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URINARY 6-SULPHATOXYMELATONIN LEVELS AND RISK OF BREAST CANCER IN PREMENOPAUSAL WOMEN: THE ORDET COHORT

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Abstract

Background—Lower urinary melatonin levels are associated with a higher risk of breast cancer in postmenopausal women. Literature for premenopausal women is scant and inconsistent.

Methods—In a prospective case–control study we measured the concentration of 6-sulphatoxymelatonin (aMT6s), in the 12-hour overnight urine of 180 premenopausal women with incident breast cancer and 683 matched controls.

Results—In logistic regression models, the multivariate odds ratio (OR) of invasive breast cancer for women in the highest quartile of total overnight aMT6s output compared with the lowest was 1.43 [95% confidence interval (CI) = 0.83–2.45; $P_{trend} = 0.03$]. Among current non-smokers no association was existent (OR, 1.00, 95% CI, 0.52–1.94; $P_{trend} = 0.29$). We observed an OR of 0.68 between overnight urinary aMT6s level and breast cancer risk in women with invasive breast cancer diagnosed >2 years after urine collection and a significant inverse association in women with a breast cancer diagnosis >8 years after urine collection (OR, 0.17, 95% CI = 0.04–0.71; $P_{trend} = 0.01$). There were no important variations in ORs by tumor stage or hormone receptor status of breast tumors.

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Conclusion—Overall we observed a positive association between aMT6s and risk of breast cancer. However, there was some evidence to suggest that this might be driven by the influence of subclinical disease on melatonin levels, with a possible inverse association among women diagnosed further from recruitment. Thus, the influence of lagtime on the association between melatonin and breast cancer risk needs to be evaluated in further studies.

Keywords

melatonin; aMT6s; premenopausal; night work; breast cancer

Introduction

Secretion of melatonin, an indoleamine hormone that is produced primarily by the pineal gland, follows a rhythm of approximately 24 hours with most production occurring during the dark phase of a light–dark cycle (1). Circadian, i.e., approximately 24-hour, rhythms (2) drive some of the most important biologic functions in humans and are regulated by a circadian pacemaker located in the hypothalamus (3), of which melatonin is thought to be a surrogate marker. The urine concentration of the major metabolite of melatonin, 6-sulphatoxymelatonin (aMT6s), is highly correlated with melatonin levels in blood and saliva (4–10).

Results of previous studies [reviewed in (11)] suggest that night-shift work, a surrogate for exposure to light at night, is associated with an increased risk of breast cancer (12). Following earlier suggestions by Cohen et al. of a role of melatonin in the induction of breast tumors (13) and on the basis of results of laboratory and animal experiments (14–16), light-induced suppression of melatonin secretion has been hypothesized as the major cause of this association; however, while associations among postmenopausal women consistently suggest a lower risk of breast cancer with higher melatonin levels (17,18) the only two prospective studies conducted to date to study associations between circulating melatonin and premenopausal breast cancer risk showed inconsistent results: one study found no evidence that 24-hour urinary levels of melatonin are strongly associated with the risk of breast cancer (19), whereas the other study reported their first morning urinary levels of melatonin to be strongly and inversely related to risk of breast cancer (20).

We used a nested case–control design to conduct a prospective study of the association between melatonin levels in 12-hour overnight urine and breast cancer risk in a large cohort of premenopausal women enrolled in ORDET. We evaluated associations between total aMT6s produced between 7:00pm and 7:00am and creatinine-adjusted aMT6s measured in overnight urine and premenopausal breast cancer risk.

Methods

The *Hormones and Diet in the Etiology of Breast Cancer Risk* (ORDET) cohort was established in northern Italy between June 1987 and June 1992, when 10,786 healthy women ages 35 to 69 years, were enrolled (21,22). They were all residents of the Varese province, — an area covered by the Lombardy Cancer Registry (23), who had heard about the study through the media, at public meetings, and at breast cancer early detection centers and who volunteered to participate. At recruitment, a number of baseline characteristics including demographics and dietary intake were queried from each participant via questionnaire, direct measurements of several anthropometric variables including height and weight were conducted, and blood and urine specimens were collected. Because of the focus of the study on endogenous hormones and their relationship with breast cancer risk, stringent inclusion criteria were established and highly standardized conditions on collecting biological samples were applied. Women were excluded if they reported a bilateral ovariectomy, were currently breast feeding or pregnant,

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used oral contraceptives or hormone replacement therapy in the last three months, were affected by, chronic or acute liver disease, or reported a history of cancer.

Cancer incidence information, available from the local cancer registry (Varese Cancer Registry) was linked to the ORDET cohort in order to identify incident breast cancer cases up to December 2003. The Varese Cancer Registry is of high quality: < 2% of breast cancer cases are known to the registry by death certificate only, and the histology and cytology of 96.3% of all cases has been confirmed through pathology reports (21,24). The ORDET file was also linked to the Varese residents' file to check participants' vital status.

After exclusion of women with a history of cancer (with the exception of non-melanoma skin cancer) and women who, immediately after baseline, were lost to follow-up (observed time=0), 10,633 participants remained to form the base population of ORDET. For this study, we further restricted the ORDET cohort to its 6,667 premenopausal participants. Women were considered premenopausal when they reported having any menstrual cycle over the past twelve months. Participants were censored at the time of cancer diagnosis, death, or loss to follow-up, whichever came first (median follow-up time, 15.4 years).

Selection of Case and Control Subjects

Case and control subjects were selected from among all 6,667 eligible premenopausal women. Case subjects were women who developed breast cancer after their recruitment into the ORDET cohort but before the end of the study period (December 31, 2003). We identified a total of 238 incident breast cancer cases. Of these, 58 women were eliminated because their FSH level was indicative of peri- or postmenopausal status (i.e., FSH >10μl/ml). Of the remaining 180 cases, 10 had in situ breast cancer.

For each case subject with breast cancer, four control subjects were randomly chosen from appropriate risk sets consisting of all cohort members who satisfied the matching criteria (age at recruitment +/− 3 years, date of recruitment +/− 180 days and laboratory batch) and were alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case. Matching characteristics were age (± 3 years) at enrollment, date of recruitment (± 180 days), and laboratory batch. A total of 683 controls were selected. An incidence density sampling protocol for control selection was used, such that controls could include subjects who became a case later (13 women), while each control subject could also be sampled more than once (77 women).

Specimen collection

Women were instructed to collect their urine over the previous night. They followed a collection protocol which called for discarding the last void at 7:00pm and collecting urine during the night up to 7:00am. Urines were collected during the luteal phase of a woman's menstrual cycle, between day 20 and 24. Overnight urine was kept at room temperature during collection. After delivery to the ORDET recruitment center the day after overnight collection between 7:30am and 9:00am, all urine samples were immediately processed and stored at −80 degrees Celsius until biochemical determinations were done. Urine was filtered and separated, and 2ml aliquots were stored. No preservatives were added either at collection or during storage. Similarly, blood samples were collected during the luteal phase of a woman's menstrual cycle, between day 20 and 24, after overnight fasting between 7:30am and 9:00am and stored at −80 degrees Celsius. This study was approved by the Ethical Review Board of the National Cancer Institute of Milan (Italy).

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Laboratory Methods

Stability and reliability of the ORDET collection method for aMT6s have been demonstrated (25) to be reasonable, although storage temperature affected specimens such as that urine stored long-term at −30 degrees Celsius had systematically lower aMT6-s levels than urine stored at −80 degrees Celsius.

Urine samples from breast cancer cases and related controls were handled identically and assayed together on the same day and in the same run. All samples were taken out of the freezer simultaneously and sent to laboratory in the same parcel on dry ice. They were stored at −80 degrees C for an average of 17 years. Laboratory personnel were blinded to case-control status. Control of analytic error was based on the inclusion of two standard samples.

Urinary aMT6s was assayed by the Hormone Research Laboratory, Fondazione IRCSS Istituto Nazionale Tumori (Milan, Italy), using the Buhlmann enzyme-linked immunosorbent assay EK-M6S (Buhlmann Laboratories AG, Allschil, Switzerland) with a lower detection limit of 0.8 ng/mL for aMT6s.

Creatinine levels were also measured for each sample by the Medical Laboratory of the Department of Oncology, Fondazione IRCSS Istituto Nazionale Tumori (Milan, Italy), with a Hitachi Modular Automatic Analyzer and optimised reagents (F. Hoffmann-La Roche Ltd, Basel, Switzerland) (26). The average between-batch coefficient of variation was 5.3% and 10.3% for urinary aMT6s (high and low standard QCs), and 2.7% and 2.1% for creatinine concentrations of 1.2 mg/dl and 4.37 mg/dl, respectively. The within-batch CVs derived from quality control urine included in the analytic runs were 1.8% and 9.8% for aMT6s (high and low standard QCs).

Plasma sex steroid measurements (testosterone, free testosterone, SHGB, and estradiol) were conducted by Centro Medico Diagnostico Emilia (Bolgona, Italy). For testosterone and free testosterone, we used Coat-A-Count procedure, a solid-phase radioimmunoassay (Diagnostic Product Corporation, Los Angeles, USA); for SHGB, IMMUNOLITE 1000 Analyzer, a solid-phase, chemiluminescent immunometric assay (Diagnostic Product Corporation, Los Angeles, USA); and for estradiol, Orion Diagnostica SPECTRIA Estradiol *Sensitive* RIA test, a coated tube radioimmunoassay (Orion Diagnostica Oy, Espoo, Finland). Quality control was done at three concentrations for SHBG and total and free testosterone and four concentrations for total estradiol. In each batch, quality control samples were evaluated in quadruplicates. Within-batch quality control coefficients of variation were 5.9 % (high concentration) and 14.0% (low concentration) for estradiol; 5.8% and 10.6%, respectively, for total testosterone; 7.0% and 9.6%, respectively, for free testosterone; and 3.1% and 3.4%, respectively, for SHBG. Average between-batch coefficients of variation were 7.4% (high) and 16.4% for estradiol (low), 8.7% and 18.5% for total testosterone, 14.9% and 17.2% for free testosterone, and .4.9% and 4.6% for SHBG.

Statistical analyses

In total, 180 case patients with invasive or in situ breast cancer and 683 matched control subjects were available for our analyses. We multiplied aMT6s concentration (ng/ml) with 12-hour urine volume to obtain total aMT6s produced between 7:00pm and 7:00am (reported as μg per 12 hours). In secondary analyses, aMT6s levels were normalized to the creatinine level of the sample to account for differences arising from variations in urine concentrations (reported as ng aMT6s per mg of creatinine).

To test for differences in hormone levels between case and control subjects, we used mixed-effects regression models for clustered data to adjust for possible confounding due to the matching factors and for any residual correlation between case and control subjects within the

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matched set (27). We used conditional regression models to estimate the relative risks of breast cancer [reported as odds ratios (ORs) with 95% confidence intervals (CIs)] by quartiles of urinary aMT6s concentrations, which were defined on the basis of the values for all control subjects. Multivariate models were adjusted for known risk factors for breast cancer [see foot note to Table 3]. In secondary analyses, we also adjusted for the sex steroids that we measured in our data set. We tested for trends by modeling natural aMT6s concentrations continuously and calculating the Wald statistic. To evaluate the presence of an interaction between smoking (binary; current versus past or never smokers) and aMT6s levels (continuously), we added an interaction term into our logistic regression model and used the likelihood ratio test for interaction to determine significance. We used SAS version 9.1.3 (Cary, NC) for all analyses. All *P* values were two-sided.

Results

Table 1 shows baseline characteristics of the 180 cases and 683 controls. The mean time between urine collection and diagnosis was 7.7 years (89 months; SD 50.5) with a range of 1–185 months. Study participants were all premenopausal with an age range of 35 to 54 years at urine collection. Most of the women's baseline characteristics did not differ by case–control status (Table 1). However, age-adjusted mean urinary aMT6s concentration of the breast cancer cases was slightly higher than that of controls [17.4 μg aMT6s versus 15.8 μg aMT6s; 29.3 ng aMT6s/mg creatinine versus 27.6 ng aMT6s/mg creatinine]. Table 2 shows age and age-adjusted baseline characteristics by quartiles of urinary overnight aMT6s (μg) among the 683 controls included in this study. Several of the women's baseline characteristics, including age, family history of breast cancer, history of benign breast disease, smoking, and BMI, differed modestly by aMT6s quartile (Table 2). From among several sex steroids, including circulating plasma testosterone, free testosterone, SHBG, and estradiol, none appeared to vary substantially by aMT6s level.

Overall, we observed a positive association between urinary aMT6s concentrations and breast cancer risk (OR for highest versus lowest quartile of urinary aMT6s concentration, 1.53; 95% CI, 0.91 to 2.56; $P_{trend} = 0.01$; Table 3), which was modestly attenuated after additional adjustment for breast cancer risk factors including current smoking status (OR 1.43; 95% CI 0.83–2.45). Night work and melatonin have been more strongly related to invasive than in situ breast cancer risk (20,28–30), and we, therefore, excluded ten cases who were diagnosed with in situ breast cancer and their matched controls. Among women with invasive breast cancer only, the association was very similar (multivariate OR for highest versus lowest quartile of urinary aMT6s concentration, 1.39; 95% CI, 0.90 to 2.41; $P_{trend} = 0.04$) and we therefore kept these 10 cases in all subsequent analyses.

When we evaluated the influence of sex steroid hormones on these associations, none of the hormones previously found to predict premenopausal breast cancer risk was correlated with urinary aMT6s to a meaningful degree (all Spearman rank correlations ≤0.08: r=−0.08, p=0.02 for testosterone; r=0.02, p=0.49 for free testosterone; r=−0.06, p=0.11 for estradiol; and r=−0.01, P=0.85 for SHBG). Further adjustment for testosterone, free testosterone, estradiol, or SHBG in our multivariate regression models did not alter our estimates substantially (data not shown).

On the basis of a previous study suggesting that the nocturnal plasma melatonin increase inversely correlates with tumor estrogen receptor (ER) concentration (31), we conducted analyses stratified on ER status. For 169 of all 180 premenopausal breast cancer cases, hormone receptor status was available, and for 164 cases HER2 status: of these, 66.9% were ER+ tumors (only 56 women had ER–breast tumors), and 79.3% were HER2– (only 34 women had HER2 + breast tumors). When we restricted analysis to women with ER+ breast tumors, the positive

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association between aMT6s and breast cancer risk was virtually the same (multivariate OR for highest versus lowest quartile of urinary aMT6s, 1.44; 95% CI, 0.67–3.08) and remained by and large unchanged when we restricted to women with HER2– tumors (multivariate OR for highest versus lowest quartile of urinary aMT6s, 1.84; 95% CI, 0.94–3.60). Similarly, though based on only 19 cases in the upper quartile, the risk of ER– breast cancer appeared highest among women in the highest quartile of aMT6s (OR, 2.16; 95% CI, 0.78–6.00).

We found no effect modification by age (stratified along the median, <43, ≥ 43 years old) or BMI (stratified along the median, 24.4). Because a previous study suggested that cigarette smoking affects melatonin production in premenopausal women (32), we further stratified by smoking status. Among never or past smokers, we observed no association between urinary melatonin levels and breast cancer risk (highest versus lowest quartile of urinary aMT6s concentration, 1.00; 95% CI = 0.52 to 1.94; $P_{trend} = 0.29$; Table 3). By contrast, we observed a positive association among women who reported cigarette smoking at the time of urine collection (highest versus lowest quartile of urinary aMT6s concentration, age-adjusted OR, 2.84; 95% CI, 0.43 to 18.8; $P_{trend} = 0.14$; χ^2 from LLH ratio test for interaction between smoking and aMT6s, 1.22, p (1df) = 0.27), although power was limited in these analyses with only 36 breast cancer cases among current smokers.

Next, to rule out the possibility of preclinical tumors influencing our aMT6s levels, we excluded cases that were diagnosed shortly after urine collection, using a stepwise approach (Table 3). In these analyses, the association between urinary aMT6s level and breast cancer risk became increasingly inverse after excluding case patients who were diagnosed with invasive breast cancer within 2 years (OR for highest versus lowest quartile of urinary aMT6s concentration and risk of breast cancer developed at least more than two years after urine collection, 0.68; 95% CI, 0.32 to 1.44; $P_{trend} = 0.63$), 4 years (OR for highest versus lowest quartile of urinary aMT6s concentration, 0.61; 95% CI = 0.26 to 1.39; $P_{trend} = 0.80$), or 8 years after urine collection (OR for highest versus lowest quartile of urinary aMT6s concentration, 0.17; 95% CI = 0.04 to 0.71; $P_{trend} = 0.01$), though the latter analysis was based on 12 cases only in the upper quartile. By contrast, when restricting to women who developed breast cancer within 3 years after urine collection (28 cases; there were only 14 and 24 cases diagnosed within 1 and 2 years post urine collection, respectively, limiting our power to explore these associations), the association with breast cancer risk was strongly positive among those with the highest melatonin levels (OR for highest versus lowest quartile of urinary aMT6s concentration, 14.8; 95% CI = 1.39 to 157; $P_{trend} = 0.03$). The lack of an association between melatonin and breast cancer risk that we observed in the overall data set (i.e., including past and current smokers) remained unchanged throughout these secondary analyses when smokers were included (data not shown). Of all tumors, 92 were histopathologically classified as localized and 51 as metastatic – the remaining 27 were of unknown tumor stage. Further analyses stratifying by localized versus metastasized breast tumors did not reveal any effect modification by tumor stage (data not shown).

In secondary analyses, we also evaluated associations between creatinine-adjusted aMT6s and breast cancer risk. Creatinine-adjusted and total aMT6s were highly correlated (Spearman $r=0.75$, $P<0.001$) and both measures also correlated well with crude aMT6s concentration ($r=0.61$ and 0.73 respectively, both $P<0.001$). In multivariate analyses, we observed a positive association between creatinine-adjusted urinary aMT6s and invasive breast cancer risk (OR for highest versus lowest quartile of total urinary aMT6s, 1.67; 95% CI, 0.99 to 2.82; $P_{trend} = 0.27$), a risk which was also markedly attenuated among never and past smokers (highest versus lowest quartile of creatinine-adjusted urinary aMT6s, 1.21; 95% CI = 0.65 to 1.25; $P_{trend} = 0.98$).

Urinary creatinine concentration is influenced by a number of factors including gender, ethnicity, age and body-mass index (BMI) (33). While our study was exclusively comprised of Caucasian women, differences in age and BMI may have biased our creatinine-adjusted aMT6s measure. There was only a modest correlation between creatinine-adjusted aMT6s and creatinine (Spearman $r=-0.15$, $P<0.001$), suggesting the potential for bias introduced by adjusting for creatinine to be small.

Discussion

Overall, we found a positive association between overnight urinary aMT6s and breast cancer risk. However, we found a significant inverse association between overnight urinary aMT6s and breast cancer risk in premenopausal women, but only in non-smokers and after allowing for sufficient (8 years) lag-time between urine collection and the diagnosis of breast cancer. These findings suggest that there might be an influence of subclinical disease on melatonin levels with a possible inverse association being seen among women diagnoses further from recruitment.

Few prior studies have evaluated the association between circulating melatonin levels and breast cancer risk in humans and most are limited by the fact that melatonin levels were measured after the subjects were diagnosed with breast cancer (6,31,34–43). The first report to evaluate an association between circulating melatonin levels and breast cancer risk in 10 women was conducted by Bartsch et al. in 1981. It found in a small sample of women with advanced breast cancer, when compared with healthy controls, that they had lower levels of urinary melatonin (36). Subsequently, Tamarkin et al. found that women with ER-positive breast cancer had a reduced nocturnal increase in melatonin, and observed an inverse correlation between ER levels and peak melatonin values (31). Several subsequent studies examined melatonin levels in cancer patients (6,31,34–43). Because blood samples for melatonin were typically collected after a diagnosis of cancer in these retrospective studies, they are limited in their ability to assess the hormone's predictive value for breast cancer risk. However, data from untreated patients with localized breast cancer provide evidence for a depression of the nocturnal surge of melatonin that parallels an increase in tumor-size and the development of distant metastases (34,35,37). Together with evidence from untreated primary prostate cancer patients, where melatonin levels were particularly high if well-differentiated G1 (incidental) carcinomas were present (44), these observations suggest complex interactions between the pineal gland and tumor growth. Melatonin has a potential role in different phases of carcinogenesis such as initial activation, inhibition of tumor growth, and re-stimulation as cancer cells disseminate; this complexity may account for apparent inconsistencies found in prospective studies.

More recently, evidence from prospective case-control studies nested in larger cohorts has been published. An Italian case-control study nested within the ORDET cohort assessed the concentration of melatonin's major metabolite, 6-sulfatoxymelatonin (aMT6s) in 178 postmenopausal women with incident invasive breast cancer and 710 matched controls. The multivariate relative risk for women in the highest quartile of total overnight aMT6s output compared with the lowest, was 0.56 (95% CI, 0.33–0.97). In this report, overnight urinary aMT6s level and breast cancer risk were more strongly associated in women who were diagnosed with invasive breast cancer more than 4 years after urine collection (OR 0.34 highest versus lowest quartile, 95% CI, 0.15–0.75).(45) A second case-control study in postmenopausal women was conducted nested within the NHS cohort (17). In that study, aMT6s levels were available for 357 postmenopausal women who developed incident breast cancer along with 533 matched control subjects. An increased concentration of urinary aMT6s was statistically significantly associated with a lower risk of breast cancer with an odds ratio

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for the highest versus lowest quartile of morning urinary 6-sulfatoxymelatonin of 0.62 (95% CI, 0.41–0.95; p for trend = 0.004).

Evidence for an association between urinary melatonin and breast cancer risk among premenopausal women is also sparse and has been less consistent, perhaps in part due to varying urine sampling methods used in these studies. Only two prospective studies have evaluated the associations, one of which did not find an increased risk (46), whereas the other one described a significantly reduced risk of breast cancer risk in women with the highest melatonin levels. In the first study, a prospective study of urinary 6MTs in 77 cases and 214 premenopausal controls matched for age, recruitment date, day of menstrual cycle, the OR for breast cancer was 0.99, (95% CI, 0.45–2.17), comparing the highest to the lowest category (46). This study utilized 24-hour urine collection, in contrast to the NHS II, which used first-morning urine samples (20). The use of 24-hour urine may decrease power to detect potential differences by case-control status, but the confidence limits of this study do not preclude an effect of melatonin on breast cancer risk. Moreover, no lag-time analyses were conducted in this study. In the NHS II cohort, finally, aMT6s levels were measured in the first-morning urine of 147 women with invasive breast cancer and 291 matched control subjects. The OR for women in the highest quartile of urinary aMT6s was 0.59 compared with those in the lowest quartile, and remained unchanged after adjusting for several important confounding factors (47). A meta-analysis of all 5 (including ours, excluding cases that were diagnosed within 2 years after urine collection) prospective studies published, to date, suggests a 34% significant risk reduction of breast cancer with the highest category of aMT6s (Figure 1).

We were able to consider most important breast cancer risk factors in our analyses. Excluding cases diagnosed with increasing years after urine collection altered our findings; we observed increasingly stronger risk reductions with longer time between urine collection and breast cancer diagnosis, suggesting that perhaps particularly in premenopausal women their typically more rapid and aggressive tumor growth that is in part attributable to differences in the prevalence of molecular breast cancer subtypes (48) may mask the predictive power of aMT6s for breast cancer risk breast cancer risk as it also appears to constitute a marker for tumor growth. Alternatively, since those women in whom we observed the strongest associations were in large part postmenopausal at the time of diagnosis, this could either argue for a stronger effect of melatonin on breast tumors with features more commonly seen in postmenopause (i.e., hormone receptor status, histology) or for a role of change in menopausal status. The apparent need to lag time between specimen collection and breast cancer diagnosis may, in addition to varying urine sampling methods (i.e., 24-hour versus overnight urine) explain the null finding in one (46) of the other two studies describing the association between melatonin and breast cancer risk in premenopausal women.

Even though we did not observe a significant interaction between smoking and aMT6s levels in this data set, it is similar in magnitude and direction as in a previous analysis (49) and further underlines the necessity to consider that various external influences including metabolization rate, possibly altered by smoking (50,51), can influence a urinary marker like aMT6s.

Our study is limited by the absence of information on light exposure at night including night work status, thus we cannot adjust for this factor. Another potential limitation of our study is that we did not have information on vitamin D status in our study subjects, another possible breast cancer risk factor (52,53). The relationship between melatonin levels and vitamin D is unclear, but if one exists it could have influenced our results. For example, it is conceivable that women with low morning melatonin levels (if due to an altered sleep-wake cycle) also have particularly low levels of vitamin D mediated by low sun exposure or differences in dietary habits.

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Our findings show that melatonin secretion, as assessed by aMT6s levels in 12-hour overnight urine, is associated with a reduced risk of developing premenopausal breast cancer but only if long enough lag times are applied. Thus, while evidence for an association between melatonin and breast cancer risk continues to accumulate, further studies are needed to evaluate the influence of lagtime on the association between melatonin and breast cancer risk.. These studies should have long enough follow-up in order to address the time between urine collection (as an apparently healthy person) and cancer diagnosis, as it is conceivable that melatonin secretion is stimulated during early sub-clinical stages of tumor development.

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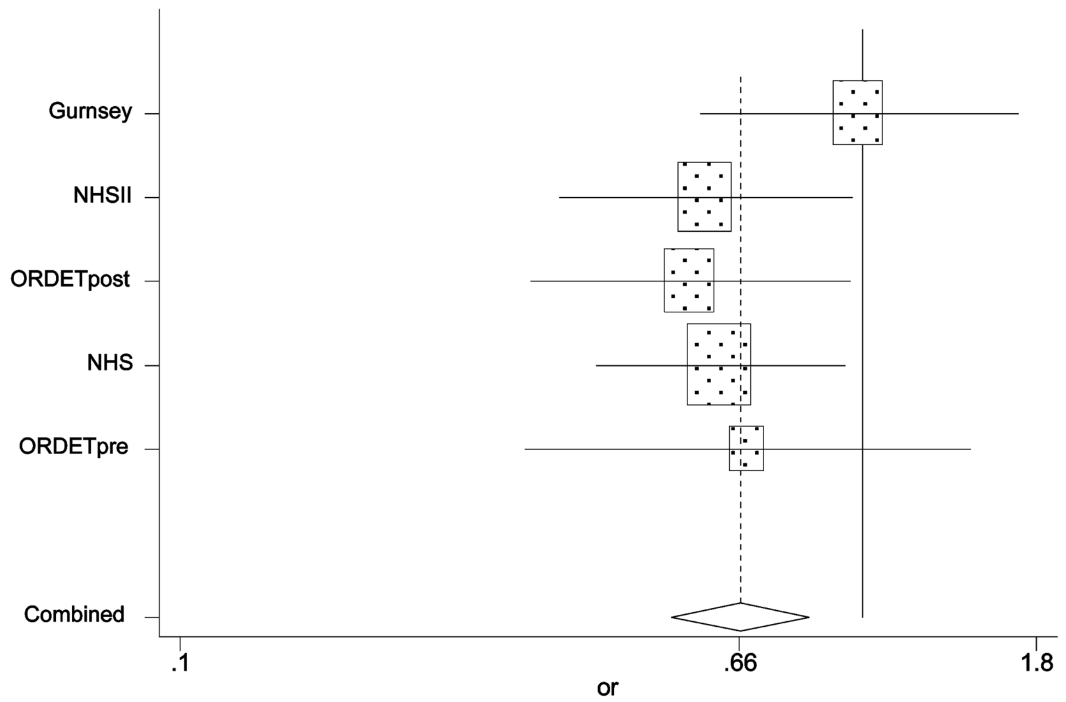

FIGURE 1. Meta-Analysis of five prospective studies examining associations between urinary melatonin secretion and breast cancer risk*

* The dashed vertical line represents the combined estimate, and the diamond-shaped box represents the confidence interval from the random-effects model. The estimates are plotted with boxes; the area of each box is inversely proportional to the estimated effect's variance in the study, hence giving more visual prominence to studies where the effect is more precisely estimated.

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Table 1

Baseline characteristics* of 180 premenopausal women with invasive (n=170) or in situ (n=10) breast cancer and their 683 matched controls.

All women	Cases (n=180)	Controls (n=683)
Age, years	43.4 (4.3)	43.1 (4.3)
Urinary aMT6s, ng/ml creatinine	29.3 (1.11)	27.6 (0.57)
Urinary aMT6s/12 hrs, μg	17.4 (0.61)	15.8 (0.31)
Age at menarche, years	12.6 (1.4)	12.6 (1.4)
Parity (among parous women only; %)	1.9 (0.8)	2.0 (0.8)
Age at first birth (among parous women only)	22.8 (9.4)	22.7 (9.0)
Family history of breast cancer (%)	9.4	7.6
OC use (%)	45.3	41.9
BMI, kg/m^2	24.3 (4.4)	24.4 (4.0)
Alcohol consumption, grams/day	9.4 (13.0)	8.8 (12.8)
History of benign breast disease (%)	31.1	27.2
Education beyond 8 years elementary school (%)	41.7	31.7
Smoking history		
Current smoker (%)	20.0	24.5
Past smoker (%)	20.0	12.5
Never smoker (%)	60.0	63.0
Pack-years among ever smokers	9.6 (9.0)	9.4 (10.1)
Sex hormone levels		
SHBG (nmol/L)	65.7 (2.1)	67.3 (1.1)
Testosterone (ng/ml)	0.29 (0.01)	0.28 (0.06)
Free testosterone (pg/ml)	0.71 (0.03)	0.69 (0.02)
Estradiol (pg/ml)	90.8 (3.9)	95.3 (2.0)
FSH (mui/ml)	4.1 (0.12)	4.0 (0.06)
LH (mui/ml)	2.5 (0.14)	2.7 (0.07)

*Mean (SD).

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Table 2

Age and age-adjusted baseline characteristics* of 683 controls by quartiles of urinary 6-sulfatoxymelatonin (aMT6s) level.

	Quartiles of 12-hour overnight urinary 6-sulfatoxymelatinin (aMT6s) output (μg)			
All control women	**Q1 (n=170)**	**Q2 (n=171)**	**Q3 (n=172)**	**Q4 (n=170)**
Range of urinary aMT6s output/12 hours (μg)	**<10.1**	**10.1–14.6**	**14.7–20.5**	**≥20.6**
Age, years	43.8	43.0	42.4	43.3
Age at menarche, years	12.8	12.6	12.6	12.5
Parity (# of children, among parous women only)	2.1	2.0	1.9	1.9
Age at first birth (among parous women only)	25.4	24.9	26.1	26.1
Family history of breast cancer (%)	8.8	7.0	5.8	8.8
OC use (%)	38.9	45.9	37.7	47.7
BMI, kg/m^2	24.6	24.4	24.5	24.2
Alcohol consumption, grams/day	8.0	7.8	11.7	7.7
History of benign breast disease (%)	29.4	29.8	25.6	24.1
Education beyond 8 years elementary school (%)	31.9	28.6	30.2	35.9
Smoking history				
Current smoker (%)	27.1	24.0	24.4	22.4
Past smoker (%)	6.5	14.0	14.0	15.3
Never smoker (%)	66.5	62.0	61.6	62.4
Pack-years among ever smokers	12.9	9.8	7.5	7.9
Sex hormone levels				
SHBG (nmol/L)	64.7	68.6	68.2	67.8
Testosterone (ng/ml)	0.30	0.29	0.29	0.25
Free testosterone (pg/ml)	0.72	0.68	0.70	0.65
Estradiol (pg/ml)	98.1	97.7	95.4	90.1
FSH (mui/ml)	4.1	4.1	4.0	3.7
LH (mui/ml)	2.8	3.0	2.8	2.3

*Mean.

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Table 3

Odds ratios (ORs) and 95% confidence intervals of breast cancer by quartile of total 12-hour overnight 6-sulfatoxymelatonin (aMT6s) output (aMT6s concentration (ng/ml) multiplied with 12-hour volume in ml).

	Quartile				
Group and parameter	**1**	**2**	**3**	**4**	P_{trend}*
Urinary aMT6s output/12 hours (µg)	<10.1	10.1–14.6	14.7–20.5	≥20.6	
No. of case patients/No. of control subjects	39/170	34/171	55/172	52/170	
Invasive and in situ breast cancer cases					
Simple OR*	1.00 (ref.)	0.91 (0.54–1.53)	1.50 (0.92–2.45)	1.53 (0.91–2.56)	0.01
Multivariate OR†	1.00 (ref.)	0.88 (0.51–1.50)	1.43 (0.85–2.42)	1.43 (0.83–2.45)	0.03
Excluding current smokers					
No. of case patients/No. of control subjects	32/96	27/107	44/106	41/114	
Simple OR†	1.00 (ref.)	0.75 (0.41–1.36)	1.24 (0.70–2.20)	1.16 (0.63–2.15)	0.19
Multivariate OR‡	1.00 (ref.)	0.72 (0.38–1.38)	1.11 (0.59–2.08)	1.00 (0.52–1.94)	0.29
Multivariate lagtime analyses among non- smokers					
No. of case patients/No. of control subjects	5/35	4/29	8/33	11/35	
Multivariate OR‡ among women diagnosed within 3 years from urine collection	1.00 (ref.)	0.90 (0.09–8.75)	1.88 (0.23–15.3)	14.8 (1.39–157)	0.03
No. of case patients/No. of control subjects	30/73	24/82	41/81	35/89	
Multivariate OR‡ 1 year lagtime	1.00 (ref.)	0.61 (0.30–1.23)	1.07 (0.54–2.12)	0.90 (0.45–1.82)	0.40
No. of case patients/No. of control subjects	28/65	24/79	38/75	30/83	
Multivariate OR‡ 2 years lagtime	1.00 (ref.)	0.58 (0.28–1.19)	0.95 (0.47–1.94)	0.68 (0.32–1.44)	0.63
No. of case patients/No. of control subjects	27/61	23/78	36/73	30/79	
Multivariate OR‡ 3 years lagtime	1.00 (ref.)	0.51 (0.24–1.08)	0.87 (0.42–1.82)	0.69 (0.32–1.48)	0.52
No. of case patients/No. of control subjects	24/53	19/73	33/67	28/68	
Multivariate OR‡ 4 years lagtime	1.00 (ref.)	0.41 (0.18–0.92)	0.76 (0.34–1.66)	0.61 (0.26–1.39)	0.80
No. of case patients/No. of control subjects	23/52	19/64	28/65	27/62	
Multivariate OR‡ 5 years lagtime	1.00 (ref.)	0.47 (0.20–1.09)	0.66 (0.29–1.48)	0.66 (0.28–1.54)	0.93
No. of case patients/No. of control subjects	20/44	17/57	25/57	23/51	

	Quartile				
Group and parameter	**1**	**2**	**3**	**4**	P_{trend}[*]
Multivariate OR[‡] 6 years lagtime	1.00 (ref.)	0.46 (0.18–1.14)	0.68 (0.28–1.64)	0.75 (0.29–1.92)	0.79
No. of case patients/No. of control subjects	19/40	16/51	22/47	16/46	
Multivariate OR[‡] 7 years lagtime	1.00 (ref.)	0.43 (0.16–1.17)	0.53 (0.19–1.47)	0.38 (0.13–1.11)	0.13
No. of case patients/No. of control subjects	18/31	15/46	18/39	12/36	
Multivariate OR[‡] 8 years lagtime	1.00 (ref.)	0.25 (0.07–0.90)	0.29 (0.07–1.10)	0.17 (0.04–0.71)	0.01

[*]We tested for trends by modeling aMT6s concentrations continuously and calculating the Wald statistic.

[†]Simple conditional logistic regression model adjusting for the matching variables [year of birth, month and year of urine collection, and laboratory batch].

[‡]Multivariate conditional logistic regression models; relative risks were adjusted for the following breast cancer risk factors: body mass index (BMI) in six categories (≤21, 21.1–23, 23.1–25, 25.1–27, 27.1–30, >30), history of benign breast disease (yes/no), family history (mother or sister) of breast cancer (yes/no), smoking history (never, past, current), age at menarche in four categories (≤12, 13, 14, 15+), alcohol consumption per day in grams, three categories (none, ≤12, >12), years of oral contraceptive use (never, ≤1 year, >1 year), parity in three categories (nulliparous, 1–2, 3+ children), age at first birth in three categories (<20, 20–24, ≥25), and participant's educational status in years of schooling, three categories (≤5 years (elementary school), 8 years (superior education), >8 years).

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