Light Exposure at Night, Urinary 6-Sulfatoxymelatonin, and Serum Estrogens and Androgens in Postmenopausal Japanese Women

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Abstract

It has been hypothesized that exposure to light at night increases the risk of breast cancer by suppressing the normal nocturnal increase in melatonin production and release, thereby resulting in increased levels of circulating estrogen. We assessed associations among concentrations of serum estrogen and androgen and the principal metabolite of melatonin in urine, 6-sulfatoxymelatonin, and exposure to light at night based on information regarding the sleeping habits and history of graveyard-shift work of 206 postmenopausal Japanese women. Serum estradiol level was significantly higher in women who were not asleep at or after 1:00 a.m. (the approximate time of the melatonin peak) than those who were asleep after controlling for covariates. Significantly increased estrone levels were observed in women who had worked graveyard shift. Serum testosterone and DHEA sulfate were unrelated to sleeping habits and history of graveyard-shift work. Urinary 6-sulfatoxymelatonin was lower in women who were not asleep at or after 1:00 a.m. on weekends than those who were asleep at this time, but the difference was of borderline significance (P = 0.08). There was no significant association between urinary 6-sulfatoxymelatonin and any serum hormone levels. These data suggest that exposure to light at night has implications for the risk of breast cancer in postmenopausal women. However, the potential role of melatonin as an intervening factor between light exposure at night and the serum concentrations of estrogen was equivocal. (Cancer Epidemiol Biomarkers Prev 2008;17(6):1418–23)

Introduction

Endogenous hormones, especially estrogens, are important determinants of breast cancer (1). It has been hypothesized that exposure to light at night increases the risk for breast cancer by suppressing the normal nocturnal increase in melatonin production and release, thereby resulting in increased levels of circulating estrogen (2). Late-night-shift work, used as a surrogate for exposure to light at night, has been associated with a risk for breast cancer in some epidemiologic studies (3).

It is well known that acute light exposure induces the suppression of melatonin (4). However, the long-term effect of exposure to light at night on melatonin levels has not been well investigated. Studies on associations between endogenous estrogen and melatonin levels are scarce. Few studies have examined the interrelationships of light-at-night exposure with melatonin and estrogen levels. Schernhammer et al. (5) assessed cross-sectional relationships of urinary melatonin to night work and steroid hormone levels in premenopausal women. They observed that the number of nights worked within the previous 2 weeks was significantly inversely associated with the urinary level of the principal metabolite of melatonin, 6-sulfatoxymelatonin, but not with the plasma estradiol level. On the other hand, the plasma estradiol level, but not the urinary 6-sulfatoxymelatonin level, was significantly positively associated with the duration of night work.

Besides estrogens, androgens have been associated with a risk for breast cancer in postmenopausal women (6). In the present study, we assessed the associations among concentrations of serum estrogens and androgens and urinary 6-sulfatoxymelatonin and exposure to light at night based on information regarding the sleeping habits and history of night-shift work of postmenopausal Japanese women. Low estrogen levels have been reported in populations at low risk for developing breast cancer such as Japanese women (7, 8). Measurements using routine RIA techniques have failed to detect estradiol among postmenopausal Japanese women (9, 10). From a case-control study of breast cancer in relation to serum hormone levels, 19 (26%) of 73 controls had estradiol levels lower than the detection limit (1.5 pg/mL) of the assay (9). We previously observed that 160 (49.4%) of 324 healthy postmenopausal Japanese women who attended a breast cancer mass screening had estradiol levels lower than the detection limit (1.4 pg/mL; ref. 10). In the present study, we were able...
to measure estrogen levels with a sensitive and reliable method using liquid chromatography–electrospray ionization tandem mass spectrometry. Cook et al. (11) reported that the level of urinary 6-sulfatoxymelatonin in morning urine was strongly correlated with the total nocturnal plasma melatonin output and the peak nocturnal melatonin value. High reproducibility of the measurement of morning urinary 6-sulfatoxymelatonin at a 5-year interval (intraclass r = 0.56; ref. 12) has been reported.

Materials and Methods

Between 2000 and 2002, women attending a breast cancer screening at a general hospital in Gifu, Japan, were recruited for a study of mammographic breast density. The hospital has been conducting a mass screening campaign for breast cancer since the early 1980s. Municipal letters inviting to the screening were mailed to women residing in the surrounding areas. Details of the study have been described elsewhere (13). The main purpose of the study was to identify the determinants of mammographic breast density. A total of 1,072 women attended the study. Another objective of the study was to examine the associations of various biomarkers with breast cancer risk factors using subsets of this population. The study period for each component study was predetermined. The present study included subpopulations in a component study conducted between June and December 2000 that included blood and morning urine collection (14). A total of 432 women who were free of breast cancer agreed to participate in the present study (response rate was 68.5%). Informed consent was obtained from each woman. The study was approved by the ethical board of the Gifu University School of Medicine.

Each woman responded to a self-administered questionnaire that was administered on the day of her visit to the screening and was designed to collect basic demographic characteristics and information regarding smoking and drinking habits, physical activity, medical history, and reproductive history. The blood sample was obtained at approximately 8:00 a.m. The urine samples taken on the next morning were returned by overnight mail with a frozen water bottle. A previous study reported that overnight shipping is acceptable for the measurement of 6-sulfatoxymelatonin (5). The means of age, alcohol intake, age at first birth, and age at menopause of these additional 49 women were similar to those of the other women. However, they were more likely to be current smokers (8.2% versus 1.3%) and to have more years of education (12.6 versus 11.0 years) and a smaller body mass index (BMI; 22.2 versus 23.5 kg/m²). The results of separately conducted analyses for the two groups were similar (see Results), and both groups were analyzed together. Thus, 206 women were included for the present analysis after excluding one woman with an estradiol level suggesting unreported estrogen use (>100 pg/mL). Of these, serum hormones and urinary 6-sulfatoxymelatonin were not measured in 9 and 12 women, respectively, because of insufficient volume. The analyses regarding sleeping habits, history of graveyard-shift work, and serum hormone or urinary 6-sulfatoxymelatonin were restricted to those women for whom such data were available (the numbers of subjects are shown in the tables).

Serum estrone, estradiol, and testosterone were measured by liquid chromatography–electrospray ionization tandem mass spectrometry method (16, 17) using reagents purchased from NIHs (estrone and estradiol) and Sigma-Aldrich (testosterone) as internal standards, 13C4-estradiol and 13C-estrone (Hayashi Pure Chemical), and 2H3-testosterone (Sigma-Aldrich). The sensitivities were 0.5 pg/mL for estrone and estradiol and 1.0 pg/mL for testosterone. The interassay coefficients of variation were less than 7.3% for estrone, less than 7.6% for estradiol, and less than 12.9% for testosterone. DHEA sulfate was measured by RIA using kits purchased from Diagnostic Product Corporation (Mitsubishi Chemical Medicine). The sensitivity was 1 µg/dL, and the sleeping (from level 1, the subject wore a mask to keep out light, to level 6, she could read comfortably). If women reported that they had ever worked the graveyard shift (11:00 p.m.-5:00 a.m.), the dates worked were also asked. One woman moved and was unreachable by mail. A total of 325 women (75.2%) returned the questionnaire. Thus, the percentage of women who responded to this additional questionnaire was 51.5% of the original target population.

The study was restricted to postmenopausal women who were not using hormone replacement therapy and were free of cancer, diabetes mellitus, chronic hepatitis, and thyroid disease. Women who had been without a menstrual cycle in the past 12 months were classified as postmenopausal. Women were excluded if they were 49 years old or younger and had had surgical menopause with ovarian conservation. We included women who had not responded to the second questionnaire sent ~3 years after the blood and urine sampling. Of 432 women, 159 fit the criteria. Of them, we excluded one who did not provide information on body height and weight. We added 49 postmenopausal women who participated in the study at another institute to increase the sample size. For these women, the same questionnaire was used to obtain information regarding exposure to light at night at the time of blood and urine sampling. Their blood samples were obtained at approximately 8:00 a.m. The urine samples taken on the next morning were returned by overnight mail with a frozen water bottle.

The blood and urine samples were frozen and stored at −80°C until assayed. Information on exposure to light at night was obtained in 2003, ~3 years after the blood and urine sampling. Each participant was sent a letter asking for a response to a mail questionnaire regarding sleeping habits and history of night-shift work. A reminding letter was sent to those who had not returned the questionnaire to us. While developing the questionnaire, we referred to the questions used in a study on light at night and breast cancer risk reported by Davis et al. (15). Questions regarding the time that a subject usually turned off the lights before going to sleep and their wake-up times on weekdays and weekends were included. Subjects were asked to answer questions regarding their habits around the time when the blood and urine samples were collected. Six response categories adopted from the study by Davis et al. (15) were provided for the question asking regarding the ambient light level in the bedroom while sleeping (from level 1, the subject wore a mask to keep out light, to level 6, she could read comfortably). If women reported that they had ever worked the graveyard shift (11:00 p.m.-5:00 a.m.), the dates worked were also asked. One woman moved and was unreachable by mail. A total of 325 women (75.2%) returned the questionnaire. Thus, the percentage of women who responded to this additional questionnaire was 51.5% of the original target population.

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### Table 1. Basic characteristics and concentrations of serum hormones and urinary metabolite of melatonin in 206 postmenopausal Japanese women

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Alcohol intake (mL/d)</th>
<th>Age at menopause (y)</th>
<th>Current smokers (%)</th>
<th>Ex-smokers (%)</th>
<th>Serum estrone (pg/mL)</th>
<th>Serum estradiol (pg/mL)</th>
<th>Serum testosterone (ng/dL)</th>
<th>Serum DHEAS (ng/mL)</th>
<th>Urinary aMT6-s (ng/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>56.9 ± 5.8</td>
<td>23.2 ± 2.9</td>
<td>5.0 ± 12.3</td>
<td>49.0 ± 4.2</td>
<td>3.0</td>
<td>4.4</td>
<td>11.0 (10.3-11.9)</td>
<td>2.1 (1.9-2.4)</td>
<td>11.3 (10.6-12.1)</td>
<td>897 (825-975)</td>
<td>30.8 (28.0-33.9)</td>
</tr>
</tbody>
</table>

NOTE: Values are mean ± SD or means (95% confidence interval). Abbreviations: DHEAS; DHEA sulfate; aMT6-s, 6-sulfatoxymelatonin.

interassay coefficient of variation was less than 15%. All samples were analyzed at the Teikoku Hormone Medical Research Center Co. Ltd. All the coefficients of variation were laboratory-reported values.

Urinary 6-sulfatoxymelatonin was measured radio-immunologically using kits purchased from the IBL Laboratories. The measurement of 6-sulfatoxymelatonin but not serum hormones was conducted later and separately by the same laboratory (SRL Inc.) for the additional 49 women; the laboratory-reported interassay coefficients of variation were less than 15.3% and 11.3%, respectively. To adjust for variation in the diluteness of urine, urinary 6-sulfatoxymelatonin levels were expressed as urine 6-sulfatoxymelatonin per urine creatinine. Urinary creatinine was measured by conventional enzymatic method. The laboratory-reported interassay coefficient of variation was less than 1.2%.

All hormone and urinary 6-sulfatoxymelatonin levels were transformed into logarithmic values for statistical analysis. The interrelationships among serum hormone and urinary 6-sulfatoxymelatonin levels and variables related to light-at-night exposure were assessed by linear regression models. The geometric means of hormone levels according to the categorized urinary 6-sulfatoxymelatonin level or exposure to light at night were provided using analysis of covariate models. A linear trend was assessed using continuous variables. As the melatonin profile reaches peak values between 1:00 and 5:00 a.m. (18), women were categorized into two groups, asleep and awake at or after 1:00 a.m., using the information on sleep habits. Age, BMI, smoking status, alcohol intake, group of subjects (additional subjects or not), and day length (the number of hours of daylight between dawn and dusk) on the day previous to the urine collection were included as covariates into the models. Smoking status was missing for three women. The three were categorized into one group in addition to those of current and former smokers. All statistical analyses were done using SAS (SAS Institute).

### Results

The characteristics of 206 women are shown in Table 1. Tables 2 and 3 show the geometric means of serum hormones and urinary 6-sulfatoxymelatonin according to variables related to light exposure at night. Serum estradiol was 113% and 70% higher in women who were not asleep at or after 1:00 a.m. on weekdays and weekends, respectively, than in those who were asleep at that time after controlling for covariates (Table 2). Urinary 6-sulfatoxymelatonin were 26.3% lower in women who were not asleep at or after 1:00 a.m. on weekends than in those who were asleep at this time, but the difference was of borderline significance ($P = 0.08$). More than 80% of the respondents reported that the hours of rising and of turning off the light to go to bed had not changed at least during 5 years before blood and urine sampling either on weekdays or weekends. No changes in the practices during 10 years before the sampling were reported in ~50% of the respondents. Even the exclusion of those who had changed their sleeping habits within 5 years before the sampling did not alter the results.

Seven women in the study had worked the graveyard shift. Only one of them was working the graveyard shift at the time of blood and urine sampling and reported that she had not been asleep at or after 1:00 a.m. on weekdays. The remaining six women reported that they had been asleep at 1:00 a.m. at the time of the sampling. The serum estrone level was significantly higher among women who had worked the graveyard shift than that among women who had not (Table 3). A nearly 2-fold increase in the estradiol level was observed among women who had worked the graveyard shift, but this association was not statistically significant. Years of graveyard-shift work were significantly positively associated with the serum estrone level ($P = 0.03$), and working the graveyard shift within 3 years before the study was significantly associated with increased serum levels of estrone (45.3 pg/mL) and estradiol (15.1 pg/mL). Urinary 6-sulfatoxymelatonin level was unrelated to history of graveyard shift. None of the serum hormones or urinary 6-sulfatoxymelatonin was not adjusted for age, BMI, smoking status, alcohol intake, group of subjects, and day length of the day previous to urine collection.

### Table 2. Geometric means of serum hormone and urinary 6-sulfatoxymelatonin levels according to being awake or asleep at or after 1:00 a.m. on weekdays and weekends

<table>
<thead>
<tr>
<th></th>
<th>Weeksdays</th>
<th></th>
<th>P</th>
<th></th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asleep (n = 168)</td>
<td>Awake (n = 12)</td>
<td></td>
<td></td>
<td>Asleep (n = 159)</td>
<td>Awake (n = 16)</td>
<td></td>
</tr>
<tr>
<td>Estrone (pg/mL)</td>
<td>11.4 (10.5-12.5)</td>
<td>14.1 (10.7-18.5)</td>
<td>0.17</td>
<td>11.5 (10.6-12.5)</td>
<td>13.4 (10.4-17.3)</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>2.2 (1.9-2.5)</td>
<td>4.7 (3.0-7.5)</td>
<td>0.003</td>
<td>2.2 (1.9-2.6)</td>
<td>3.7 (2.4-5.8)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>11.8 (10.9-12.8)</td>
<td>12.5 (9.6-16.3)</td>
<td>0.68</td>
<td>11.9 (10.9-12.9)</td>
<td>12.2 (9.6-15.6)</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>DHEAS (ng/mL)</td>
<td>939 (846-1,043)</td>
<td>942 (669-1,327)</td>
<td>0.99</td>
<td>932 (839-1,036)</td>
<td>979 (713-1,345)</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>aMT6-s (ng/mg creatinine)</td>
<td>30.5 (27.2-34.2)</td>
<td>27.3 (18.4-40.5)</td>
<td>0.60</td>
<td>31.8 (28.4-35.6)</td>
<td>23.3 (16.8-32.3)</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age, BMI, smoking status, alcohol intake, group of subjects, and day length of the day previous to urine collection.
significantly associated with the ambient light level in bedroom (data not shown).

Table 4 presents the geometric means of hormone levels according to the tertile of urinary 6-sulfatoxymelatonin levels. None of the hormones measured was associated with urinary 6-sulfatoxymelatonin level.

Additional adjustment for other potential breast risk factors such as parity, age at menarche, years of education, age at first birth, and age at menopause did not substantially alter the results. When we reanalyzed data separately for the 157 and additional 49 participants, the results were similar except that the associations between urinary 6-sulfatoxymelatonin and sleep habits were somewhat strengthened in the first group. In the first group, the mean estradiol levels were 2.0 and 4.0 pg/mL in women who were asleep and awake, respectively, at or after 1:00 a.m. on weekdays (P = 0.06). The corresponding values for weekends were 1.9 and 6.9 pg/mL, respectively (P < 0.01). The means of urinary 6-sulfatoxymelatonin were 31.1 and 13.5 ng/mg creatinine in women who were asleep and awake at or after 1:00 a.m. on weekdays, respectively (P = 0.02). The corresponding values for weekends were 31.8 and 21.0 ng/mg creatinine, respectively (P = 0.009). The means of estrone were 10.3 and 15.7 pg/mL for those who had not worked the graveyard-shift work and for those who had, respectively (P = 0.08). In the additional 49 participants, the means of estradiol were 2.4 and 5.6 pg/mL in women who were asleep and awake, respectively, at or after 1:00 a.m. on weekdays. Despite the small sample size, this difference was statistically significant (P = 0.02). The corresponding values for weekends were 2.6 and 3.0 pg/mL, respectively. The means of urinary 6-sulfatoxymelatonin were 30.0 and 36.9 ng/mg creatinine in women who were asleep and awake, respectively, at or after 1:00 a.m. on weekdays. The corresponding values for weekends were 32.8 and 25.6 ng/mg creatinine, respectively. Because only one woman had worked the graveyard shift before, the associations between history of graveyard shift work and urinary melatonin and hormone levels were not assessed.

We obtained information on the use of diuretics and b-blockers, which can affect urinary 6-sulfatoxymelatonin levels, from the additional 49 women. However, we obtained only information of the medical history of hypertension for the rest of the women. When we reanalyzed data after excluding four women using these medications and 39 women with a diagnosis of hypertension, the association between serum estrogen levels and sleeping habits were somewhat strengthened; the mean estradiol levels were 2.2 and 5.6 pg/mL for women who were asleep and awake, respectively, at or after 1:00 a.m. on weekdays (P < 0.001). The corresponding values for weekends were 2.2 and 4.9 pg/mL, respectively. The association between serum estrone and sleeping habits was of borderline significance after controlling for covariates; the mean estrone levels were 11.2 and 15.3 pg/mL for women who were asleep and awake, respectively, at or after 1:00 a.m. on weekends (P = 0.06). The corresponding values for weekends were 11.2 and 15.0 pg/mL (P = 0.07), respectively. Urinary 6-sulfatoxymelatonin was unrelated to sleep habits and graveyard-shift work; the mean 6-sulfatoxymelatonin levels were 33.3 and 30.5 ng/mg creatinine in women who were asleep and awake, respectively, at or after 1:00 a.m. on weekends (P = 0.65). The mean 6-sulfatoxymelatonin levels were 31.7 and 33.8 ng/mg creatinine for those who had not worked the graveyard-shift work and for those who had, respectively (P = 0.86).

The exclusion of women who had worked the graveyard shift did not alter the results; the mean estradiol levels were 2.2 and 3.6 pg/mL for women who were asleep and awake, respectively, at or after 1:00 a.m. on weekends (P = 0.04). The corresponding values for weekends were 2.1 and 3.8 pg/mL, respectively (P = 0.009). The mean urinary 6-sulfatoxymelatonin levels were 31.8 and 22.1 ng/mg creatinine for women who were asleep and awake, respectively, at or after 1:00 a.m. on weekends (P = 0.06). The exclusion of current and ex-smokers also did not essentially alter the results; the mean estradiol levels were 2.2 and 5.2 pg/mL for women who were asleep and awake, respectively, at or after 1:00 a.m. on weekends (P = 0.004). The corresponding values for weekends were 2.2 and 4.2 pg/mL, respectively (P = 0.01). The mean urinary aMT-6 levels were 31.3 and 25.5 ng/mg creatinine in women who were asleep and awake, respectively, at or after 1:00 a.m. on weekends (P = 0.26). The mean estrone levels were 11.2 and 20.3 pg/mL for those who had not worked

### Table 3. Geometric means of serum hormone and urinary 6-sulfatoxymelatonin levels according to graveyard-shift work

<table>
<thead>
<tr>
<th>Ever worked graveyard</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (n = 170)</td>
<td></td>
</tr>
<tr>
<td>Estrone (pg/mL)</td>
<td>11.5 (10.6-12.5)</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>2.3 (2.0-2.7)</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>11.8 (10.9-12.7)</td>
</tr>
<tr>
<td>DHEAS (ng/mL)</td>
<td>925 (836-1,024)</td>
</tr>
<tr>
<td>aMT6s (ng/mg creatinine)</td>
<td>30.0 (26.9-33.5)</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age, BMI, smoking status, alcohol intake, group of subjects, and day length of the day previous to urine collection.

### Table 4. Geometric means of serum hormone levels according to tertile of urinary 6-sulfatoxymelatonin

<table>
<thead>
<tr>
<th>Urinary aMT6-s tertile</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone (pg/mL)</td>
<td>11.8 (10.3-13.4)</td>
<td>10.9 (9.6-12.3)</td>
<td>12.7 (11.1-14.6)</td>
<td>0.79</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>2.4 (1.9-3.0)</td>
<td>1.9 (1.5-2.3)</td>
<td>3.0 (2.4-3.7)</td>
<td>0.48</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>11.3 (9.9-12.7)</td>
<td>11.7 (10.4-13.2)</td>
<td>12.3 (10.8-14.0)</td>
<td>0.22</td>
</tr>
<tr>
<td>DHEAS (ng/mL)</td>
<td>991 (848-1,160)</td>
<td>973 (835-1,135)</td>
<td>877 (743-1,034)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age, BMI, smoking status, alcohol intake, and day length of the day previous to urine collection.
the graveyard-shift work and for those who had, respectively \( P = 0.006 \).

**Discussion**

Serum estradiol level was significantly increased in women who were not asleep at or after 1:00 a.m. as compared with those who were asleep. Serum estrone level was significantly higher among women with a history of the graveyard shift. In addition, there was a suggestion of decreased urinary 6-sulfatoxymelatonin level in women who were not asleep at or after 1:00 a.m. on weekdays. However, the implication of melatonin as a link between light exposure at night and estrogen was not evident because we failed to find direct associations between urinary 6-sulfatoxymelatonin and serum estrogen levels.

To our knowledge, two studies have evaluated the interrelationships between estrogen, melatonin, and exposure to light at night in women. Using an experimental study design, Graham et al. (19) observed the delay of the peak of the nocturnal melatonin level after acute exposure to bright light (for two nights) in premenopausal women. However, there was no change in plasma estradiol levels after the exposure. They also noted that there was no association between the total amount of melatonin excretion and plasma estradiol levels under the nonexposed control condition. Schernhammer et al. (5) examined the cross-sectional relationships of shift work, urinary 6-sulfatoxymelatonin, and plasma hormone levels in premenopausal women who participated in the Nurses’ Health Study. In their study, serum estrogen and urinary 6-sulfatoxymelatonin levels were associated with some variables (not identical) related to a history of shift work. However, they failed to find a significant association between serum estrogen and urinary 6-sulfatoxymelatonin after controlling for age and BMI. Their findings were not contradictory with our results. The cross-sectional relations between serum estrogen and urinary melatonin levels may not fully reflect the long-term effects of exposure to light at night on estrogen and melatonin.

The effect of melatonin administration on estrogen levels among postmenopausal women has been reported in two experimental studies. Serum estradiol level was decreased in one study (20) but not in another study (21) after 6 months of melatonin treatment with comparable doses.

Few studies have addressed the effect of chronic exposure to light at night on melatonin levels among normal women. Davis et al. (22) reported that neither the ambient nighttime light nor the number of times a light was turned on at night was associated with the urinary 6-sulfatoxymelatonin levels in 203 women living in Washington State. Levallois et al. (23) reported no association of nocturnal bedroom light exposure and light use at night with urinary 6-sulfatoxymelatonin in 416 Canadian women. We also did not observe a significant association between urinary 6-sulfatoxymelatonin and the ambient light level in a bedroom. However, our results suggest that the habit of being awake around the time of the melatonin peak may be associated with a decreased urinary 6-sulfatoxymelatonin level. Hansen et al. (24) found lower levels of 6-sulfatoxymelatonin in the urine from nurses during a workday on the night shift than in that of those working a fixed schedule on the day shift. However, Borugian et al. (25) reported that rotating-shift workers have a tendency to show increased melatonin levels upon arising and during work.

The average 6-sulfatoxymelatonin level in morning spot urine in this study population seems to be higher than those reported among other populations. For example, the mean of the 6-sulfatoxymelatonin levels was 19.3 ng/mg creatinine in 203 women living in Washington State who were 20 to 74 years old (22). Similar values have been reported in 195 Canadian women in the same age group (18.4 ng/mg creatinine; ref. 23), in 40 postmenopausal Dutch women (13.4 ng/mg creatinine; ref. 12), and in 459 primarily premenopausal women from the Nurses’ Health Study (19.6 ng/mg creatinine; ref. 26). To our knowledge, the highest value reported was 35.2 ng/mg creatinine in U.S. women who were 20 to 45 years old (27). Although a low BMI may partially contribute to high levels of 6-sulfatoxymelatonin among the Japanese population, the endogenous melatonin level might vary across ethnic groups. This possibility is of interest in the context of the potential involvement of melatonin in longevity.

We should consider several limitations of the present study. With few subjects who had worked the graveyard shift, we were unable to assess the effects of duration and the time frame engaged in the shift work separately. The number of women who were not asleep at or after 1:00 a.m. was also small.

The low response rate may have affected the results. It is unlikely that hormone or urinary melatonin levels are directly associated with participation in this study. However, melatonin has been implicated in sleep quality (28). If women who were not asleep at or after 1:00 a.m. were more likely to have participated in the present study when they felt a low quality of sleep due to low melatonin level than those who were asleep at the time, our results on sleep habits and urinary 6-sulfatoxymelatonin level might have arisen through selection bias.

Data regarding sleeping habits and other surrogates for light exposure at night were obtained ~3 years after the blood and urine sampling with the exception of the 49 subjects who were added later. It should be especially difficult to recall the sleeping habits. Although many of the participants reported that their sleeping habits had been stable, this recollection must have resulted in a measurement error.

Blood samples for estrogen and androgen measurements were obtained at an almost fixed time (8:00 a.m. or 2:00 p.m.). These hormones did not show great circadian variations but tended to be high in the morning (29, 30). The time of blood sampling may have been critical to find meaningful correlations between first-void morning 6-sulfatoxymelatonin and hormone levels.

In conclusion, using the bedtime hour and history of graveyard-shift work as surrogates of exposure to light at night created a significant association with serum estrogen levels. We failed to find evidence of melatonin as an intervening factor between light exposure at night and serum concentrations of estrone, estradiol, testosterone, and DHEA sulfate. These data suggest that exposure to light at night has implications for the risk for breast cancer in postmenopausal women. It seems important to encourage further studies that use strong assessment
methods for measuring exposure to light at night, include appropriate adjustment, and are large enough to detect associations.

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Light Exposure at Night, Urinary 6-Sulfatoxymelatonin, and Serum Estrogens and Androgens in Postmenopausal Japanese Women

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