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Original Contribution

First-Morning Urinary Melatonin and Breast Cancer Risk in the Guernsey Study

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It has been hypothesized that suppressed nocturnal melatonin production is associated with an increased risk of breast cancer, but results from several small prospective studies of the association have been inconclusive. We examined the association between nocturnal melatonin and breast cancer risk in a case-control study nested within the Guernsey III Study, a British prospective cohort study (1977–2009). Concentrations of 6-sulfatoxymelatonin were measured in prediagnostic first-morning urine samples from 251 breast cancer cases and 727 matched controls. Conditional logistic regression models were used to calculate odds ratios for breast cancer in relation to 6-sulfatoxymelatonin level. No significant association was found between 6-sulfatoxymelatonin level and breast cancer risk, either overall (for highest third vs. lowest, multivariable-adjusted odds ratio = 0.90, 95% confidence interval: 0.61, 1.33) or by menopausal status. However, in a meta-analysis of all published prospective data, including 1,113 cases from 5 studies, higher 6-sulfatoxymelatonin levels were associated with lower breast cancer risk (for highest fourth vs. lowest, odds ratio = 0.81, 95% confidence interval: 0.66, 0.99). In summary, we found no evidence that 6-sulfatoxymelatonin level in a first-morning urine sample was associated with breast cancer risk among British women. However, overall the published data suggest a modest inverse association between melatonin levels and breast cancer risk. Further data are needed to confirm this association.

breast cancer; cohort studies; melatonin; nested case-control studies; 6-sulfatoxymelatonin

Abbreviations: CI, confidence interval; OR, odds ratio.

The International Agency for Research on Cancer has classified shift work involving disruption of circadian rhythm as a probable carcinogen (1) on the basis of sufficient experimental evidence in animals and limited evidence in humans, primarily for risk of breast cancer. The underlying biological mechanisms surrounding this potential association with night work are generally unknown, but a leading hypothesis involves the suppression of nocturnal production of the pineal hormone melatonin (*N*-acetyl-5-methoxytryptamine) (2, 3) by exposure to light at night. Results from research in animal models support a protective association between melatonin and the development of breast cancer, showing that a pinealectomy increases tumorigenesis and shortens survival time, whereas administration of melatonin reverses these trends and inhibits tumor growth (4–7). To date, however, only 5 small prospective epidemiologic studies have evaluated the relationship between endogenous melatonin levels and subsequent risk of breast cancer, with inconclusive findings (8–12).

6-Sulfatoxymelatonin is the main metabolite of melatonin in urine and is a reliable biomarker of melatonin concentrations in the blood for the period during which the urine sample was collected. We previously investigated the relationship between melatonin and breast cancer risk in a nested case-control study of 6-sulfatoxymelatonin in 24-hour urine samples from women in the Guernsey Study and found no significant association (8). In contrast, 3 studies with first-morning or 12-hour overnight urine specimens found some evidence of an inverse association (9–11), and 1 study of 6-sulfatoxymelatonin levels in overnight urine samples found a significant positive association (12). It has been suggested that the differences in these results may be due to the timing of the urine specimen collection, because melatonin is mainly produced at night, with peak production occurring in the early hours of the

morning (12); differences in nocturnal production may be less detectable in 24-hour samples than in overnight urine samples. To specifically address this issue, we have conducted a second investigation among women from the Guernsey Study, in which we have measured melatonin (6-sulfatoxymelatonin) levels in first-morning urine samples that were collected immediately prior to the start of collection of the 24-hour specimen. In this report, we assess the relationship between melatonin levels in first-morning voids and subsequent risk of breast cancer among British women and describe the correlation between melatonin levels measured in first-morning voids and 24-hour urine samples. We also present results of a meta-analysis that combines our results with those of published studies.

METHODS

Participants and data

Between April 25, 1977, and October 28, 1985, a total of 5,093 women living on the island of Guernsey (Channel Islands, United Kingdom) were recruited into a prospective study of hormones and breast cancer, known as the Guernsey III Study. Approval was given by the local ethics committee, and each woman gave written informed consent to study participation and follow-up. Height and weight were measured at interview, and a questionnaire was completed with details on reproductive history, menopausal status, and past use of oral contraceptives and other hormones.

Shortly after recruitment, a first-morning void and a 24-hour urine sample were collected from each woman (median time after recruitment, 16 days; interquartile range (25th–75th percentiles), 9–29). Women were asked to collect their first-morning void and to keep it in the refrigerator. In a second container, women collected all urine voided during the subsequent 24 hours. Samples were then collected, frozen, and stored at −20°C. In premenopausal women, samples were collected irrespective of the stage of the menstrual cycle, but the dates of onset of menses preceding and following urine collection were recorded (the latter by postcard).

Follow-up for the diagnosis of breast cancer was done by searching pathology reports and death certificates on Guernsey and records of the Wessex Cancer Registry for participants in the study. Eligible case patients were women who had been diagnosed with carcinoma of the breast between their enrollment in the Guernsey III Study and October 31, 2009. Case patients diagnosed as a result of mammographic screening at recruitment were excluded. Patients with carcinoma in situ of the breast ($n = 26$) were included as case patients in the main analyses; the analyses were then repeated after exclusion of these patients.

We previously reported findings based on measurements made in 24-hour urine samples from 127 women with breast cancer and 353 matched control participants whose urine samples were assayed in 2002; the closure date for this study period was October 31, 2001 (8). For the current study, we extended the nested case-control series to also include patients with breast cancer diagnosed more recently, between 2001 and 2009, and their matched controls. It was possible to retrieve first-morning urine samples and conduct 6-sulfatoxymelatonin assays for a total of 251 cases and 727 controls (including 100 cases and 256 controls who were also included in the earlier study of 24-hour urine samples).

Women were eligible for this study if they were not using any exogenous sex hormones at the time of recruitment, had not previously had cancer (other than nonmelanoma skin cancer), had provided a first-morning urine specimen on a known date, and had a known menopausal status. Women were classified as premenopausal if they reported at interview that they had menstruated in their usual pattern during the previous 6 months and had a cycle length not longer than 42 days. Women were classified as being postmenopausal if they had not had a menstrual period for at least 1 year, had undergone a hysterectomy without bilateral oophorectomy before menopause, or were aged 60 years or older at recruitment.

Each case patient was matched to 3 control participants randomly selected from the cohort according to the following matching criteria: age (within 2 years), date of recruitment (within 1 year), and menopausal status (premenopausal or postmenopausal). To be consistent with matching criteria used previously (13, 14), premenopausal case patients were also matched on the day of the menstrual cycle on which blood had been collected, and postmenopausal case patients with a natural menopause were matched to control participants on the number of years they had been postmenopausal. For the case patients who had undergone a hysterectomy, control participants were matched on this criterion when possible, but when it was not possible, the control participants were selected to be 3 or more years naturally postmenopausal, because these case patients were aged 60 years or older at recruitment.

Once a control participant had been matched to a case patient, she was unavailable for matching with further case patients. Women who had previously been studied as control participants (8) were included in this study as case patients if they were subsequently diagnosed with breast cancer. Control participants were women who were not known to have died or been diagnosed with breast cancer by the date on which the case patient to whom they were matched was diagnosed with breast cancer.

Hormone assays

Assays for 6-sulfatoxymelatonin and creatinine in first-morning urine voids were conducted in the Cancer Epidemiology Unit Laboratory at the University of Oxford (Oxford, United Kingdom) in 2011. Samples from each case-control set were assayed in the same laboratory batch. Samples were assayed in duplicate, and the mean of the duplicate results was used for statistical analyses. 6-Sulfatoxymelatonin was assayed using the Bühlmann enzyme-linked immunosorbent assay (product code EK-M6S; Bühlmann Laboratories AG, Schönenbuch, Switzerland). Creatinine was measured by the Jaffé method using the Cayman Creatinine Colorimetric Assay Kit (product code 500701; Cayman Chemical Company, Ann Arbor, Michigan).

The assays for 6-sulfatoxymelatonin included low and high quality-control samples in each batch. Coefficients of variation were 15.8% and 21.0% for the low and high quality controls (mean values of 3.9 ng/mL and 22.2 ng/mL, respectively). We also included additional quality-control

samples from 2 pooled first-morning urine samples (mean 6-sulfatoxymelatonin concentrations of 10.4 ng/mL and 19.0 ng/mL) in each batch, and the coefficients of variation were 14.8% and 21.0%, respectively.

Details on the assays conducted in 2002 for 6-sulfatoxymelatonin and creatinine levels in 24-hour urine samples by Stockgrand Ltd. (University of Surrey, Guildford, United Kingdom) for the nested case-control study of breast cancer have been published previously (8, 15–17). A random selection of 24-hour urine samples from 66 women were re-assayed in 2 batches in the Cancer Epidemiology Unit Laboratory in 2011 using the enzyme-linked immunosorbent assay method described above for first-morning voids to enable interassay comparisons; the correlation coefficients for correlations between the 24-hour urine measurements made in 2001 and 2011 were 0.90 and 0.95 for 6-sulfatoxymelatonin and creatinine, respectively.

Statistical analyses

The hormonal values were logarithmically transformed for statistical analyses to approximately normalize their frequency distributions, and geometric means and 95% confidence intervals were calculated. Differences in baseline characteristics of cases and controls were compared using weighted paired-samples *t* tests for continuous variables (18) and conditional logistic regression models for categorical variables. Pearson correlation coefficients were calculated to assess the correlations between 6-sulfatoxymelatonin levels in first-morning samples and 24-hour urine samples. Analyses of covariance, adjusting for age at urine collection and assay batch, were used to examine whether breast cancer risk factors and other subject characteristics were associated with the level of 6-sulfatoxymelatonin excreted.

Conditional logistic regression analyses were applied to calculate the odds ratios for breast cancer in tertile groups (thirds) of 6-sulfatoxymelatonin excretion using tertile cutpoints among control participants, with the lowest category designated the reference group and adjustment for age as an a priori confounder to account for small differences in age between cases and matched controls. A linear trend for breast cancer risk was calculated using the logarithm of 6-sulfatoxymelatonin as a continuous variable.

The influence of potential confounders—duration of urine storage (years; continuous), body mass index (weight (kg)/height $(m)^2$; continuous), first-degree family history of breast cancer at recruitment (yes, no), age at menarche (<13, 13, or ≥14 years), parity and age at first birth (nulliparous, parous with age at first birth <25 years, or parous with age at first birth ≥25 years), previous use of oral contraceptives (yes, no), previous use of other hormones (yes, no), season of urine collection (spring (March–May), summer (June–August), autumn (September–November), or winter (December–February)), stage of the menstrual cycle in premenopausal women (early follicular, late follicular, midcycle, early luteal, or late luteal, defined as ≥22, 16–21, 12–15, 3–11, and ≤2 days before the next menstrual period, respectively), and age at menopause in postmenopausal women (years; continuous)—was examined by including these variables in the conditional logistic regression models. Any missing values were assigned to a separate category. No data on cigarette smoking, alcohol consumption, or other dietary factors were available; therefore, no adjustment could be made for these potential confounders.

Conditional logistic regression analyses were also performed for premenopausal and postmenopausal women separately because mechanisms by which melatonin may influence the risk of breast cancer, such as interaction with ovarian hormone levels, may differ by menopausal status. Likelihood ratio tests were used to examine the heterogeneity of the associations of 6-sulfatoxymelatonin levels with risk of breast cancer for participants categorized by menopausal status (premenopausal vs. postmenopausal) and by time to diagnosis (<4 years after blood collection vs. ≥4 years after blood collection).

Statistical analyses were performed using the Stata 12 statistical software package (StataCorp LP, College Station, Texas). All tests of statistical significance were 2-sided, and *P* values below 0.05 were considered significant.

Meta-analysis

To put the results of the present study into the context of previous research, we conducted a fixed-effects meta-analysis of our results together with the results of previously published prospective studies of the association between melatonin and breast cancer risk. We followed standard criteria for the reporting of meta-analyses (PRISMA guidelines (19)). Relevant publications were identified from reviews and computer-aided literature searches (using PubMed, with the keywords breast cancer (incidence or mortality), melatonin, and 6-sulfatoxymelatonin) up to October 31, 2012. Relative risk estimates for incident breast cancer (in situ and invasive cancers combined) and 95% confidence intervals were extracted for the highest exposure category compared with the lowest. Summary relative risks were estimated by calculating the weighted average of the study-specific logarithms of the relative risks, with weights proportional to the inverses of the variances of the study-specific log relative risks. Results from individual studies are presented as squares and lines, representing odds ratios for breast cancer and corresponding 95% confidence intervals, respectively, among women in the highest category of 6-sulfatoxymelatonin exposure compared with the lowest category (referent). The position of the square indicates the value of the odds ratio, where the size is inversely proportional to the variance of the logarithm of the odds ratio and indicates the amount of statistical information available for that particular estimate. The diamond (the lateral points of which are the 95% confidence intervals) represents the overall odds ratio.

RESULTS

Characteristics of case patients and control participants

Breast cancer diagnosis followed urine collection by a mean of 15.5 years (range, 0.07–31.4 years). A lower proportion of case patients than of control participants was parous ($P = 0.007$), and among parous women, cases were younger at first birth ($P = 0.003$) (Table 1). Cases were also more likely to have a first-degree family history of breast cancer than

Table 1. Characteristics of Women Included in an Analysis of Urinary 6-Sulfatoxymelatonin Concentration and Breast Cancer Risk, by Case-Control Status, Guernsey Study, 1977–2009

	Controls ($n = 727$)		Cases ($n = 251$)		*P* Value[a]
	Mean (SD)	%	Mean (SD)	%	
Age at urine collection, years	45.6 (0.3)		45.7 (0.6)		
Body mass index[b]	24.7 (0.1)		24.8 (0.2)		0.8
Urine storage time, years	30.5 (0.08)		30.6 (0.1)		0.06
Age at menarche, years	13.1 (0.06)		13.1 (0.1)		0.9
Parous		89.6		82.9	0.007
Age at first birth in parous women, years	24.9 (0.2)		24.3 (0.3)		0.003
Postmenopausal at recruitment[c]		24.6		24.7	
Age at natural menopause, years[c]	49.1 (0.3)		48.5 (0.6)		0.4
Ever use of OCs		52.1		53.8	0.5
Ever use of non-OC hormones[c]		14.9		12.5	0.4
First-degree family history of breast cancer[d]		5.9		13.2	0.0002

Abbreviations: OC, oral contraceptive; SD, standard deviation.

[a] Weighted paired-sample *t* tests for the comparison of mean values (continuous variables) and conditional logistic regression for the comparison of proportions (categorical variables). Cases and controls were matched on age at recruitment, recruitment date, and menopausal status.

[b] Weight (kg)/height $(m)^2$.

[c] Postmenopausal women only.

[d] Family history of breast cancer in a mother, sister, or daughter.

controls ($P = 0.0002$). Case patients and control participants were similar with respect to other characteristics (Table 1).

Correlation between 6-sulfatoxymelatonin levels measured in first-morning samples and in 24-hour urine samples

The levels of 6-sulfatoxymelatonin (adjusted for creatinine) in first-morning urine samples (measured for the current study) and 24-hour urine samples (measured previously) were moderately correlated (based on 256 controls, $r = 0.66$, $P < 0.0001$) (Figure 1). When control participants were grouped into thirds based on the distribution of 6-sulfatoxymelatonin in first-morning and 24-hour urine samples (Table 2), 58% of participants were categorized into the same third (weighted $\kappa = 0.49$, $P < 0.001$), with a clear increasing trend in first-morning 6-sulfatoxymelatonin levels across increasing categories of 24-hour 6-sulfatoxymelatonin ($P < 0.0001$).

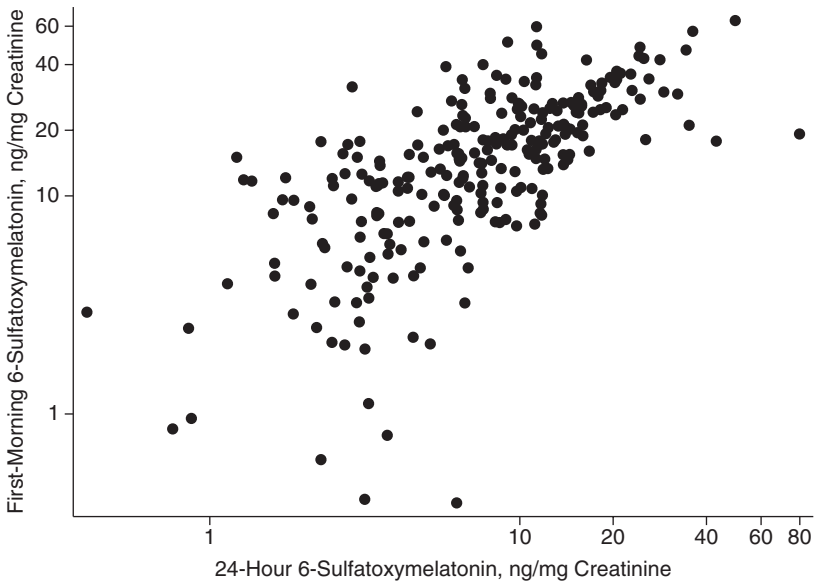

Figure 1. Correlations between 6-sulfatoxymelatonin concentrations in first-morning urine samples and 24-hour urine samples among 256 control participants from the Guernsey Study, 1977–2009.

Table 2. Cross-Classification of 256 Control Participants According to Tertile Group (Third) of 6-Sulfatoxymelatonin Levels in 24-Hour and First-Morning Urine Samples, Guernsey Study, 1977–2009

Third of 24-Hour 6-Sulfatoxymelatonin Level[a]	Third of First-Morning 6-Sulfatoxymelatonin Level[a]						First-Morning 6-Sulfatoxymelatonin Level by Third of 24-Hour 6-Sulfatoxymelatonin Level	
	1		2		3		Geometric Mean, ng/mg creatinine	95% Confidence Interval
	No.	%[b]	No.	%[b]	No.	%[b]		
1	55	21.5	29	11.3	2	0.8	6.3	5.5, 7.3
2	21	8.2	38	14.8	26	10.2	14.9	12.9, 17.2
3	5	2.0	25	9.8	55	21.5	23.6	20.4, 27.3

[a] Adjusted for creatinine concentration.
[b] Percentage of the total number of samples from controls with measurements of 6-sulfatoxymelatonin level in both 24-hour and first-morning urine samples.

Association between 6-sulfatoxymelatonin and other variables in control participants

Urinary 6-sulfatoxymelatonin levels were lower in older women at the time of urine collection (assay-adjusted decrease of 2% per 1-year increase in age; $P < 0.0001$) (Table 3). After adjustment for age and assay batch, there were no statistically significant associations between 6-sulfatoxymelatonin excretion and any of the other characteristics of the study participants and their samples (Table 3).

Urinary 6-sulfatoxymelatonin excretion in case patients and control participants

Table 4 shows geometric mean concentrations of 6-sulfatoxymelatonin excreted by case patients and control participants, adjusted for age at urine collection and assay batch and then further adjusted for other factors that may confound the relationship between 6-sulfatoxymelatonin and breast cancer risk. The mean concentrations of 6-sulfatoxymelatonin were similar between cases and controls, both before and after adjustment for potential confounders, and did not differ appreciably by menopausal status.

Table 5 shows the relative risk of breast cancer according to tertile group (third) of 6-sulfatoxymelatonin excretion. The age-adjusted odds ratio for breast cancer for the highest third of 6-sulfatoxymelatonin versus the lowest third was 0.89 (95% confidence interval (CI): 0.61, 1.30), and odds ratios in premenopausal and postmenopausal women were similar (odds ratio (OR) = 0.95 (95% CI: 0.60, 1.52) and OR = 0.90 (95% CI: 0.43, 1.88), respectively; P for interaction = 0.5). Adjustment for other potential confounders did not materially alter these findings, and there was no significant heterogeneity in the findings by time to diagnosis (P for interaction = 0.5). When the hormone data were divided into quartile groups or fourths (instead of thirds), similar results were noted for all women: Compared with women with levels in the lowest fourth, the multivariable-adjusted odds ratios for women with levels in the second, third, and highest fourths of 6-sulfatoxymelatonin concentration were 1.37 (95% CI: 0.92, 2.06), 0.77 (95% CI: 0.49, 1.21), and 1.01 (95% CI: 0.64, 1.58), respectively.

Findings were similar when analyses were confined to invasive breast cancer only (225 case patients and 652 matched controls); compared with women with levels in the lowest third, the multivariable-adjusted odds ratios for women with levels in the middle and highest thirds of 6-sulfatoxymelatonin concentration were 0.88 (95% CI: 0.60, 1.30) and 0.80 (95% CI: 0.53, 1.21), respectively (P-trend = 0.7).

Meta-analysis of prospective observational studies of melatonin and breast cancer risk

We identified 7 prospective studies, including the current study, of the risk of breast cancer in relation to the major urinary metabolite of melatonin, 6-sulfatoxymelatonin (8–12, 20). One study used measurements of urinary 6-sulfatoxymelatonin levels in spot urine samples (20) and was not considered eligible for the current meta-analysis because randomly timed, spot-urine-derived 6-sulfatoxymelatonin levels are not informative as surrogates of nocturnal melatonin production. Two of the studies included women from the Guernsey cohort (the study by Travis et al. (8) and the current study); however, for consistency with other published studies in terms of the type of urine sample assayed and to avoid including women twice, we included in the meta-analysis only the data from the current Guernsey study (6-sulfatoxymelatonin in first-morning voids). Altogether, the meta-analysis included data from 1,113 women with incident breast cancer (in situ and invasive cancers combined) and 2,944 matched control participants (Figure 2). When results from these 5 studies were combined, the aggregate odds ratio was 0.81 (95% CI: 0.66, 0.99) for women in the highest fourth of urinary 6-sulfatoxymelatonin concentration versus women in the lowest fourth, with no significant heterogeneity in estimates between the studies (P for heterogeneity = 0.09). There was no significant heterogeneity in the association between premenopausal and postmenopausal women (P for heterogeneity = 0.09) based on data from the current study and 3 other published studies (10–12); 6-sulfatoxymelatonin levels were not significantly associated with risk among premenopausal women (for highest fourth vs. lowest fourth, aggregate OR = 1.05, 95% CI: 0.71, 1.54), while there was a significant inverse association in postmenopausal women (OR = 0.68, 95% CI: 0.49, 0.92).

DISCUSSION

In this study of British women, the risk of breast cancer was not significantly associated with levels of urinary

Table 3. Relationships Between First-Morning Urinary 6-Sulfatoxymelatonin Excretion (Natural Logarithmic Values) and Selected Characteristics of Control Participants, Guernsey Study, 1977–2009

	No. of Controls	6-Sulfatoxymelatonin Level[a]				*P* Value[a,b]
		% Change per Unit[c]	95% CI	Geometric Mean, ng/mg creatinine	95% CI	
Age at urine sample collection, years[d]	727	−2.0	−2.8, −1.2			<0.0001
Body mass index[e]	727	−0.8	−2.4, 0.8			0.3
Storage time, years	727	0.05	−4.9, 5.0			1.0
Age at menarche, years	724	0.3	−3.7, 4.4			0.9
Age at natural menopause, years	178	1.8	−2.6, 6.2			0.4
Parity						0.7
Nulliparous	76			12.7	10.5, 15.4	
Parous	651			13.2	12.4, 14.0	
Age at first birth among parous women, years						0.05
<25	324			12.3	11.2, 13.5	
≥25	327			14.0	12.8, 15.3	
Menstrual cycle phase among premenopausal women						0.9
Early follicular	60			14.1	11.3, 17.6	
Late follicular	113			13.9	11.7, 16.5	
Midcycle	75			11.9	9.7, 14.7	
Early luteal	156			13.2	11.4, 15.4	
Late luteal	55			13.6	10.7, 17.2	
Menopausal status						0.9
Premenopausal	465			13.2	11.9, 14.7	
Postmenopausal	179			13.2	10.7, 16.2	
Perimenopausal	83			12.5	10.2, 15.3	
Ever use of oral contraceptives						0.4
No	348			12.7	11.6, 14.0	
Yes	349			13.5	12.3, 14.8	
Ever use of other hormones						0.7
No	611			13.2	12.4, 14.1	
Yes	107			12.7	10.8, 15.0	
First-degree family history of breast cancer						0.8
No	684			13.1	12.3, 13.9	
Yes	43			13.4	10.5, 17.2	
Season of urine collection						0.6
Spring	191			12.9	11.4, 14.5	
Summer	214			12.4	11.1, 13.9	
Autumn	165			13.6	12.0, 15.4	
Winter	157			13.9	12.2, 15.8	

Abbreviation: CI, confidence interval.
[a] All values were adjusted for age at urine collection and assay batch unless otherwise specified.
[b] *P* value for test of linear trend (continuous variables) or test of heterogeneity (categorical variables) from analyses of covariance.
[c] Change in 6-sulfatoxymelatonin concentration (ng/mg creatinine) per unit increase in the specified variable.
[d] Values were adjusted for assay batch only.
[e] Weight (kg)/height $(m)^2$.

6-sulfatoxymelatonin, as measured in first-morning urine voids. These results are consistent with those from our previous investigation of 6-sulfatoxymelatonin in 24-hour urine samples (8). However, the results from the meta-analysis suggest that an inverse association between melatonin and breast cancer risk cannot be ruled out and should be investigated further. To our knowledge, this is the first prospective study to have assessed the relationship between melatonin

Table 4. Urinary 6-Sulfatoxymelatonin Excretion Among Case Patients and Control Participants, Overall and by Menopausal Status, Guernsey Study, 1977–2009

	No. of Women	Age- and Batch-Adjusted 6-Sulfatoxymelatonin Level			Multivariable-Adjusted 6-Sulfatoxymelatonin Level[a]		
		Geometric Mean, ng/mg creatinine	95% CI	*P* Value[b]	Geometric Mean, ng/mg creatinine	95% CI	*P* Value[b]
All women							
Cases	251	12.8	11.6, 14.2	0.7	12.9	11.6, 14.2	0.8
Controls	727	13.1	12.4, 14.0		13.1	12.4, 13.9	
Premenopausal women							
Cases	160	14.5	12.9, 16.3	1.0	14.6	13.0, 16.4	1.0
Controls	465	14.6	13.6, 15.6		14.6	13.6, 15.6	
Postmenopausal women							
Cases	62	10.5	8.4, 13.2	0.9	10.6	8.5, 13.3	1.0
Controls	179	10.7	9.4, 12.2		10.7	9.4, 12.2	

Abbreviation: CI, confidence interval.
[a] Adjusted for age, assay batch, body mass index, storage time, season of urine collection, parity, and age at first birth.
[b] *P* for heterogeneity of case and control mean values from analyses of covariance.

excreted overnight (i.e., measured in a first-morning void) and melatonin excreted over a 24-hour period and to have examined the relationship with breast cancer risk using both measures.

Our null results in the Guernsey III Study are comparable with those from 2 previous studies of melatonin and breast cancer risk, which after multivariable adjustment also found no significant trend in breast cancer risk with increasing 6-sulfatoxymelatonin level and no significant reduction in risk among women in the highest fourth of 6-sulfatoxymelatonin (9, 10). However, 2 other studies have found significant but opposing trends in risk of breast cancer in relation to nocturnal melatonin level (11, 12). It has been suggested that some of these inconsistencies might be attributable, in part, to the varying sample collection methods (21), with power to detect an association between breast cancer risk and nocturnal melatonin levels possibly having been limited in the previous Guernsey study (8) because the measurement of 6-sulfatoxymelatonin

Table 5. Risk of Breast Cancer According to Tertile Group (Third) of Urinary 6-Sulfatoxymelatonin Excretion, Overall and by Menopausal Status, Guernsey Study, 1977–2009

6-Sulfatoxymelatonin Level, ng/mg Creatinine	No. of Cases	No. of Controls	Age-Adjusted		Multivariable-Adjusted[a]		
			OR	95% CI	OR	95% CI	*P* Trend[b]
All women							0.8
<10.8	88	243	1.00	Referent	1.00	Referent	
10.8–20.4	84	242	0.95	0.67, 1.35	0.88	0.61, 1.27	
>20.4	79	242	0.89	0.61, 1.30	0.90	0.61, 1.33	
Premenopausal women							0.9
<12.1	55	155	1.00	Referent	1.00	Referent	
12.1–21.7	54	155	1.00	0.64,1.56	0.89	0.56, 1.41	
>21.7	51	155	0.95	0.60, 1.52	0.86	0.53, 1.41	
Postmenopausal women							0.7
<8.3	21	60	1.00	Referent	1.00	Referent	
8.3–17.0	23	60	1.11	0.55, 2.24	1.20	0.57, 2.55	
>17.0	18	59	0.90	0.43, 1.88	1.06	0.47, 2.38	

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] ORs were conditioned on the matching variables and adjusted for age at urine collection, body mass index, season of urine collection, urine storage time, first-degree family history of breast cancer, age at menarche, ever use of oral contraceptives, ever use of other hormones, parity, and age at first birth. Values for premenopausal women were additionally adjusted for menstrual cycle phase, and values for postmenopausal women were additionally adjusted for age at menopause.
[b] *P* for trend from a multivariate model with ln(6-sulfatoxymelatonin) as a continuous variable.

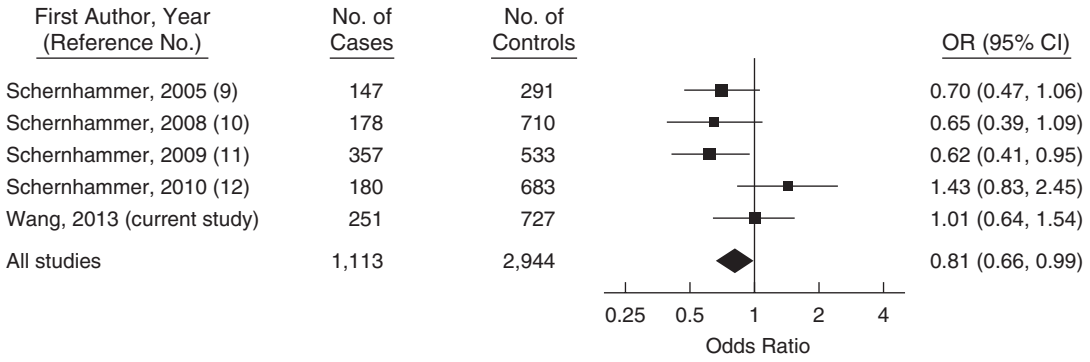

Figure 2. Results from prospective studies of 6-sulfatoxymelatonin level and breast cancer risk and from a meta-analysis of breast cancer odds ratios (ORs) among women in the highest 6-sulfatoxymelatonin quartile group (fourth) versus the lowest fourth, 1977–2009. The area of each square is inversely proportional to the variance of the logarithm of the OR and hence proportional to the amount of statistical information available for that particular estimate. The diamond (the lateral points of which are the 95% confidence intervals (CIs)) represents the overall OR. For each study, the quartile cutpoints were defined according to the distribution of urinary 6-sulfatoxymelatonin levels among controls. The lower and upper quartile cutpoints, respectively, and the units of measurement for each study were as follows: Nurses' Health Study II (9), <11.5 ng and ≥29.0 ng of 6-sulfatoxymelatonin per mg of creatinine in first-morning urine samples; Hormones and Diet in the Etiology of Breast Cancer Risk (ORDET) Study (postmenopausal women) (10), <6.5 μg and ≥16.5 μg of urinary 6-sulfatoxymelatonin output per 12 hours; Nurses' Health Study (11), <10.2 ng/mL and ≥34.3 ng/mL of 6-sulfatoxymelatonin per mg of creatinine in first-morning urine samples; ORDET Study (premenopausal women) (12), <10.1 μg and ≥20.6 μg of urinary 6-sulfatoxymelatonin output per 12 hours; and Guernsey Study (current study), <8.4 ng and ≥23.9 ng of 6-sulfatoxymelatonin per mg of creatinine in first-morning urine samples.

was made in 24-hour urine samples rather than in early-morning or overnight urine collections. However, data from the current study showed a moderate correlation between 6-sulfatoxymelatonin measured in first-morning samples and that from 24-hour urine samples ($r = 0.66$), suggesting that differences in urine collection are unlikely to explain the different associations found between studies.

An alternative explanation for the inconsistent findings between studies of 6-sulfatoxymelatonin and breast cancer risk may be chance, given the small sample sizes in the individual studies. The current study, for example, had approximately 80% power at a statistical significance level of 0.05 to detect a breast cancer relative risk of 0.6 among women in the highest third of 6-sulfatoxymelatonin concentration versus women in the lowest third, but it had limited ability to detect a more modest association. In contrast, the meta-analysis conducted here, with a total of 1,113 breast cancer cases, had 80% power to detect an odds ratio of 0.77 for the highest quartile of 6-sulfatoxymelatonin versus the lowest quartile (given a 1:3 ratio of cases to controls). Another consideration when interpreting the findings of the meta-analysis is the potential influence of publication bias. For example, we are unable to exclude the possibility that the observed inverse association was due to bias arising from systematic differences between published and unpublished data, with null results from other studies being less likely to have been published. It is unlikely, however, that there are many unpublished data on the relationship between melatonin levels and breast cancer, given that there have been relatively few large cohort studies with the necessary urine specimens to be able to assess the association (prediagnostic 24-hour, overnight, or first-morning samples).

Taken together, the findings from the current meta-analysis suggest that women with relatively high levels of melatonin (within the normal physiological range) may have a lower risk of breast cancer. Several mechanisms have been proposed through which melatonin might reduce breast cancer development, including the direct growth-inhibitory and oncostatic effects of melatonin as well as indirect pathways involving altered endogenous hormone levels, but there are limited data in humans (22–25). Our finding in the meta-analysis of no significant heterogeneity in the 6-sulfatoxymelatonin–breast cancer relationship by menopausal status provides limited support for a role of hormone-related mechanisms linking melatonin to breast cancer risk, and more data are needed for robust subgroup analyses.

There is limited understanding of the genetic and lifestyle determinants of melatonin concentration. Several studies have found lower melatonin levels among night workers (reviewed by Davis et al. (26)). Previously, we (8) and others have variously reported lower levels of 6-sulfatoxymelatonin in nulliparous women, in older women, and in women with a higher body mass index (reviewed by Dopfel et al. (27)). In the current study, however, while 6-sulfatoxymelatonin levels were lower in older women, we did not observe strong associations with body mass index or parity. Although it has been hypothesized that some other lifestyle and dietary factors, such as smoking and intakes of alcohol, caffeine, and selected nutrients (28–30), may affect circulating melatonin levels, we were unable to adjust for these factors, as such information was not available in the Guernsey III Study.

It was estimated that approximately half of the occupational burden of cancer in British women may be attributable to the apparent increase in breast cancer risk among shift workers, if there is a causal relationship (31). Given that circadian disruption involving suppressed melatonin in night workers is the primary hypothesized mechanism for the association, there is a need to establish the role of melatonin levels, if any, in

the development of breast cancer. Clearly, further prospective data on the relationship of melatonin to breast cancer risk are required, although this is currently limited by the low numbers of large cohort studies that have collected overnight or early-morning urine samples. One recent study investigated the use of morning serum samples as a suitable medium for assessing nocturnal melatonin levels and found serum measurements to be moderately correlated with 24-hour urine values ($r = 0.46$) (32). Further work is needed on the development and validation of these methods, which might make it possible to study melatonin levels in cohorts with stored blood specimens. Finally, considering the null findings in this British study and the modest association in the meta-analysis, further research into other possible explanations for an association between shift work and breast cancer, such as residual confounding (33) and other putative mechanisms, is warranted—including, for example, the roles of shift-work-associated sleep disturbance and lifestyle changes (34).

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