Epidemiology of Urinary Melatonin in Women and Its Relation to Other Hormones and Night Work

Eva S. Schernhammer,[1,2,6] Bernard Rosner,[1,3] Walter C. Willett,[1,2,4] Francine Laden,[1,2,5] Graham A. Colditz,[1,2,7,8] and Susan E. Hankinson[1,2]

[1]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; Departments of [2]Epidemiology, [3]Biostatistics, [4]Nutrition, and [5]Environmental Health, Harvard School of Public Health, Boston, Massachusetts; [6]Ludwig Boltzmann-Institute for Applied Cancer Research, KFJ-Spital, Vienna, Austria; [7]Epidemiology Program, Dana Farber/Harvard Cancer Center, Boston, Massachusetts; and [8]Harvard Center for Cancer Prevention, Boston, Massachusetts

Abstract

Objective: Light exposure during night work suppresses melatonin production, and night work has been associated with an increased cancer risk. There is little information, however, about the interrelationships of night work, urinary melatonin levels, and levels of plasma steroid hormones in women. Method: We examined the reproducibility of morning urinary measurements of 6-sulfatoxymelatonin over a 3-year period in 80 premenopausal women. We assessed correlations between average urinary melatonin and plasma steroid hormone levels and evaluated potential associations between night work and hormone levels, using current and long-term shift work information from two large, prospective cohorts, the Nurses' Health Study cohorts. Results: The intraclass correlation for creatinine-adjusted 6-sulfatoxymelatonin was 0.72 (95% confidence interval, 0.65, 0.82). We found significantly increased levels of estradiol after longer durations of night work (geometric mean levels of estradiol, 8.8 pg/mL for women who never worked night shifts versus 10.1 pg/mL for women who worked 15 or more years of night shifts; P for trend = 0.03). We observed a significant inverse association between increasing number of nights worked within the 2 weeks preceding urine collection and urinary melatonin levels ($r = -0.30$, $P = 0.008$), but no association of recent night work with estradiol ($r = 0.10$, $P = 0.41$). Conclusion: A single morning urinary melatonin measurement is a reasonable marker for long-term melatonin levels among premenopausal women. Women who work on rotating night shifts seem to experience changes in hormone levels that may be associated with the increased cancer risk observed among night-shift workers. (Cancer Epidemiol Biomarkers Prev 2004;13(6):936–43)

Introduction

More than 35 years ago, it was hypothesized that light at night can alter a woman's risk of breast cancer through the melatonin pathway (1). The physiologic production of melatonin, the ''hormone of the dark,'' follows a circadian rhythm that is heavily determined by day/night light exposure, with a peak of production in the middle of the night and relatively low circulating levels during the day (2). For a healthy, young adult, daytime melatonin values lie around 10 ng/mL, whereas they peak at nighttime to average values of 60 ng/mL (3-5). Melatonin has gained considerable attention for its oncostatic properties that may link changes in the level of the hormone with cancer risk (6). Exposure to light at night, such as frequently encountered by night workers, most powerfully suppresses nightly melatonin production and has been associated with an increased cancer risk in several observational studies (7-10). Stevens hypothesized that exposure to light at night not only suppresses melatonin production, which was confirmed in laboratory settings (11), but is also paralleled by an increase in estrogen levels (12), which in turn may induce a higher breast cancer risk among women who are frequently exposed to light at night. However, to date, few efforts have been made to clarify pathways, including variations of steroid hormone levels associated with melatonin levels or night work.

Serum melatonin has a very short half-life and is rapidly metabolized, mainly in the liver. Measurements of the principal metabolite of melatonin, 6-sulfatoxymelatonin (aMT6-s), which is excreted in urine, reflect pineal function; various studies have shown a high correlation between measurements of aMT6-s in urine and plasma (13-18). Both morning urinary melatonin and aMT6-s account for approximately 70% of the total plasma melatonin measured from the previous night (19). In particular, peak nocturnal levels of plasma melatonin have been significantly related to morning levels of urinary melatonin (19). Thus, even though morning urinary aMT6-s measurements lose the temporal pattern of nighttime circulating melatonin levels, these measurements are still a reasonably good indicator of nocturnal plasma melatonin, especially in most epidemiologic applications. Large epidemiologic studies usually collect only one blood and/or urine sample per participant for reasons of cost and logistics. However, long-term hormone levels are often of greatest interest, particularly

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Requests for reprints: Eva S. Schernhammer, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115. Phone: (617) 525-4648; Fax: (617) 525-2008. E-mail: eva.schernhammer@channing.harvard.edu

in etiologic studies of cancer with long latency periods. It is, therefore, important to assess whether a single measure accurately reflects levels over extended periods for a given individual. Previous studies have evaluated reproducibility of aMT6-s either over short periods (9, 20, 21) or among postmenopausal women only (22), and all of them reported reasonable reliability of aMT6-s measurements over time [from day to day, $r = 0.85$-0.92 (20, 21), over a period of 6 months, $r = 0.75$ (23), and over 5.1 years, postmenopausal women only, $r = 0.56$ (22)].

In this report, we assess whether shipping urine samples by overnight courier is feasible for the assay of aMT6-s in epidemiologic research. We further evaluate the reproducibility of morning urinary measurements of aMT6-s melatonin over a 3-year period in premenopausal women. Finally, we examine the interrelationships of shift work, urinary melatonin, and plasma steroid hormone levels in women.

Materials and Methods

Study Cohorts

Nurses' Health Study. In 1976, the Nurses Health Study (NHS) was established, enrolling 121,700 female registered U.S. nurses to assess risk factors for cardiovascular disease and cancer (24). Since then, the women have been followed biennially by a mailed questionnaire. In 1988, study participants were asked how many years in total they had worked rotating night shifts for at least three nights per month in addition to days or evenings in that month (7). Between 1989 and 1990, blood samples were collected and archived from 32,826 women participating in the NHS. The effect of transport condition on steroid hormones in blood (25) and the reproducibility of plasma hormone levels over several years have been reported (26). To assess the relationship between plasma steroid levels and shiftwork, we used controls from a nested case-control study of breast cancer (27). Six hundred sixty-three of these control women (who at blood were free of cancer, postmenopausal, and had not used hormone replacement therapy within 3 months of blood draw) had answered the 1988 question on lifetime years having worked rotating night shifts and had several endogenous hormone levels measured as part of the previous study of endogenous steroid hormones and breast cancer risk (27). Further details of the NHS are described elsewhere (24, 28).

Nurses' Health Study II. The Nurses' Health Study II (NHS II) is a prospective cohort study that started in 1989, when 116,671 registered female U.S. nurses ages 25 to 42 were enrolled. Since then, they have been followed biennially by a mailed questionnaire. Further details of the cohort are described elsewhere (29). 19,092 of these women participated in the blood and urine collection study for the NHS II cohort from 1996 to 1999. A total of 113 random participants from this pool of women in the cohort who were (1) premenopausal (i.e., still having menstrual periods); (2) had not used oral contraceptives or other hormonal preparations (e.g., for infertility) in the previous 6 months and had no plans to begin using them; (3) had not been pregnant or lactating in the previous 6 months and were not planning to become pregnant in the next year; and (4) had no history of cancer (except non-melanoma skin cancer) provided three complete sets of luteal urine over a 3-year period—the initial sample collection and two additional samples in 1998 and 1999. For these 113 women, several plasma steroid hormones were measured for an ongoing reproducibility study. To minimize cost while still maintaining sufficient power, we randomly chose 80 of the 113 women for aMT6-s level measurements. Roughly 18% of these 80 women reported having worked at least one night shift within the 2 weeks preceding urine collection.

Both studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Urine Collection. A spot morning urine sample was collected an estimated 7 to 9 days before the anticipated start of the next menstrual cycle (luteal phase) in all three instances, and urine samples were timed (nurses provided information on the onset of their period following urine collection). The samples were then returned by overnight mail, with a frozen water bottle to keep them cool. On arrival in the laboratory, the samples were aliquoted into labeled cryotubes of 4.5 mL volume each. Without the addition of any preservative, all of the tubes were then stored in liquid nitrogen freezers.

Each woman was sent a questionnaire on which to record the dates of collection of the urine sample. In addition, information was gathered on the numbers of nights worked in the 2 weeks before urine collection, the participant's current weight, and other lifestyle variables. Information on more detailed lifestyle factors associated with health, including reproductive history, were available for each participant from the NHS II questionnaires completed in 1997.

We conducted a small pilot study to investigate whether levels of urinary aMT6-s would remain stable when processing was delayed for 24 and 48 hours. Fifteen premenopausal women from the greater Boston area were recruited, and a first morning spot urine sample from the luteal phase of their menstrual cycle was sampled for each. The unpreserved samples were split (to assess laboratory reproducibility) and processed immediately or exactly as described above (which included simulating transport in a cooler with a frozen water bottle). In this pilot, there was significant batch-to-batch variation; batch-wise within-batch coefficients of variation for aMT6-s splits were 10.6%, 15.3%, and 19.0%.

Laboratory Methods

NHS. Hormone fractions of estradiol, estrone, estrone sulfate, testosterone, androstendione, DHEA, DHEAS, and prolactin were assayed in up to five different batches. Estrone sulfate from batches 1 and 2 and prolactin from batches 1 to 3 were assayed in the laboratory of Dr. C. Longcope (University of Massachusetts Medical Center, Worcester, MA). Prolactin from batches 4 and 5 was assayed in the laboratory of Dr. P. Sluss (Massachusetts General Hospital, Boston, MA). All other analyses were done by Nichols Institute (San Juan Capistrano, CA). Methods for plasma hormone assays and information about laboratory precision and reproducibility have been published (26, 28, 30, 31). The within-batch laboratory coefficient of variation was $\leq 15\%$.

NHS II. Urinary aMT6-s was assayed by the Endocrine Core Laboratory of Dr. M. Wilson (Yerkes National Primate Research Center, Emory University, Atlanta, GA), using commercially available ELISA kits (ALPCO, Windham, NH). The Bühlmann 6-SMT ELISA is a competitive immunoassay using an antibody-capture technique with a lower detection limit of 0.8 ng/mL for aMT6-s. Because urine samples were not collected over a 24-hour period and total urinary output was unknown, creatinine levels also were measured for each sample by the same laboratory, using Sigma Diagnostics creatinine reagents. All aMT6-s levels are creatinine-standardized (aMT6-s concentration divided by concentration of creatinine) to account for differences arising from variations in urine concentrations. The 240 specimens from repeated urinary collections of 80 women (three samples per subject) were analyzed in 10 batches. Laboratory personnel who did the assays were blinded to prevent them from identifying which samples were from the same woman. To validate laboratory methods, 10% of the total samples were blinded quality-control samples. For those, the coefficient of variation was 15% for the urinary melatonin metabolite and 4.5% for creatinine. All hormone fractions of estradiol, estrone, estrone sulfate, progesterone, DHEA, DHEAS, testosterone, and androstendione were assayed by Quest Laboratory (San Juan Capistrano, CA). Further details of the laboratory methods used for plasma hormones have been described in a previous report (28). The overall within-batch laboratory coefficients of variation ranged from 5.7% to 20%. The coefficient of variation was high only for progesterone, (40.2%) but was lowered to 14.9% after removal of a single, improbable outlier value.

Statistical Analysis

NHS. Women were first categorized according to their night-work status; the groupings were selected to provide approximately equal 15-year categories: never having worked rotating night shifts, 1 to 14 years, and 15 or more years. A 15-year interval was chosen based on findings from our cohort that have been published previously, indicating an increased cancer risk after 15 or more years of night-shift work (7, 8). Within the subset of women who were eligible for the hormone analyses, hormone values greater than three interquartile ranges were treated as outliers and excluded (estradiol, $n = 1$; free estradiol, $n = 2$; testosterone, $n = 2$; androstendione, $n = 2$; DHEA, $n = 2$; DHEAS, $n = 5$). Because of insufficient plasma, not all hormone fractions were measured for all women. We fitted analysis of covariance models using PROC GLM of SAS (32) to evaluate associations between years of night work and circulating hormone levels, controlling for age, body mass index (BMI) at blood draw, time of blood draw, and laboratory batch. Natural logarithms of the plasma hormone values were used in these analyses to reduce the skewness of the regression residuals. Differences in hormonal levels between varying numbers of years having worked rotating night shifts were evaluated, with women who reported never having worked night shifts as the reference group. Hormone levels were interpreted as percentage age differences relative to the reference job type by subtracting 1.0 from the appropriate exponentiated linear combination of coefficients from the mixed regression models. All statistical tests are two-sided. We used the SAS statistical package for all analyses (32).

NHS II. Four samples (of a total of 240 measurements) from two different subjects with aMT6-s concentrations were below the detectable limit of the assay (0.8 pg/mL), and we assigned half of the value of the detectable limit of the assay (0.4 pg/mL) to these women before the value was normalized to creatinine levels. To assess reproducibility of the urinary melatonin metabolite over time, we used SAS PROC MIXED to calculate intraclass correlations (ICC), in which $ICC = \sigma_B^2/(\sigma_B^2 + \sigma_W^2)$.

95% Confidence intervals were calculated for the ICCs (26). To assess the influence of season of the year (correlated with melatonin levels) on the ICC, we conducted analyses adjusting for season. We created a binary variable based on the month of urine collection, women whose urine was collected during the darker periods of the year (October through March), and women whose urine was collected during the brighter months of the year (April through September), and report the P value from a partial F test, using PROC MIXED models. Because three measures (obtained over a 3-year period) were available for all hormones from each woman and because these three measures were reasonably well correlated over at least a 1-year period (ref. 33 and Table 2), we generally used the mean of these three measures for all hormones to assess correlations. Among those, aMT6-s values of four women were detected as outliers when the *generalized ESD many outlier procedure* (34) was used, and none of the steroid hormone values, with the exception of testosterone ($n = 1$), was identified as an outlier. We excluded the testosterone value that was detected as an outlier from subsequent analyses but kept the four women with extreme aMT6-s values in the analyses because similar low aMT6-s levels have been observed previously (refs. 17, 23 for example). However, we log-transformed creatinine-adjusted aMT6-s and report medians instead of means, when applicable. We also used the natural logarithms of all steroid hormone measurements in the analyses because the transformed values were slightly more normally distributed. We calculated Pearson correlation coefficients and Pearson's partial correlation coefficients for comparisons of the log-transformed measure of melatonin with the log-transformed continuous values for steroid hormones, with or without adjustment for several other covariates.

Twelve percent of the women had either explicitly stated that the collected urine was not their first morning spot urine or had not stated the time of collection on the questionnaire that accompanied the urine collection kit, and we excluded these women in subanalyses; however, because correlations remained essentially unchanged after their exclusion, we kept them in the main analyses. Number of nights worked in the 2 weeks before the first sample collection was used for the correlations between number of nights worked and urinary aMT6-s as well as plasma steroid hormone levels, because there was very little variation in women's night-work status over time. Night work was assessed in prespecified categories, and we used the midpoints of these categories (e.g., 1.5 nights for the category ''1-2'' nights) to obtain a continuous measure. All but 1 of the 80 women provided information on night work. For hormonal correlations with

number of nights worked (a cardinal variable), we used Spearman rank correlations and tested for trend with ordinal linear regression models. Generalized linear model analyses of variance provided estimates of the mean steroid hormone values for each quartile of urinary melatonin, adjusted for potential confounders. Two-sided P values are reported for all correlation coefficients.

Results

Characteristics of the 80 premenopausal women at time of first urine collection are summarized in Table 1. The mean number of months between the collection of the first and third urine samples was 34 (range, 23 to 46). At the time of the first urine collection, the average age of the women was 42 years, ranging from 34 to 50 years. Approximately 18% of the women had worked some nights in the 2 weeks before the first and second blood collections, and almost all of the women who had reported night work on the first urine questionnaire (follicular blood collection) also reported some night work on the second questionnaire (luteal phase blood and urine collection), which were approximately 2 weeks apart.

Age was not correlated with aMT6-s levels (Pearson $r = 0.02$, $P = 0.83$), whereas both current BMI (Spearman $r = -0.31$, $P = 0.01$) and BMI at age 18 (Spearman $r = -0.16$, $P = 0.17$) appeared to be inversely related with urinary melatonin levels. After exclusion of four aMT6-s outliers, these associations became slightly stronger (Spearman $r = -0.38$, $P = 0.001$; $r = -0.21$, $P = 0.07$, respectively). Average alcohol consumption [number of drinks in last month; Spearman $r = 0.25$ ($P = 0.03$)] and times per week a woman had perspired heavily through activity [a measure for physical exercise; Spearman $r = 0.35$ ($P = 0.001$)] were both positively correlated with aMT6-s levels. These associations were slightly attenuated after the exclusion of four outliers.

When three different processing methods were used (see Materials and Methods), the ICC was 0.94 (95% confidence interval, 0.90, 0.99). There was, however, a 20% decline of mean aMT6-s levels associated with longer processing duration (geometric mean, aMT6-s levels adjusted for laboratory batch, 11.7 ng/mL when processed immediately, 10.4 ng/mL when processed after 24 hours, and 9.4 ng/mL when processed after 48 hours).

Table 2 shows mean urinary creatinine and aMT6-s levels from 80 premenopausal women at the three sampling collections. The graphical display [(Fig. 1)] of the relationship between first and third sampling (33.9 months apart) reveals a fairly symmetrical distribution of aMT6-s values, showing that urinary melatonin levels track well over time. We did not observe an influence of season of urine collection on these estimates (partial F test from PROC MIXED model, with season as a fixed effect, $F = 0.20$, $P = 0.65$).

For all 80 women, we had previously measured progesterone, estrone, androstendione, DHEAS, and DHEA. For 79, we had measured estradiol; for 78, testosterone; for 76, estrone sulfate; and for 62, bioavailable estradiol.

Table 1. Baseline characteristics of 80 premenopausal* women who donated three luteal urine samples over approximately a 3-year period

Characteristics	Distribution
At time of first urine collection	
Age (years; mean/SD)	42.4 (3.7)
BMI† at age 18 (mean/SD)	21 (2.5)
BMI at urine collection (kg/m^2; mean/SD)	24 (4.6)
Weight change of ≥10 pounds in past 6 months (%)	11%
Cigarettes smoked per day in last month (median/range)	1.0 (1-4)
Times/week perspired heavily through activity (median/range)	2 (1-6)
Average alcohol consumption in last month (median/range)	2 (1-7)
Time between urine sample collection (months, mean/range):	
1st and 2nd	13.2 (5-18)
2nd and 3rd	20.7 (10-35)
1st and 3rd	33.9 (23-46)
Creatinine-adjusted melatonin level (ng/mg creatinine; median/range)	22.5 (1-71)
Estradiol (pg/mL; median/range)‡	144 (69-317)
Bioavailable estradiol (pg/mL; median/range)‡	35 (15-66)
Estrone sulfate (pg/mL; median/range)‡	1465 (453-7619)
Progesterone (ng/dL; median/range)‡	1157 (375-2315)
DHEA (ng/dL; median/range)‡	291 (82-942)
DHEAS (ng/dL; median/range)‡	157 (30-375)
Number of night shifts worked in last 2 weeks; n (%)	
0	65 (82%)
1-2	5 (6.5%)
3-4	2 (2.5%)
5-6	5 (6.5%)
>6	2 (2.5%)

*Includes only women who were premenopausal at all three times of urine collection.
†BMI is the weight in kilograms divided by the square of the height in meters.
‡Plasma hormone levels.

Table 2. Mean (SD) urinary creatinine and melatonin metabolite levels from 80 premenopausal women at the three sample collections*, and ICCs and 95% confidence intervals based on these three collections

Mean (SD)					
Urinary metabolite	Sample 1	Sample 2	Sample 3	ICC	95% Confidence interval
Creatinine (mg/dL)	119 (59)	125 (55)	130 (63)	0.42	(0.33-0.58)
aMT6-s (ng/mL)	32.2 (24)	31.5 (23)	33.2 (25)	0.65	(0.56-0.77)
Creatinine-adjusted aMT6-s (ng/mg creatinine)	28.5 (19)	26.8 (18)	27.2 (18)	0.72	(0.65-0.82)

*Samples were collected over a 3-year period.

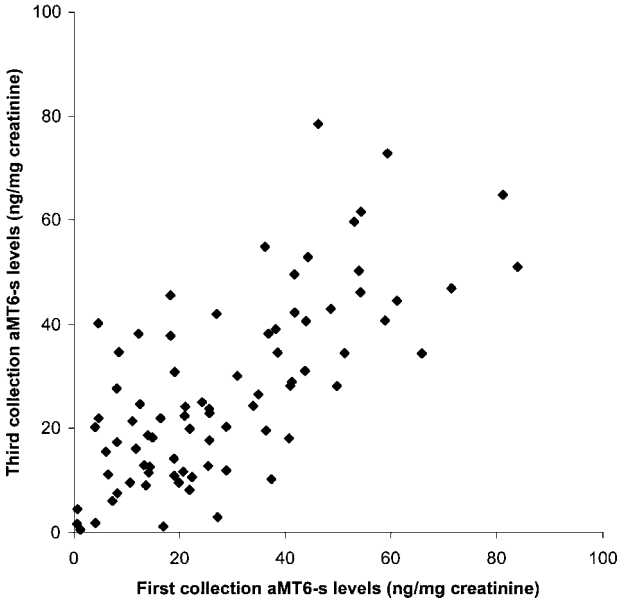

Figure 1. Graphical display of urinary creatinine adjusted melatonin metabolite levels from 80 premenopausal women at first and third sample collections.

We observed a stepwise decline of levels of bioavailable estradiol with increasing quartiles of aMT6-s levels (top versus bottom quartile, 23% difference, Table 3). There was a significant inverse association between bioavailable estradiol and aMT6-s and a significant positive correlation between urinary aMT6-s and progesterone but no association between aMT6-s and total estradiol levels. We further noted positive associations with DHEA and DHEAS. Adjustment for age and current BMI slightly attenuated all estimates (e.g., partial Pearson coefficient for bioavailable estradiol, $r = -0.16$, $P = 0.25$; progesterone, $r = 0.17$, $P = 0.23$); BMI appeared to be the primary confounding factor, with little effect of age on these correlations. After the removal of four women with progesterone levels below 300 ng/mL, levels generally indicating an anovulatory cycle in the luteal phase, these correlations remained essentially unchanged (($n = 72$) data not shown).

Table 4 shows median aMT6-s and steroid hormone levels according to numbers of nights worked in the 2 weeks before urine collection. We observed a significant inverse association between increasing numbers of nights worked and urinary melatonin levels. Women who reported not having worked night shifts had 125% higher levels of aMT6-s than women who worked more than four nights in the 2 weeks preceding urine collection (27 ng/mL versus 12 ng/mL; $r = -0.30$, $P = 0.008$). There were only very modest and statistically insignificant associations between bioavailable estradiol ($r = -0.14$, $P = 0.32$) and total estradiol ($r = 0.10$, $P = 0.41$) and numbers of nights worked. These correlations remained essentially unchanged when we excluded women with progesterone levels below 300 ng/mL.

Table 5 shows associations between lifetime years of rotating night work and geometric mean plasma levels of steroid hormones among postmenopausal women (NHS). Those who worked 1 to 14 years and 15 or more years of rotating night shifts were of similar age and BMI to women who never worked rotating night shifts. More detailed differences about breast cancer risk factors according to night-work status have been reported previously for the total cohort (7) and, overall, were negligible. Similar to the total cohort, 37% of the women never worked a rotating night shift, 54% worked night shifts for 1 to 14 years, and 9% for more than 15 years (corresponding numbers, total cohort: 40%, 52%, and 7.4%). Compared with women who never worked rotating night shifts, women who reported having worked 15 or more years had statistically elevated levels of estradiol in all fractions (total estradiol, +14.3%,

Table 3. Median luteal steroid hormone levels by creatinine-adjusted aMT6-s (ng/mL) quartiles (Q) among 80 premenopausal women

Steroid hormone†	Median (range*) steroid hormone levels by aMT6-s (ng/mL) quartiles				Pearson correlation coefficient‡ (P)
	Q1	Q2	Q3	Q4	
	Inter-quartile range				
	(1.0-15)	(15.1-22.5)	(22.6-37)	(37.1-71.4)	
	$n = 19$	$n = 21$	$n = 21$	$n = 19$	
Estradiol (pg/mL)	140 (210)	153 (160)	151 (208)	137 (247)	−0.04 (0.75)
Bioavailable estradiol (pg/mL)	40 (46)	35 (36)	35 (44)	31 (42)	−0.25 (0.05)
Estrone (pg/mL)	90 (83)	73 (107)	81 (79)	76 (129)	−0.08 (0.50)
Estrone sulfate (pg/mL)	1490 (7028)	1142 (6491)	1349 (2589)	1772 (3893)	0.03 (0.78)
Progesterone (ng/dL)	1003 (1778)	1099 (1637)	1227 (1617)	1358 (1643)	0.31 (0.01)
Testosterone (ng/dL)	27 (26)	29 (45)	27 (28)	24 (25)	−0.12 (0.29)
Androstendione (ng/dL)	127 (183)	122 (255)	142 (177)	120 (194)	0.07 (0.52)
DHEA (ng/dL)	284 (860)	284 (534)	342 (716)	286 (641)	0.19 (0.10)
DHEAS (ng/dL)	145 (344)	130 (280)	197 (292)	177 (190)	0.17 (0.15)

*The range is the difference between the largest and smallest observations in the sample.
†The actual n per aMT6-s quartiles varies slightly between each steroid hormone.
‡Pearson correlation coefficient between the continuous measure of log-transformed creatinine-adjusted aMT6-s and the continuous level of log-transformed steroid hormones, after exclusion of four aMT6-s outliers.

Table 4. Median creatinine-adjusted aMT6-s and steroid hormone levels (range*) by night work status in the 2 weeks before first urine/blood collection among 79† premenopausal women

	Number of night shifts worked in the last 2 weeks			Spearman correlation coefficient‡ (*P*)
	0 (*n* = 65)	1-4 (*n* = 7)	>4 (*n* = 7)	
Creatinine-adjusted aMT6-s (ng/mL)				
Mean of three measures	27 (81)	18 (40)	12 (37)	−0.30 (0.008)
1st collection§	24 (81)	12 (40)	8 (37)	−0.29 (0.01)
2nd collection§	21 (80)	29 (64)	8 (38)	−0.01 (0.95)
3rd collection§	21 (77)	17 (46)	18 (33)	−0.10 (0.36)
Steroid hormones				
Estradiol (pg/mL)	143 (200)	160 (211)	139 (346)	0.10 (0.41)
Bioavailable estradiol (pg/mL)	32 (72)	30 (23)	30 (17)	−0.14 (0.32)
Estrone (pg/mL)	83 (127)	83 (70)	88 (148)	0.15 (0.19)
Estrone sulfate (pg/mL)	1371 (7377)	1157 (1742)	1969 (2488)	0.02 (0.85)
Progesterone (ng/dL)	1336 (2686)	1616 (1644)	1587 (3336)	0.17 (0.14)
Testosterone (ng/dL)	26 (67)	26 (15)	28 (17)	0.08 (0.51)
Androstendione (ng/dL)	140 (275)	139 (209)	164 (196)	0.08 (0.49)
DHEA (ng/dL)	282 (1078)	266 (656)	536 (674)	0.20 (0.09)
DHEAS (ng/dL)	154 (412)	114 (250)	204 (160)	−0.01 (0.95)

*The range is the difference between the largest and smallest observations in the sample.
†Night work information was missing for one woman.
‡Spearman correlation coefficient between the continuous numbers of night work (using midpoints of the prespecified categories), and the continuous levels of creatinine, aMT6-s, creatinine-adjusted aMT6, and steroid hormones.
§Individual, creatinine-adjusted aMT6-s levels at the respective collection time, rather than the mean of these three measures, are presented.

P = 0.04; bioavailable estradiol, +29%, *P* = 0.03; free estradiol, +29%, *P* = 0.02). No significant differences were found for estrone, androgens, progesterone, and prolactin.

Discussion

Previous reports on the effects of transport of whole blood on several analytes have shown that overnight shipping is feasible (25). In this study, we found that delayed processing of urine samples also had only a modest effect on aMT6-s levels, with slightly lower levels of aMT6-s associated with delayed processing. When measured 3 years apart, the ICC for creatinine-adjusted aMT6-s was 0.72 in a sample of premenopausal women. To our knowledge, this is the first report assessing aMT6-s reproducibility among premenopausal women. The ICCs observed in this study are comparable in magnitude to those of some other exposure measures commonly used in epidemiology and indicate that a single morning spot urine sample can reasonably represent aMT6-s levels over at least a 3-year period. Thus, first spot morning urine melatonin measurements are a feasible biomarker in observational studies.

The hypothalamus and the hypophysis constitute a complex axis that regulates a variety of circulating steroid hormone levels through feedback loops. It is unclear, however, to what extent, if any, melatonin, a hormone produced in the pineal gland, is involved in

Table 5. Geometric mean hormone levels* by number of years worked rotating night shifts up to 1988 for 663 postmenopausal women without current use of postmenopausal hormones at blood draw (1989)†

Hormone	Geometric mean by number of years on rotating night shifts‡			*P* for trend
	Never (*n* = 244)	1-14 (*n* = 357)	≥15 (*n* = 62)	
Age (years; mean)	61.8	61.6	62.1	
BMI at blood collection (kg/m^2; mean)	25.9	26.3	26.6	
Estrone (pg/mL)	32 (234)	34 (339)	34 (59)	0.34
Estrone sulfate (pg/mL)	236 (227)	246 (343)	266 (61)	0.20
Estradiol (pg/mL)	8.8 (237)	9.2 (347)	10.1 (60)	0.03
Bioavailable estradiol (pg/mL)	1.7 (165)	1.8 (241)	2.2 (43)	0.04
Free estradiol (pg/mL)	0.14 (167)	0.15 (238)	0.18 (42)	0.02
Testosterone (ng/dL)	21 (236)	22 (344)	22 (58)	0.34
Androstendione (ng/dL)	59 (231)	63 (341)	57 (58)	0.07
DHEA (ng/dL)	198 (226)	211 (328)	177 (54)	0.06
DHEAS (g/dL)	84 (239)	91 (345)	75 (59)	0.08
Prolactin (ng/mL)	8.0 (229)	7.9 (337)	8.3 (61)	0.90
Progesterone (ng/mL)	3.3 (188)	3.3 (284)	3.4 (53)	0.80

*Controlling for age (five age groups), BMI (continuous), time of blood draw, and laboratory batch.
†Never night workers are the reference group for all comparisons.
‡Number varies due to missing data and removal of outliers.

these feedback loops. The observation of an increased breast cancer risk among women exposed to light at night has fueled speculations about reduced melatonin levels among night workers and an inverse correlation between melatonin and estrogen production. The results of our study revealed several interesting associations and shed new light on these hypotheses.

We found recent night work (within the last 2 weeks) to be associated with a 56% significant reduction of urinary aMT6-s, and aMT6-s levels were inversely associated with levels of bioavailable estradiol. There was no direct association between recent night work and estrogen levels; long-term night work, however, did seem to increase estrogen levels among postmenopausal women. We further noted a positive association between aMT6-s and progesterone levels, which is novel and needs further evaluation.

A potential limitation of our study is that urine collections were done during a woman's luteal phase. We did not observe important variations of aMT6-s levels within the luteal phase. According to a previous report (35), the correlation of aMT6-s excretion with plasma melatonin is higher during the follicular phase ($r = 0.89$) than during the luteal phase ($r = 0.73$) of a woman's cycle. However, these data need to be confirmed before any conclusions can be drawn about the optimal timing of urine collection for aMT6-s studies in premenopausal women. We also cannot rule out the possibility of individual susceptibility to the effects of exposure to light at night that we were not able to account for. For example, those with lower baseline melatonin levels may be more susceptible to the additional effects of light exposure at night in lowering their melatonin levels. Finally, season of the year is known to affect the increase of melatonin (36). However, we were able to take into account the effects of season of the year in our analyses and did not detect a significant influence.

Our results provide some evidence for a potential link between night-shift work (a surrogate for exposure to light) and melatonin suppression, including possible long-term effects of night work on circulating estrogen levels. Further studies are needed to determine the optimal timing within the menstrual cycle to measure urinary aMT6-s among premenopausal women. Future research should address individual susceptibility and the question whether women with lower melatonin levels choose to do night work more often than do women with higher melatonin levels. Finally, associations between melatonin levels and breast cancer risk need to be evaluated.

In summary, our data support a potential association between night work and cancer risk through the melatonin pathway. Breast cancer, in particular, may be influenced by relations between melatonin and estrogen levels.

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References

1. Joechle W. Trends in photophysiologic concepts. Ann NY Acad Sci 1964;117:88-104.
2. Czeisler CA, Duffy JF, Shanahan TL, et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 1999;284:2177-81.
3. Lynch HJ, Wurtman RJ, Moskowitz MA, Archer MC, Ho MH. Daily rhythm in human urinary melatonin. Science 1975;187:169-71.
4. Waldhauser F, Dietzel M. Daily and annual rhythms in human melatonin secretion: role in puberty control. Ann NY Acad Sci 1985; 453:205-14.
5. Waldhauser F, Weiszenbacher G, Frisch H, Zeitlhuber U, Waldhauser M, Wurtman RJ. Fall in nocturnal serum melatonin during prepuberty and pubescence. Lancet 1984;18:362-5.
6. Brzezinski A. Melatonin in humans. N Engl J Med 1997;336:186-95.
7. Schernhammer ES, Laden F, Speizer FE, et al. Rotating night shifts and risk of breast cancer in women participating in the Nurses' Health Study. J Natl Cancer Inst 2001;93:1563-8.
8. Schernhammer ES, Laden F, Speizer FE, et al. Night-shift work and risk of colorectal cancer in the Nurses' Health Study. J Natl Cancer Inst 2003;95:825-88.
9. Davis S, Mirick DK, Stevens RG. Night shift work, light at night, and risk of breast cancer. J Natl Cancer Inst 2001;93:1557-62.
10. Hansen J. Increased breast cancer risk among women who work predominantly at night. Epidemiology 2001;12:74-7.
11. Czeisler CA, Dijk DJ. Chapter 20: Human circadian physiology and sleep-wake regulation. In: Takahashi JS, Turek FW, Moore RY, editors. Handbook of behavioral neurobiology: circadian clocks. New York: Plenum Publishing Co.; 2001. p. 531-569.
12. Stevens RG. Electric power use and breast cancer: a hypothesis. Am J Epidemiol 1987;125:556-61.
13. Wetterberg L. Melatonin in humans physiological and clinical studies. J Neural Transm Suppl 1978;13:289-310.
14. Nowak R, McMillen IC, Redman J, Short RV. The correlation between serum and salivary melatonin concentrations and urinary 6-hydroxymelatonin sulphate excretion rates: two non-invasive techniques for monitoring human circadian rhythmicity. Clin Endocrinol 1987; 27:445-52.
15. Lang U, Kornemark M, Aubert ML, Paunier L, Sizonenko PC. Radioimmunological determination of urinary melatonin in humans: correlation with plasma levels and typical 24-hour rhythmicity. J Clin Endocrinol & Metab 1981;53:645-50.
16. Arendt J, Bojkowski C, Franey C, Wright J, Marks V. Immunoassay of 6-hydroxymelatonin sulfate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. J Clin Endocrinol & Metab 1985;60:1166-73.
17. Cook MR, Graham C, Kavet R, Stevens RG, Davis S, Kheifets L. Morning urinary assessment of nocturnal melatonin secretion in older women. J Pineal Res 2000;28:41-7.
18. Baskett JJ, Cockrem JF, Antunovich TA. Sulphatoxymelatonin excretion in older people: relationship to plasma melatonin and renal function. J Pineal Res 1998;24:58-61.
19. Graham C, Cook MR, Kavet R, Sastre A, Smith DK. Prediction of nocturnal plasma melatonin from morning urinary measures. J Pineal Res 1998;24:230-8.
20. Levallois P, Dumont M, Touitou Y, et al. Effects of electric and magnetic fields from high-power lines on female urinary excretion of 6-sulfatoxymelatonin. Am J Epidemiol 2001;154:601-9.
21. Stevens RG, Davis S, Mirick DK, Kheifets L, Kaune W. Alcohol consumption and urinary concentration of 6-sulfatoxymelatonin in healthy women. Epidemiology 2000;11:660-5.
22. Travis RC, Allen NE, Peeters PHM, van Noord PAH, Key TJ. Reproducibility over 5 years of measurements of 6-sulphatoxymelatonin in urine samples from postmenopausal women. Cancer Epidemiol Biomarkers 2003;12:806-8.
23. Davis S, Kaune WT, Mirick DK, Chen C, Stevens RG. Residential magnetic fields, light-at-night, and nocturnal urinary 6-sulfatoxymelatonin concentration in women. Am J Epidemiol 2001;154:591-600.
24. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. J Womens Health 1997;6:49-62.
25. Hankinson SE, London SJ, Chute CG, et al. Effect of transport conditions on the stability of biochemical markers in blood. Clin Chem 1989;35:2313-6.
26. Hankinson SE, Manson JE, Speigelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3 year period. Cancer Epidemiol, Biomarkers & Prev 1995;4:649-54.
27. The Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002;94:606-16.

28. Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90:1292-9.
29. Rockhill B, Willett WC, Hunter DJ, et al. Physical activity and breast cancer risk in a cohort of young women. J Natl Cancer Inst 1998;90: 1155-60.
30. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351:1393-6.
31. Hankinson SE, Willett WC, Michaud DS, et al. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1999;91:629-34.
32. Inc. SAS/STAT user's guide version 6. 4. Cary, NC: SAS Institute.
33. Michaud DS, Manson JE, Spiegelman D, et al. Reproducibility of plasma and urinary sex hormone levels in premenopausal women over a one-year period. Cancer Epidemiol, Biomarkers & Prev 1999;8: 1059-64.
34. Rosner B. Percentage points for a generalized ESD many-outlier procedure. Technometrics 1983;25:165-72.
35. Fernandez B, Malde JL, Montero A, Acuna D. Relationship between adenohypophyseal and steroid hormones and variations in serum and urinary melatonin levels during the ovarian cycle, perimenopause and menopause in healthy women. J Steroid Biochem 1990;35: 257-62.
36. Beck-Friis J, von Rosen D, Kjellman BF, Ljunggren JG, Wetterberg L. Melatonin in relation to body measures, sex, age, season and the use of drugs in patients with major affective disorders and healthy subjects. Psychoneuroendocrinology 1984;9:261-77.