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Research Article

Urinary Levels of Melatonin and Risk of Postmenopausal Breast Cancer: Women's Health Initiative Observational Cohort

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Abstract

Background: Results from prospective studies on the association between urinary levels of melatonin and risk of postmenopausal breast cancer have been mixed. Several although not all studies have found lower urinary levels of melatonin in women who developed breast cancer compared with cancer-free women.

Methods: We examined the association between urinary levels of melatonin and breast cancer risk in postmenopausal women in a case–control study nested in the Women's Health Initiative Observational Cohort. Levels of 6-sulfatoxymelatonin were measured in first morning voids from 258 women who later developed breast cancer and from 515 matched controls. Multivariable conditional logistic regression was used to calculate ORs and 95% confidence intervals (CI).

Results: Fully adjusted risk estimates of breast cancer, relative to the lowest quartile level of creatinine-adjusted melatonin, were 1.07 (95% CI, 0.67–1.71), 1.26 (95% CI, 0.79–2.01), and 1.25 (95% CI, 0.78–2.02) for women in the second, third, and highest quartile ($P_{trend} = 0.27$). Comparable results for cases diagnosed less than four years after urinary collection and matched controls were 1.0, 1.25 (95% CI, 0.51–3.06), 1.85 (95% CI, 0.75–4.57), and 1.94 (95% CI, 0.75–5.03; $P_{trend} = 0.11$). Melatonin levels and breast cancer were not associated in cases diagnosed four or more years after urinary collection and matched controls ($P_{trend} = 0.89$).

Conclusions: We found no evidence that higher urinary levels of melatonin are inversely associated with breast cancer risk in postmenopausal women.

Impact: Accumulating discrepancies in results across studies warrant further exploration. *Cancer Epidemiol Biomarkers Prev; 23(4); 629–37. ©2014 AACR.*

Introduction

Elevated circulating levels of the hormone melatonin may have a role in the prevention of estrogen-related breast cancer. Produced by the pineal gland in a circadian pattern, melatonin is normally very low throughout the day, higher in the evening, and at a peak in the early morning hours. The presence of darkness, although not sleep, is necessary for melatonin production (1). As night shift work may be a marker of increased light exposure at night, suppression of melatonin production among women working in the night shift might explain the putative association between night shift work and increased breast cancer risk (2, 3). In addition to light at night, other factors have been associated with lower melatonin levels, including higher body mass index, increased parity, and older age among adults (4–6).

Laboratory studies suggest several potential mechanisms by which melatonin may block the effects of estrogen on breast cancer development and progression (7). First, elevated circulating levels of melatonin may prevent pituitary gonadotropin release, thereby downregulating production of ovarian estrogens (8). Second, melatonin has been shown to reduce breast tumor cell growth by interfering with estrogen signaling pathways. Finally, melatonin can inhibit aromatase activity, and by this reduce the peripheral conversion of androgens to estrogens. As reviewed by Viswanathan and colleagues (9), melatonin may also alter breast cancer risk through other mechanisms, including immune, and the antioxidant pathways.

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Results from prospective studies on the association between urinary levels of melatonin and risk of breast cancer in postmenopausal women have been mixed. Several have found lower urinary levels of melatonin in women who went on to develop breast cancer compared with those who did not (10, 11). Others have not observed an inverse association (12, 13), although the null results observed in these studies may be related to issues surrounding the timing of urine collection. Prospective studies of premenopausal breast cancer have also been inconsistent, showing inverse (14), positive (15), and null associations (12). We therefore examined the relationship between urinary levels of melatonin and risk of postmenopausal breast cancer in the Women's Health Initiative Observational Cohort (WHI-OS) with its unique resource of prediagnostic first morning urine specimens.

Materials and Methods

The Observational Study arm of the WHI recruited women in 40 clinical centers from September 1993 to December 1998, enrolling a total of 93,676 women. Eligible women were between the ages of 50 to 79 years old, postmenopausal at enrollment (no menstrual cycles for at least 12 months before enrollment if 54 years old or younger and 6 months if 55 years old or older) with the intention to reside in the area for at least 3 years after enrollment. Exclusion criteria included any medical condition that had a predicted survival rate of less than 3 years, as well as any condition that may have limited the ability to comply or stay within the study, such as alcohol or drug dependency, mental illness, dementia, or active participation in another randomized control trial (16). Written informed consent was obtained from study participants and Institutional Review Boards at all clinics approved the WHI-OS. The Institutional Review Board at the University of Massachusetts Amherst (Amherst, MA) approved this ancillary analysis.

Analyses for this study are restricted to women who enrolled at three clinical centers (Birmingham, Pittsburgh, and Tucson) that participated in a bone density substudy requiring a urine collection at or near the time of enrollment in the WHI-OS. A total of 329 centrally adjudicated invasive breast cancer cases occurred in the three sites through September 2010.

We established a nested case–control study of invasive postmenopausal breast cancer, selecting two controls for each case from the risk set at the time of the case's event. Cases and controls were matched on age at enrollment (within 2 years), enrollment date (within 120 days), and randomization clinic. The matching algorithm was allowed to select the closest match based on criteria to minimize an overall distance measure (17). The overall distance measure was 3,402, with date of enrollment between the cases and the controls as the main contributor. A total of 24 cases and 712 controls were excluded for inadequate urine volume. A total of 46 cases and 834 controls were also excluded that had a self-reported history of any cancer except nonmelanoma skin cancer before WHI-OS enrollment. In addition, as part of the WHI standard protocol to preserve biologic specimens, controls were excluded that had diagnosis of a primary WHI outcome (coronary heart disease, stroke, hip fracture, and colon cancer) up until the end of follow-up. There were a total of 6,874 study participants without breast cancer at the three sites, and the total number of eligible controls was 4,724. The final nested case–control study consisted of 260 cases and 519 controls. No *in situ* breast cancer cases were included in the control group.

Determination of breast cancer outcomes, including adjudication protocols, within the WHI has been described previously (18). Briefly, participants self-reported cancer diagnoses on annual questionnaires. Physician adjudicators confirmed these self-reported outcomes using pathology reports and additional medical records. Data on demographics, family history of cancer, reproductive factors, recreational activity, history of smoking and alcohol intake, and other risk factors were collected through self-administered questionnaires at entry into the study. Weight and height were measured at baseline using standardized protocols.

Information on sleep duration was obtained at baseline by asking "about how many hours of sleep did you get in a typical night over the past 4 weeks?" Responses were given as integers, with choices of 5 or less, 6, 7, 8, 9, and 10 or more. Women were additionally asked to report their difficulty falling asleep, waking up several times at night, waking up earlier than desired, and difficulty falling back asleep after early waking over the past 4 weeks. Responses were given as integers, with choices of 0 (no, not in past 4 weeks), 1 (yes, less than one time per week), 2 (yes, 1–2 times per week), 3 (yes, 3–4 times per week), and 4 (yes, 5–6 times per week). Women also rated how restful their sleep typically was using a five-point scale ranging from 0 "very sound or restful" to 4 "very restless." On the basis of responses to the latter five questions, women were scored using the validated Women's Health Initiative Insomnia Rating Scale where higher values indicate greater perceived insomnia symptoms (19). A score of nine or higher indicates clinical insomnia.

The standard procedure at these three designated clinical centers participating in the bone density study specified the collection of the first morning midstream urine void after 5 am. Study participants were instructed to collect urine specimens at home and to keep the container on ice or refrigerated until leaving for the clinic visit. Urine samples were centrifuged for 5 minutes within 30 minutes of receipt at the clinic, and then frozen immediately at −70°C, or if necessary, frozen at −20°C for no more than 2 days and then transferred to a −70° C freezer.

Urinary 6-sulfatoxymelatonin was assessed through a competitive ELISA (Buhlmann Laboratories AG) and adjusted for creatinine levels to control for urine volume. Urinary creatinine was measured colorimetrically. A total of 40 masked duplicates were included across the three

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batches. The intra-assay coefficient of variation and inter-assay coefficient of variation for unadjusted melatonin and creatinine levels were 12.5% and 12.7%, and 9.1% and 5.8%, respectively. Cases and matched controls were arranged within the same batch. One case had a missing melatonin level and one control had a melatonin level that was an obvious outlier (1,971.28 ng/mg). These participants were removed from analysis, along with their matched pairs. Creatinine-adjusted melatonin level was classified into quartiles based on the distribution in the controls. We also conducted analyses using unadjusted melatonin levels, and results were essentially the same as findings based on creatinine-adjusted melatonin (data not shown).

Multivariable conditional logistic regression was used to calculate ORs and 95% confidence intervals (CI). To address potential confounding, we included covariates identified in previous studies as known risk factors for breast cancer established within WHI as well as covariates included in other studies assessing the relationship between melatonin levels and breast cancer risk. An initial multiple predictor conditional logistic regression model was fit with the following predictors: ethnicity, education level, family history of breast cancer, body mass index, age at first live birth, number of live births, age of menopause, bilateral oophorectomy, history of prior breast biopsy, energy expenditure from recreational activities [measured as metabolic equivalent of task (METs)/week], duration of estrogen and progesterone use, and time since quitting hormone replacement therapy. A variable was retained for inclusion in subsequent models if its associated likelihood ratio test was statistically significant at $P < 0.10$ or if the factor was considered clinically significant.

We assessed linear trend by testing the significance of melatonin categorized into quartiles and modeled as an ordinal variable.

Results

As shown in Table 1, mean creatinine-adjusted melatonin levels were similar in cases and controls: 16.3 ng/mg (SD = 11.9) for cases and 16.1 ng/mg (SD = 12.9 for controls. As shown in Table 1, cases were slightly heavier, more educated, more likely to have a family history of breast cancer, more likely to have had a prior breast biopsy, and less likely to have had a bilateral oophorectomy than controls (Table 1). A total of 12.0% of cases were Black or African American compared with 11.6% of controls. Comparable figures for Hispanic/Latinos were 3.5% and 6.2%. Of the 258 invasive breast cancer tumors, 176 (68.2%) were estrogen receptor–positive (ER^+), 36 (14.0%) were ER^-, and 46 (17.8%) had either uncertain or missing receptor status information. Mean creatinine-adjusted melatonin levels for ER^+ tumor cases ($n = 176$) and their

Table 1. Nested case–control study: baseline characteristics for 258 cases and 515 control subjects

	Cases, $n = 258$	Controls, $n = 515$	P
Mean urinary melatonin (ng/mg of creatinine; SD)	16.3 (11.9)	16.1 (12.9)	0.80
Mean urinary melatonin (ng; SD)	11.6 (11.1)	12.3 (12.5)	0.43
Mean creatinine (mg; SD)	0.79 (.46)	0.76 (0.45)	0.48
Mean age at enrollment (y; SD)	63.6 (7.2)	63.5 (7.1)	0.82
Mean body mass index (kg/m^2; SD)	28.5 (6.1)	27.5 (6.1)	0.04
Mean total energy expenditure from recreational activity (MET h/wk; SD)	11.9 (12.8)	12.9 (14.6)	0.37
Mean alcohol intake (servings/wk; SD)	2.4 (6.1)	2.1 (4.6)	0.41
Mean age at menopause[a] (y; SD)	48.8 (6.6)	48.1 (6.8)	0.16
% White	81.8	81.2	0.87
% >High school education	65.9	57.3	0.002
% Bilateral oophorectomy	12.4	20.6	0.02
% Family history of breast cancer in first-degree relative	22.1	14.6	0.02
% Prior breast biopsy	33.1	22.0	0.001
% Age of menarche <12 y	23.3	22.5	0.46
% Nulliparous	14.3	11.1	0.55
% Age at first birth <20 y[b]	21.1	17.1	0.38
% Ever oral contraceptive users	35.3	37.9	0.48
% Ever estrogen and progesterone use	27.5	24.7	0.39
% Ever smoked	51.6	54.0	0.33
% $\leq$ 6 hours versus 9+ hours of sleep	33.7 vs. 5.4	33.0 vs. 5.4	0.93
% Insomnia	29.7	28.6	0.70

[a]Restricted to 726 women with natural menopause.
[b]Restricted to 608 parous women.

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matched controls ($n = 351$) were 15.5 (SD = 12.0) and 15.9 ng/mg (SD = 12.3), respectively ($P = 0.71$].

The distribution of demographic and lifestyle factors by quartile level of median creatinine-adjusted melatonin in control subjects is presented in Table 2. The proportion of women who were overweight decreased with increasing quartile of creatinine-adjusted melatonin, whereas the proportion of control subjects that were more educated tended to increase. The proportion of White women also increased from the lowest quartile to the highest quartile level of creatinine-adjusted melatonin. In contrast, the proportion of Black/African American women tended to decline with increasing quartile of melatonin: 12.6%, 16.9%, 10.8%, and 6.2%. A similar decrease in the percentage of Hispanic/Latino women was observed with increasing quartile level of melatonin: 7.1%, 7.7%, 6.2%, and 3.9%. Among parous women, the proportion of women with three or more children tended to increase with increasing melatonin level (58.8%, 61.4%, 78.2%, and 75.2% in the lowest to highest quartile level).

The fully adjusted risk estimates of breast cancer, relative to the lowest quartile level of creatinine-adjusted melatonin, were 1.07 (95% CI, 0.67–1.71), 1.26 (95% CI, 0.79–2.01), and 1.25 (95% CI, 0.78–2.02) for women in the second, third, and highest quartile of creatinine-adjusted melatonin, respectively ($P_{trend} = 0.27$; Table 3). No trend in risk emerged with increasing quartile level of melatonin in analyses restricted to 176 ER^+ cases and the 351 matched control subjects. After excluding women who were current smokers, the comparable risk estimates and 95% CI were 1.0, 1.22 (0.72–2.06), 1.45 (0.86–2.44), and 1.46 (0.48–1.68) for all breast cancer and 1.0, 0.90 (0.48–1.68), 1.15 (0.60–2.22), and 1.03 (0.53–2.01) for ER^+ breast cancer only. In additional analyses restricted to White women only, and in analyses restricted to women with no prior oophorectomy, results were essentially unchanged from the data presented in Table 3.

As shown in Table 4, further analyses examined risk estimates according to two time intervals between urinary collection and diagnosis (≤4 years, >4 years). We selected this cutpoint based on a prior analysis that observed a more substantial decrease in risk of developing breast cancer when this lag time was applied (11). To aid in comparison, we present these results using same quartile cutpoints for melatonin levels as in the main analysis; however, results were nearly identical when we used cutpoints developed separately for the ≤4 and >4 years analyses (data not shown). Among 80 cases diagnosed within 4 years of their urinary collection and 160 matched control subjects, fully adjusted risk estimates were 1.0, 1.25 (95% CI, 0.51–3.06), 1.85 (95% CI, 0.75–4.57), and 1.94 (0.75–5.03) for women in the lowest, second, third, and highest quartiles of creatinine-adjusted melatonin level, respectively ($P_{trend} = 0.11$). No pattern of decreasing risk

Table 2. Control subjects: baseline characteristics for 515 control subjects by quartile level of melatonin (ng/mg of creatinine)

	Q1	Q2	Q3	Q4	
	<6.7	[6.7–12.8)	[12.8–22.2)	≥22.2	P_{trend}
Mean age at enrollment (y; SD)	63.4 (7.4)	63.9 (7.5)	63.8 (6.3)	62.7 (7.3)	0.43
Mean body mass index (kg/m^2; SD)	28.8 (5.7)	27.6 (6.0)	28.0 (6.9)	25.9 (5.2)	≤.01
Mean total energy expenditure from recreational activity (MET h/wk; SD)	10.9 (13.5)	14.8 (17.1)	11.5 (13.2)	14.4 (13.9)	0.22
Mean alcohol intake (servings/wk; SD)	2.4 (5.4)	1.7 (3.4)	2.0 (5.1)	2.2 (4.2)	0.18
Mean age at menopause (y; SD)[a]	47.3 (7.7)	48.3 (6.4)	48.7 (6.1)	48.0 (6.8)	0.36
% White	78.7	74.6	82.2	89.2	0.02
% > High school education	50.0	57.4	52.4	67.4	0.02
% Bilateral oophorectomy	25.2	20.0	16.3	20.9	0.31
% Family history of breast cancer in first-degree relative	14.2	13.8	17.0	13.2	0.71
% Prior breast biopsy	28.0	22.2	19.0	19.2	0.08
% Age of menarche <12 y	23.6	22.3	23.3	21.1	0.69
% Nulliparous	10.2	13.3	10.9	10.2	0.48
% Age at first birth <20 y[b]	20.6	16.5	12.6	18.8	0.82
% Ever oral contraceptive user	37.8	44.6	33.3	35.7	0.35
% Ever estrogen and progesterone use	26.8	30.8	17.8	23.3	0.17
% Ever smoked	44.4	42.6	34.7	59.4	0.05
% ≤ 6 h of sleep	34.7	41.7	26.4	30.5	0.09
% Insomnia	29.2	28.2	29.2	23.6	0.56

[a]Restricted to 726 women with natural menopause.
[b]Restricted to 608 parous women.

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Table 3. Adjusted RRs for quartile levels of creatinine-adjusted melatonin (ng/mg of creatinine) for all breast cancer combined and for ER^+ breast cancer only

	Cases	Controls	RR (95% CI)	P_{trend}	RR[a] (95% CI)	P_{trend}
All cases						
Q1 (<6.69)	58	127	1.0 (ref.)	0.41	1.0 (ref.)	0.27
Q2 [6.69–12.81)	60	130	1.02 (0.66–1.56)		1.07 (0.67–1.71)	
Q3 [12.81–22.18)	74	129	1.26 (0.82–1.94)		1.26 (0.79–2.01)	
Q4 (≥22.18)	66	129	1.13 (0.73–1.75)		1.25 (0.78–2.02)	
ER^+ cases						
Q1 (<6.69)	48	89	1.0 (ref.)	0.84	1.0 (ref.)	0.80
Q2 [6.69–12.81)	40	90	0.83 (0.55–1.38)		0.82 (0.48–1.42)	
Q3 [12.81–22.18)	47	87	0.99 (0.58–1.69)		0.93 (0.53–1.65)	
Q4 (≥22.18)	41	85	0.89 (0.53–1.51)		0.88 (0.50–1.56)	

Abbreviation: RR, relative risk.

[a]Adjusted for body mass index, education, and bilateral oophorectomy.

with increasing quartile level of melatonin was observed for cases diagnosed more than 4 years after urinary collection and their matched controls, or even when we lagged exposure 7 or more years (data not shown). In ER^+ breast cancer cases diagnosed within 4 years of urinary collection and matched control subjects, fully adjusted risk estimates were 1.0, 0.64 (95% CI, 0.20–2.04), 1.49 (95% CI, 0.50–4.43), and 0.90 (95% CI, 0.27–2.95) with increasing quartile levels of creatinine-adjusted melatonin level, respectively ($P_{trend} = 0.72$). Results were similarly null for ER^+ breast cancer diagnosed more than 4 years after urinary collection and matched control subjects. Similar patterns were observed in the two time intervals for all cancers and ER^+ cancer when results were restricted to nonsmokers only (data not shown).

In secondary analyses, we applied the same exclusion criteria used for selecting control subjects to the breast cancer case subjects (i.e., exclusion of women with

Table 4. Adjusted RR for quartile levels of creatinine-adjusted melatonin (ng/mg of creatinine) for all breast cancer combined and for ER^+ breast cancer alone by time interval between urinary collection and diagnosis

	Cases	Controls	RR (95% CI)	P_{trend}	RR[a] (95% CI)	P_{trend}
All cases, ≤4 y						
Q1 (<6.69)	17	46	1.0 (ref.)	0.18	1.0 (ref.)	0.11
Q2 [6.69–12.81)	21	43	1.37 (0.63–3.01)		1.25 (0.51–3.06)	
Q3 [12.81–22.18)	21	35	1.71 (0.77–3.83)		1.85 (0.75–4.57)	
Q4 (≥22.18)	21	36	1.69 (0.74–3.84)		1.94 (0.75–5.03)	
All cases, >4 y						
Q1 (<6.69)	38	74	1.0 (ref.)	0.93	1.0 (ref.)	0.89
Q2 [6.69–12.81)	36	79	0.89 (0.52–1.53)		0.97 (0.54–1.75)	
Q3 [12.81–22.18)	48	84	1.12 (0.65–1.91)		1.09 (0.60–1.97)	
Q4 (≥22.18)	37	80	0.90 (0.52–1.56)		1.00 (0.55–1.83)	
ER^+ cases, ≤ 4 y						
Q1 (<6.69)	14	30	1.0 (ref)	0.65	1.0 (ref)	0.72
Q2 [6.69–12.81)	13	31	0.94 (0.36–2.46)		0.64 (0.20–2.04)	
Q3 [12.81–22.18)	15	22	1.49 (0.55–4.03)		1.49 (0.50–4.43)	
Q4 (≥22.18)	12	25	1.10 (0.39–3.13)		0.90 (0.27–2.95)	
ER^+ cases, >4 y						
Q1 (<6.69)	28	51	1.0 (ref.)	0.71	1.0 (ref.)	0.72
Q2 [6.69–12.81)	32	65	0.90 (0.49–1.64)		0.94 (0.50–1.79)	
Q3 [12.81–22.18)	34	69	0.89 (0.47–1.66)		0.82 (0.41–1.63)	
Q4 (≥22.18)	28	58	0.88 (0.46–1.68)		0.92 (0.46–1.83)	

Abbreviation: RR, relative risk.

[a]Adjusted for body mass index, education, and bilateral oophorectomy.

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incident coronary heart disease, stroke, colon cancer, and hip fracture through September 2010). Of the 258 cases, a total of 12, 6, 2, and 11 were diagnosed with coronary heart disease, stroke, colon cancer, and hip fracture, respectively. The mean baseline creatinine-adjusted melatonin level in the 258 cases tended to be slightly lower in those who developed these conditions compared with those that did not but the difference was not statistically significant (14.1 vs. 16.6 ng/mg; $P = 0.27$). Results based on the restricted case group and their matched controls were very similar to those presented in our main analysis. For example, the fully adjusted risk estimate comparing the highest to lowest quartile level of melatonin was 1.38 (95% CI, 0.82–2.33) for all cases and 0.95 (95% CI, 0.51–1.76) for ER^+ cases.

We also examined associations between measures of sleep duration and quality and breast cancer risk (Table 5). From the fully adjusted model, we found that compared with women who slept 6 hours or less, the risk of breast cancer was 1.00 (95% CI, 0.68–1.47), and 0.91 (95% CI, 0.59–1.41) for those who slept 7 hours and 8 or more hours, respectively. Results were similar when we restricted our analyses to ER^+ tumors and matched controls. No association was observed between insomnia and overall breast cancer risk. Results did not change for sleep-related variables after adjusting for levels of creatinine-adjusted melatonin or body mass index (data not shown).

For all breast cancer cases and their matched controls, there was no evidence of multiplicative interaction between quartile levels of creatinine-adjusted melatonin and three categories of sleep duration ($P = 0.46$). In matched pairs without insomnia, patterns in the adjusted risk estimates with increasing quartile levels of creatinine-adjusted melatonin were generally similar to those presented in our main analysis. Among the 78 cases and 157 controls without insomnia, the risk estimates and 95% CI with increasing quartile levels of melatonin were 1.0, 0.55 (0.23–1.31), 0.83 (0.38–1.82), and 0.49 (0.21–1.18) for all cases ($P_{trend} = 0.25$). Comparable estimates were 1.0, 0.73 (0.28–1.91), 0.96 (0.41–2.26), and 1.04 (0.35–3.14; $P_{trend} = 0.99$) for ER^+ cancers based on 59 cases and 102 controls. There were too few women with insomnia to examine associations in this group separately.

Discussion

We found no evidence that higher urinary levels of melatonin were inversely associated with breast cancer risk in postmenopausal women. Our results conflict with two prior prospective studies that found inverse associations between urinary levels of melatonin and breast cancer risk in postmenopausal women (10, 11). Similar to our findings, results have been null in two other prospective studies of postmenopausal women (12, 20). However, the latter two studies relied on 24-hour urine collections or untimed spot urines for assessing melatonin levels. Studies that have observed an inverse association between urinary levels of melatonin and risk of postmenopausal breast cancer have used either a 12-hour overnight urine (10) or a spot morning void (11), both of which are considered to be more suited to capturing peak nighttime melatonin levels. Thus, strength of our study is that the first morning midstream spot void was the standard WHI protocol for urine collection. We note that results from studies of premenopausal women have also been mixed in the two studies that did have either a first

Table 5. Adjusted RR for measures of sleep for all breast cancer combined and for ER^+ breast cancer only

	Cases	Controls	RR (95% CI)	P_{trend}	RR (95% CI)[a]	P_{trend}
All cases						
Sleep duration						
<6 h	88	170	1.0 (ref.)	0.74	1.0 (ref.)	0.70
7 h	107	211	0.99 (0.70–1.42)		1.00 (0.68–1.47)	
≥8 h	63	132	0.93 (0.62–1.38)		0.91 (0.59–1.41)	
Insomnia						
No insomnia	177	354	1.0 (ref.)	0.73	1.0 (ref.)	0.72
Insomnia	77	148	1.06 (0.76–1.47)		0.93 (0.65–1.34)	
ER^+ cases						
Sleep duration						
<6 h	58	115	1.0 (ref.)	0.98	1.0 (ref.)	0.67
7 h	71	139	1.02 (0.66–1.59)		0.96 (0.60–1.54)	
≥8 h	47	96	0.99 (0.61–1.60)		0.89 (0.53–1.50)	
Insomnia						
No insomnia	129	238	1.0 (ref.)	0.32	1.0 (ref.)	0.28
Insomnia	45	104	0.81 (0.54–1.22)		0.83 (0.54–1.28)	

Abbreviation: RR, relative risk.

[a]Adjusted for education, bilateral oophorectomy, and first-degree relative with breast cancer.

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morning or 12-hour overnight urine collection, with both inverse (14) and positive (15) associations observed. Results were null in a third prospective study that used a 24-hour urine collection (12).

We found an elevated but not statistically significant risk of breast cancer among women who had higher urinary levels of melatonin and whose breast cancer occurred within 4 years of urine collection. In our study, the number of cases on whom urine was collected within 4 years before diagnosis was relatively low and the suggestive increased risk may have been a chance of subgroup finding. Alternatively, this finding may be indicative of a preclinical effect of breast cancer on urinary levels of melatonin, a possibility that has been raised previously in the only study to report an overall positive association between urinary levels of melatonin and premenopausal breast cancer risk (15). Contrary to the ORDET cohort study findings (15), we found no indication of a decreased risk of breast cancer with higher urinary levels of melatonin when we restricted our analyses to nonsmokers, lagged exposure by 4 or more years after urinary collection, or both.

An early cross-sectional study found that peak night time plasma melatonin levels were lower in patients with breast cancer who had ER^+ tumors than in healthy controls or in patients who had ER^- tumors (20). However, prospective studies conducted in postmenopausal women have not observed heterogeneity in risk estimates according to tumor receptor status (10, 11). We also found no evidence that higher urinary levels of melatonin are associated with lower risk of ER^+ tumors. One study has suggested that the association between urinary levels of melatonin and postmenopausal breast cancer risk is somewhat stronger for *in situ* tumors than invasive tumors (11), but our study did not include *in situ* breast cancer cases.

One possible limitation of our study is that, according to the WHI processing manual, urines were to be collected at the time of the study visit if a woman forgot to collect a first morning void, but we were unable to identify women whose urines, if any, may have been affected. An additional caveat is that many postmenopausal women may void during the night so that "first morning" urine may not reflect a true overnight first void. Providing support for the adequate classification of melatonin levels in our study, however, we did observe the expected associations between urinary melatonin levels with obesity, education, and increased parity that have been previously reported (5). Another potential limitation is that we had a one-time measure of melatonin, although urinary melatonin concentrations have been previously shown to be reasonably stable over 3 to 5 years (21, 22).

The methods of control selection in this nested case–control study deserve further consideration. To preserve biologic specimens for future studies, the WHI protocol required that individuals who went on to develop certain conditions (coronary heart disease, stroke, hip fracture, and colon cancer) were excluded from potential control selection. Although epidemiologic evidence is still limited, lower levels of melatonin may be associated with increased risk of cardiovascular disease (23, 24) and related conditions including diabetes and hypertension (25, 26), and osteoporosis (27). As a result, it is possible that our control group that is depleted of women who went on to develop coronary heart disease, stroke, and hip fracture, had, on average, higher levels of melatonin levels than if the WHI exclusionary criteria for matched control selection had not been applied. Under this scenario, the control selection procedure applied in tour study would have tended to exaggerate an inverse association between urinary levels of melatonin and risk of breast cancer. Our failure to find an inverse association, therefore, is unlikely to be explained by the control selection procedure. It is also reassuring that our findings were unchanged when we excluded breast cancer cases who went on to develop coronary heart disease, stroke, colon cancer, and hip fracture.

We also observed no association between sleep duration or with symptoms of insomnia and risk of breast cancer. Results from several prior cohort studies that have focused on duration of sleep have been conflicting, with two suggesting an inverse association (28, 29) and two finding no association (30, 31). In our study, sleep measurements were reflective of recent sleep patterns assessed only at a single time point and thus our inability to consider long-term sleep quality could have masked a true effect.

In summary, we observed no link between urinary levels of melatonin and breast cancer risk in postmenopausal women in a well-designed study using the first morning urine to assess melatonin levels, even when we lagged exposure measurements. We are uncertain why our results differ from two previous similar studies in postmenopausal women that found inverse associations (10, 11). However, we note that the number of invasive breast cancer cases in the highest quartile level of melatonin in each of these studies was smaller than ours (37 and 59, respectively) and that the observed reductions in risk comparing the highest to lowest quartile of melatonin were small to moderate (risk estimates ranging from 0.56 to 0.74). Furthermore, as outlined above, several prospective studies have reported results similar to ours (12, 13, 15), although these have tended to receive less weight because of potential concerns related to timing of urine collection and preclinical disease effects. In summary, the link between melatonin exposure and breast cancer development is an intriguing biologic hypothesis, but the accumulating discrepancies in results across studies warrant further exploration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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For a list of all the investigators who have contributed to WHI science, please visit: https://cleo.whi.org/researchers/SitePages/Write%20a%20Paper.aspx

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References

1. Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. Science 1980;210:1267–9.
2. Jia Y, Lu Y, Wu K, Lin Q, Shen W, Zhu W, et al. Does night work increase the risk of breast cancer? A systematic review and meta-analysis of epidemiological studies. Cancer Epidemiol 2013;37:197–206.
3. Kamdar BB, Tergas AI, Mateen FJ, Bhayani NH, Oh J. Night-shift work and risk of breast cancer: A systematic review and meta-analysis. Breast Cancer Res Treat 2013;138:291–301.
4. Dopfel RP, Schulmeister K, Schernhammer ES. Nutritional and lifestyle correlates of the cancer-protective hormone melatonin. Cancer Detect Prev 2007;31:140–8.
5. Schernhammer ES, Kroenke CH, Dowsett M, Folkerd E, Hankinson SE. Urinary 6-sulfatoxymelatonin levels and their correlations with lifestyle factors and steroid hormone levels. J Pineal Res 2006;40:116–24.
6. Stevens RG, Schernhammer E. Epidemiology of urinary melatonin in women and its relation to other hormones and night work. Cancer Epidemiol Biomarkers Prev. 2005;14:551.
7. Proietti S, Cucina A, Reiter RJ, Bizzarri M. Molecular mechanisms of melatonin's inhibitory actions on breast cancers. Cell Mol Life Sci 2013;70:2139–57.
8. Cohen M, Lippman M, Chabner B. Role of pineal gland in aetiology and treatment of breast cancer. Lancet 1978;2:814–6.
9. Viswanathan AN, Schernhammer ES. Circulating melatonin and the risk of breast and endometrial cancer in women. Cancer Lett 2009;281:1–7.
10. Schernhammer ES, Berrino F, Krogh V, Secreto G, Micheli A, Venturelli E, et al. Urinary 6-sulfatoxymelatonin levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2008;100:898–905.
11. Schernhammer ES, Hankinson SE. Urinary melatonin levels and postmenopausal breast cancer risk in the Nurses' Health Study cohort. Cancer Epidemiol Biomarkers Prev 2009;18:74–9.
12. Travis RC, Allen DS, Fentiman IS, Key TJ. Melatonin and breast cancer: A prospective study. J Natl Cancer Inst 2001;96:475–82.
13. Wu AH, Stanczyk FZ, Wang R, Koh WP, Yuan JM, Yu MC. Sleep duration, spot urinary 6-sulfatoxymelatonin levels and risk of breast cancer among Chinese women in Singapore. Int J Cancer 2013;132:891–6.
14. Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. J Natl Cancer Inst 2005;97:1084–7.
15. Schernhammer ES, Berrino F, Krogh V, Secreto G, Micheli A, Venturelli E, et al. Urinary 6-sulphatoxymelatonin levels and risk of breast cancer in premenopausal women: The ORDET cohort. Cancer Epidemiol Biomarkers Prev 2010;19:729–37.
16. Design of the Women's Health Initiative Clinical Trial and Observational Study. The Women's Health Initiative Study Group. Control Clin Trials 1998;19:61–109.
17. Bergstralh EJ, Kosanke JL. Computerized matching of cases to controls. Mayo Clinic, Rochester MN: Department of Health Sciences Research; 1995.
18. Curb JD, McTiernan A, Heckbert SR, Kooperberg C, Stanford J, Nevitt M, et al. Outcomes ascertainment and adjudication methods in the Women's Health Initiative. Ann Epidemiol 2003;13:S122–8.
19. Levine DW, Dailey ME, Rockhill B, Tipping D, Naughton MJ, Shumaker SA. Validation of the Women's Health Initiative Insomnia Rating Scale in a multicenter controlled clinical trial. Psychosom Med 2005;67:98–104.
20. Tamarkin L, Danforth D, Lichter A, DeMoss E, Cohen M, Chabner B, et al. Decreased noctural plasma peak in patients with estrogen receptor positive breast cancer. Science 1982;216:1003–5.
21. Schernhammer ES, Rosner B, Willett WC, Laden F, Colditz GA, Hankinson SE. Epidemiology of urinary melatonin in women and its relation to other hormones and night work. Cancer Epidemiol Biomarkers Prev 2004;13:936–43.
22. Travis RC, Allen NE, Peeters PH, van Noord PA, Key TJ. Reproducibility over 5 years of measurements of 6-sulphatoxymelatonin in urine samples from postmenopausal women. Cancer Epidemiol Biomarkers Prev 2003;12:806–8.
23. Brugger P, Marktl W, Herold M. Impaired nocturnal secretion of melatonin in coronary heart disease. Lancet 1995;345:1408.
24. Kitkhuandee A, Sawanyawisuth K, Johns NP, Kanpittaya J, Johns J. Pineal calcification is associated with symptomatic cerebral infarction. J Stroke Cerebrovasc Dis 2013;23:249–253.
25. McMullan CJ, Schernhammer ES, Rimm EB, Hu FB, Forman JP. Melatonin secretion and the incidence of type 2 diabetes. JAMA 2013;309:1388–96.
26. Forman JP, Curhan GC, Schernhammer ES. Urinary melatonin and risk of incident hypertension among young women. J Hypertens 2010;28:446–51.
27. Sanchez-Barcelo EJ, Mediavilla MD, Tan DX, Reiter RJ. Scientific basis for the potential use of melatonin in bone diseases: Osteoporosis and adolescent idiopathic scoliosis. J Osteoporos 2010;˙2010:830231.

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28. Verkasalo PK, Lillberg K, Stevens RG, Hublin C, Partinen M, Koskenvuo M, et al. Sleep duration and breast cancer: A prospective cohort study. Cancer Res 2005;65:9595–600.
29. Kakizaki M, Kuriyama S, Sone T, Ohmori-Matsuda K, Hozawa A, Nakaya N, et al. Sleep duration and the risk of breast cancer: The Ohsaki Cohort Study. Br J Cancer 2008;99:1502–5.
30. Wu AH, Wang R, Koh WP, Stanczyk FZ, Lee HP, Yu MC. Sleep duration, melatonin and breast cancer among Chinese women in Singapore. Carcinogenesis 2008;29:1244–8.
31. Pinheiro SP, Schernhammer ES, Tworoger SS, Michels KB. A prospective study on habitual duration of sleep and incidence of breast cancer in a large cohort of women. Cancer Res 2006;66:5521–5.

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