</td>
</tr>
<tr>
<td></td>
<td>No. Mean (SD)</td>
<td>No. Mean (SD)</td>
</tr>
<tr>
<td>Day-shift workers</td>
<td>19 29.1 (15.4)</td>
<td>19 7.9 (7.9)</td>
</tr>
<tr>
<td>Asian</td>
<td>White 115 36.2 (18.1)</td>
<td>113 7.9 (6.0)</td>
</tr>
<tr>
<td>Night-shift workers</td>
<td>32 33.5 (24.4)</td>
<td>31 18.5 (13.6)</td>
</tr>
<tr>
<td>Asian</td>
<td>White 110 25.9 (25.8)</td>
<td>109 20.3 (18.1)</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.

Table 3. Results From Regression Analyses of Melatonin Levels, Asian and White Night-Shift Workers Relative to All Day-Shift Workers, Seattle, Washington, 2003–2008

<table>
<thead>
<tr>
<th>Comparison</th>
<th>% Decrease in NSW 6-Sulfatoxymelatonin Levels Relative to DSW Levels*</th>
<th>95% Confidence Intervalb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day sleep (NSW) vs. night sleep (DSW)†</td>
<td>Unadjusted: Asian −73.5*, White −58.3*</td>
<td>Adjusted: Asian −72.5**, White −60.0**</td>
</tr>
<tr>
<td>Night sleep (NSW vs. DSW)†</td>
<td>Asian −15.0, White −46.5</td>
<td>Asian −44.9, 9.2</td>
</tr>
<tr>
<td>Night work (NSW) vs. night sleep (DSW)†</td>
<td>Asian −58.8, White −72.3</td>
<td>Night work (NSW) vs. night sleep (NSW)†</td>
</tr>
<tr>
<td>Asian</td>
<td>−59.6**, −72.8, −46.4</td>
<td>Asian −44.1, −46.5**</td>
</tr>
<tr>
<td>White</td>
<td>−71.6**, −77.8, −65.4</td>
<td>White −50.3, −50.1**</td>
</tr>
</tbody>
</table>

Abbreviations: DSW, day-shift worker; NSW, night-shift worker.
* P < 0.05 (2-sided t test for difference between racial categories of percent decrease in 6-sulfatoxymelatonin levels); **P < 0.0001 (2-sided t test for percent decrease in 6-sulfatoxymelatonin levels, relative to DSW levels).
† Analyzed by using the natural log transformation.
‡ Adjusted for the effects of age, hours of darkness, body mass index, number of pregnancies, number of alcoholic beverages consumed, and use of psychotherapeutics.

Table 4. Results From Regression Analyses of Melatonin Levels, Within Asian and White Night-Shift Workers, Seattle, Washington, 2003–2008

<table>
<thead>
<tr>
<th>Comparison</th>
<th>% Decrease in 6-Sulfatoxymelatonin Levels*</th>
<th>95% Confidence Intervalb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day sleep (NSW) vs. night sleep (NSW)†</td>
<td>Unadjusted: Asian −55.0, White −29.9</td>
<td>Adjusted: Asian −56.2**, White −31.6**</td>
</tr>
<tr>
<td>Night sleep (NSW vs. DSW)†</td>
<td>Asian −15.0, White −46.5</td>
<td>Night work (NSW) vs. night sleep (NSW)†</td>
</tr>
<tr>
<td>Asian</td>
<td>−59.6**, −72.8, −46.4</td>
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<td>White −50.3, −50.1**</td>
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</table>

Abbreviation: NSW, night-shift worker.
* P < 0.05 (2-sided t test for difference between racial categories of percent decrease in 6-sulfatoxymelatonin levels); **P < 0.0001 (2-sided t test for percent decrease in 6-sulfatoxymelatonin levels, relative to NSW night sleep levels).
† Analyzed by using the natural log transformation.
‡ Adjusted for the effects of age, hours of darkness, body mass index, number of pregnancies, number of alcoholic beverages consumed, and use of psychotherapeutics.

whites relative to Asians could offset any biologically detrimental effects of reduced levels of melatonin with respect to cancer risk. However, Hrushesky and Blask argued that the “circadian temporal organization of melatonin availability” is more likely to be associated with cancer risk, rather than the amount of melatonin secreted over a 24-hour period (18, p. 888), and evidence suggests that they may be correct. Three studies observed decreased risks of breast cancer with increasing concentrations of 6-sulfatoxymelatonin in morning postsleep urine samples (19–21), whereas the one study that examined 24-hour 6-sulfatoxymelatonin levels observed no association with breast cancer risk (22). In conjunction with these findings, our results suggest that Asian night-shift workers may incur less suppression of melatonin and, as a result, reduced risk of cancer.

A limitation of this study is a lack of sufficient numbers in other racial categories, which would have allowed us to evaluate the impact of other racial backgrounds on melatonin levels. Even with the limited number of Asian participants, however, we were able to observe statistically significant differences in 6-sulfatoxymelatonin concentrations. Another limitation of this study is the lack of data to determine what aspect of race is involved in the observed effects, because race is considered a proxy for cultural and/or genetic differences. Although race is often a proxy for socioeconomic status, this was not the case in our study where all participants were health-care workers of similar socioeconomic status. Genetic differences in the core circadian regulatory genes may be an important consideration, for example, in a recent study, the evening rise in melatonin was found to be significantly suppressed by blue-enriched polychromatic light at 6,500 K among individuals homozygous for the longer allele of a variable number tandem repeat polymorphism in the period circadian clock 3 gene (PER3) but not among those that were homozygous for the shorter allele (23). Interestingly, the shorter allele has been found to occur at a greater frequency among Asians than Caucasians (24), which may potentially explain our findings. Finally, this study did not evaluate health effects, specifically cancer, in association with the different reductions in 6-sulfatoxymelatonin levels. Thus, we cannot address specifically whether racial differences in melatonin levels in response to night-shift work are responsible for the differing results reported regarding night-shift work and breast cancer risk between Western and Asian populations. 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, future studies may consider evaluating biomarkers of risk (e.g., DNA damage). Despite these limitations, this is the first study to evaluate the impact of race on differences in melatonin levels between day-shift and night-shift workers. A major strength of this study was the collection of multiple urine samples at critical time points throughout a shift worker’s typical work day and subsequent sleep, allowing for a comprehensive evaluation of the effect of race on melatonin levels.

The results of this study provide evidence that, with respect to disrupted melatonin levels, Asians may be protected from the negative effects of shift work relative to whites. However, this evidence needs to be examined more extensively in future studies, with detailed genetic, demographic, and lifestyle information to investigate what aspects of race are responsible for the observed differences in melatonin disruption associated with night-shift work. Examination of other biomarkers in addition to 6-sulfatoxymelatonin would also be useful in this endeavor. Collectively, such studies will broaden our understanding of the differences in cancer risk with night-shift work observed between populations thus far.

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REFERENCES


