

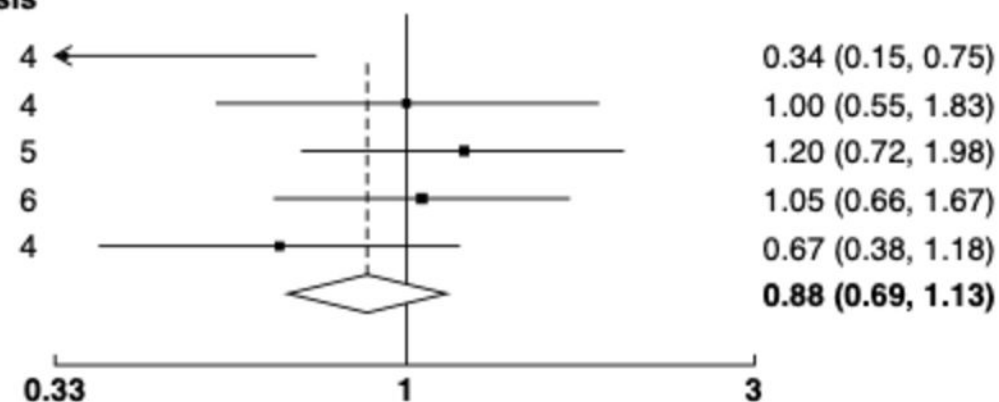
Figure 1.

Year Study

2008 The ORDET cohort (Postmenopausal women) (1)
 2014 Women's Health Initiative Observational Cohort (WHI) (4)
 2015 Nurses' Health Study II (NHS II) (5)
 2017 Nurses' Health Study (NHS) (6)
 2020 The DOM study

All

Years lagged for analysis



**RR (95% CI) of breast cancer for
 the highest vs lowest levels of
 6-sulfatoxymelatonin
 with exclusion of cases
 diagnosed soon after baseline**

Figure 2.

Urinary melatonin in relation to breast cancer risk: nested case-control analysis in the DOM study and meta-analysis of prospective studies

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Abstract (250 words)

Background: Exposure to higher levels of melatonin may be associated with lower breast cancer risk, but epidemiological evidence has been limited. We examined the relationship in a case-control study nested within the Diagnostisch Onderzoek Mammacarcinoom (DOM) study, and conducted a meta-analysis of prospective studies.

Methods: Concentrations of 6-sulfatoxymelatonin in pre-diagnostic first-morning urine voids were measured in 274 postmenopausal women diagnosed with breast cancer and 274 matched controls from the DOM study. Conditional logistic regression models were used to estimate multivariable-adjusted odds ratios (ORs) of breast cancer for thirds of 6-sulfatoxymelatonin. Meta-analysis of the current and previous prospective studies of urinary melatonin with breast cancer risk estimated the inverse-variance weighted averages of study-specific log-relative risks (RRs) of breast cancer for the highest versus lowest levels of 6-sulfatoxymelatonin.

Results: In the DOM study, the ORs of breast cancer for the middle and highest versus lowest thirds of 6-sulfatoxymelatonin were 0.70(95%CI:0.45-1.09) and 0.72(0.44-1.19), respectively. In the meta-analysis of the DOM study with six previous studies(2,296 cases), RR of breast cancer for the highest versus lowest levels of 6-sulfatoxymelatonin was 0.87(0.76-1.01).

Conclusion: Results from the DOM study, together with the published prospective data, do not support a strong association of melatonin with breast cancer risk.

Impact: This study adds to the relatively scarce prospective data on melatonin in relation to breast cancer risk. The totality of the prospective evidence does not clearly show an association between melatonin and breast cancer risk, but further data are needed to be able to exclude a modest association.

Introduction

Higher levels of melatonin are hypothesised to be associated with a lower risk of breast cancer (1-10). Night shift work, which probably reduces melatonin production in women, has been categorised by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans, mainly based on evidence from animal experiments on the carcinogenicity of alterations of the light-dark schedule (11, 12). Experimental evidence has supported pro-apoptotic, anti-proliferative, anti-metastatic, and anti-angiogenic actions of melatonin (13).

Of particular relevance to breast cancer, melatonin may interact with oestrogen-signalling pathways through various mechanisms, for example inhibiting local synthesis of oestrogens by repressing its expression and transactivation and preventing binding of oestrogen to oestrogen receptor alpha (ER α) (13). In postmenopausal women, the major source of oestradiol is from the conversion of oestrone, which is produced primarily in extra-glandular tissues from androgen precursors (14); melatonin may also inhibit the expression and activity of aromatase through regulating gene expression of specific aromatase promotor regions (13).

6-sulfatoxymelatonin (aMT6s), the major metabolite of melatonin, in first morning urine voids is a reliable marker of the nocturnal peak and the total nocturnal output of circulating plasma melatonin, (15, 16) which allows epidemiological investigations into disease risks associated with endogenous melatonin. The most recent meta-analysis published in 2017 of a total of six cohorts did not find an association between urinary melatonin and breast cancer risk (RR=0.97, 0.88–1.08) (10). With 1,824 cases and 3,954 controls, however, the data available were relatively limited.

We examined urinary aMT6s levels in relation to risk of breast cancer using a case-control analysis nested within the prospective Diagnostisch Onderzoek Mammacarcinoom (DOM) study. To put the results from the DOM study in the context of the totality of prospective evidence, we conducted a meta-analysis of prospective studies on first morning or 12-hour overnight urinary melatonin levels in relation to breast cancer risk.

Materials and Methods

The DOM study

The DOM study is a population-based, prospective breast cancer screening study in Utrecht and surroundings, The Netherlands. The details of the study have been described elsewhere (17). During 1974-1986, the DOM study recruited 27,718 women who were born between 1911 and 1945 for mammography screening (18, 19). Mailed self-administrated questionnaires were returned when women attended the physical examinations (20). In addition to mammography screening, physical measurements (for example, height and weight) were performed at the screening centres and women were asked to donate a first morning urine void on the day of the examination (19). Urine samples were stored at -20°C in 250 ml polypropylene jars, without preserving agents (19). Urine samples were obtained between 1974 and 1978.

The cases and controls for this study were those who had been selected for a prior investigation of oestrogen receptor and breast cancer risk (21); these were DOM study participants who were postmenopausal women (defined by no menstrual period for at least 12 months, after spontaneous stopping of their menstrual cycles), who did not have a prior history of breast cancer, who were not using exogenous hormones at baseline, who were diagnosed with incident breast cancer at the end of the follow-up period in 1996 (for cases), and who had urine samples available for melatonin analyses. In this sample, 20 women had missing data on melatonin or creatinine, or were excluded because they were no longer in a complete matched set. Women were followed for occurrence of breast cancer until January 1996, through general practitioners and from 1986 onwards through linkage to the regional cancer registry (19, 22). The initial screenings of the DOM study was carried out at the time when institutional review was not required, but all women gave oral informed consent in accord with the good clinical practice rules at that time (21). The subsequent follow-up study was approved by the Institutional Review Board (20).

Definitions of cases and controls

During follow-up, incident breast cancer cases were identified. Each case was matched to one control according to the following criteria: age at recruitment (within 1 year), date of

recruitment (within 1 month), number of donated urine samples (some participants also provided a urine void in the next round of screening), and time interval between urine samples (within 1 month). The analysed sample included 274 cases and 274 matched controls.

Hormone assays

Assays for aMT6s and creatinine were performed at the laboratories of Stockgrand Limited (University of Surrey, Guildford, United Kingdom) in 2016, and case and control participants from the same sets were assayed in the same batch. Urinary aMT6s was assayed in eleven batches using the Bühlmann enzyme-linked immunosorbent assay (product code EK-M6S; Bühlmann Laboratories AG, Schönenbuch, Switzerland). Urinary creatinine was assayed in three batches by the Jaffé reaction using the Cayman Creatinine Colorimetric Assay Kit (product code 500701; Cayman Chemical Company, Ann Arbor, Michigan). The measurements were done in duplicate and the values for analysis were taken by averaging the two measurements. When urine samples were available from an individual for two time points, equal volumes of the samples were mixed to form a pooled aliquot for that individual. This process was applied to both cases and controls within the same matched sets.

Pooled quality controls, produced by combining residual samples from some control participants, were inserted across study samples; in total, 56 quality controls were assayed in duplicate. Laboratory staff were blinded to the status of the samples. The coefficients of variation by batch ranged from 3% to 16% for aMT6s and from 4% to 28% for creatinine.

Statistical analyses

The aMT6s results were reported in ng/mg creatinine (15, 16). Creatinine-adjusted aMT6s levels were divided into thirds using tertile cut-points in the control participants. Crude summary statistics of personal characteristics were presented by case control status. The hormone values were logarithmically transformed, and geometric means and 95% confidence intervals (95% CI) of aMT6s levels were estimated for selected categorical personal characteristics in control participants using analyses of covariance, and tests for heterogeneity were assessed. Percentage changes in logarithmically transformed aMT6s levels and 95% CIs

per unit change in the continuous variables were estimated by adjusted linear regression and test for trend was assessed by Wald's test.

Conditional logistic regression was used to estimate odds ratios (ORs) and 95% CIs of breast cancer by thirds of aMT6s levels. Based on results from previous studies (10), the regression models were adjusted for the following potential confounders: age at recruitment (continuous), body mass index (kg/m^2 ; continuous), family history of breast cancer (yes, no), age at menarche (11-12, ≥ 13 years, unknown), age at menopause (<45, 45-54, ≥ 55 years), parity and age of first birth (nulliparous, 1-2/<25 years, 1-2/ ≥ 25 years, ≥ 3 /<25 years, ≥ 3 / ≥ 25 years), ever use of oral contraceptives (ever, never), ever use of hormone replacement therapy (ever, never), and smoking status (ever, never). We tested for linear trend using a pseudo-continuous variable equal to medians of the thirds of aMT6s levels. As a sensitivity analysis, we excluded cases that were diagnosed within the first four years of follow-up, leaving 204 cases and 204 matched controls. Creatinine-adjusted aMT6s levels were redivided into thirds using data from the 204 controls. The analyses were also repeated using the original creatinine-adjusted aMT6s cut-offs.

All statistical analyses were conducted using Stata 15.1 (StataCorp, College Station, TX, USA). A two-sided p-value of 5% was considered to be statistically significant.

Meta-analysis

Article search and screening

English articles were searched with search terms (Supplementary Table S1) from inception to 22nd October 2019 in Embase and MEDLINE. Duplicates were firstly excluded. Titles and abstracts of studies were screened by two independent reviewers (ATYW and TYNT) according to the following exclusion criteria: (i) the study was not an original study in humans (e.g. systematic reviews, comments, letters, and animal studies), (ii) 12-hour overnight or first morning urinary melatonin was not the exposure (melatonin levels derived from randomly timed spot urine is not informative as a proxy for nocturnal melatonin production (3, 23)), (iii) breast cancer was not the outcome, (iv) the study was not prospective in design, or (v) there was insufficient information (only age-adjusted estimates or published

as an abstract only and with insufficient information on the multivariable-adjusted estimates, study population, analysis design or covariate adjustment). In the second stage, full-texts were screened again using the same exclusion criteria. Reference lists of the included articles were screened for additional articles. To avoid including participants from the same cohort twice, only data from the analyses with the larger number of cases and/or a longer follow-up period were included for the meta-analysis. This applied to Brown et al.(5) and Schernhammer et al.(7), in which women were recruited from the Nurses' Health Study II (NHS II) cohort, as well as to Devore et al.(6) and Schernhammer et al.(8), which analysed data collected from the Nurses' Health Study (NHS) cohort. Any disagreement on screening results was resolved by discussion. The flow diagram of the article search is displayed in Supplementary Figure S1.

Data extraction

The following study characteristics were extracted, wherever available: the first author's surname, study cohort, country, publication year, menopausal status at urine collection, mean age, year of urinary melatonin collection, number of cases and controls, matching criteria, type of urine samples, cut-offs or mean levels of the highest and the lowest levels of urinary melatonin, outcome ascertainment, follow-up period, covariates adjusted for, and multivariable-adjusted ORs and 95% CIs for breast cancer risk for the highest level versus the lowest level of urinary melatonin. The ORs for breast cancer for the highest third versus lowest third of urinary melatonin were the main results in this study and Wang et al.(3), and the ORs for the highest fourth and lowest fourth of urinary melatonin were the main results in the remaining included studies.

The Newcastle-Ottawa Scale for cohort studies was used to assess the risk of bias of the included studies based on the design of cohort selection, comparability, and outcome assessment (24).

Statistical analyses

ORs were regarded as relative risks (RRs) based on the rare disease assumption. The overall RR of the meta-analysis was estimated using inverse-variance weighted averages of the

logarithmic RR values in the separate studies. Sensitivity analyses were performed with the exclusion of the cases diagnosed soon after baseline and their matched controls. The p-values for the Cochran's Q-test and I^2 estimate were presented for assessing heterogeneity in the estimates across studies. For this sensitivity analysis, it was not possible to include corresponding results for premenopausal women in the ORDET cohort (2) because the analyses were further restricted to participants who also did not smoke. Analyses excluding cases diagnosed in early follow-up were not available for the Guernsey III study (3). Subgroup analysis by menopausal status at urine collection was also performed. The meta-analyses were performed using the “metafor” package and forest plots were generated in R (Version 1.1.463) (Foundation for Statistical Computing, Vienna, Austria) (25).

Results

The DOM study

Breast cancer cases were diagnosed after a median of 10 years (IQR, 4-15 years). The median follow-up period for controls was 20 years (IQR, 19-20 years). The mean age at recruitment was 59 (SD, 4) years old. The median aMT6s levels for breast cancer cases and controls were 28.1 (IQR, 14.8-55.3) and 33.8 (16.9-55.6) ng/mg of creatinine, respectively. The prevalence of family history of breast cancer and younger age at menarche were higher in cases than controls, but other personal characteristics were similar by case-control status (Table 1). Among the controls, women with higher aMT6s levels were more likely to be younger and be parous, but were similar to women with lower aMT6s levels with respect to other personal characteristics (Table 2).

In the age-adjusted model, ORs (95% CI) of breast cancer for the middle and highest thirds of aMT6s levels, versus the lowest third, were 0.67 (0.45-1.01) and 0.69 (0.44-1.08), respectively (Table 3). In the multivariable-adjusted models, ORs (95% CI) for the middle and highest thirds versus the lowest third were 0.70 (0.45-1.09) and 0.72 (0.44-1.19) (p for trend=0.2), respectively. Findings were similar in sensitivity analyses excluding cases diagnosed in the first four years of follow-up (ORs for the middle and the highest thirds of melatonin versus the lowest third were 0.61 [0.37-1.00] and 0.67 [0.38-1.18] using new cut-

offs defined after the exclusion; and 0.62 [0.37 -1.03] and 0.67 [0.38-1.19] using the original cut-offs, respectively).

Meta-analysis

We identified 95 articles from the two databases, of which 86 articles were excluded based on duplicates, titles, and abstracts, and a further three articles were excluded after screening for full texts.

Prior to the DOM results, during the past 12 years, six other prospective studies from the US and Europe were published (Supplementary Table S2). Melatonin data, collected from first-morning urine samples (5 studies including the DOM study) or 12-hour overnight urine samples (2 studies), were available for a total of 2,296 breast cancer cases and 4,494 controls. Women were followed up for invasive breast cancer (2 studies) or both invasive breast cancer and *in situ* breast carcinoma (5 studies). Most of the studies adjusted for various established risk factors for breast cancer, such as, age at menarche, parity, age at first birth, family history of breast cancer, body mass index, and alcohol use. The risk of bias of the included studies are similar and low (Supplementary Table S3).

In the meta-analysis, the summary RR (95% CI) for breast cancer associated with the highest versus the lowest aMT6s levels was 0.87 (0.76-1.01) (p for heterogeneity=0.2, I^2 =27%) (Figure 1). The ORDET study in postmenopausal women (1) also provided an estimate of further adjustment for testosterone in the multivariable-adjusted model, and our results were materially unchanged when we included this estimate in the meta-analysis (RR=0.87, 95% CI: 0.75-1.00).

The sensitivity analysis based on five studies that reported estimates after excluding cases diagnosed in the first few years of follow-up showed similar findings (RR=0.88 [0.69-1.13], p for heterogeneity=0.08, I^2 =53%) (Figure 2). Six studies provided estimates separately for premenopausal and/or postmenopausal women at baseline, and we found no evidence of heterogeneity in the association of breast cancer risk with urinary melatonin by menopausal status (test for heterogeneity, p=0.2).

Discussion

In this study nested within the DOM cohort, levels of melatonin in first morning urine samples were not associated with the risk of breast cancer in postmenopausal women, and our meta-analysis of seven prospective studies, including the DOM study, does not support an overall association of breast cancer risk with 12-hour overnight or first-morning urinary melatonin in women.

Compared with the most recently published meta-analysis in 2017 (10), this current meta-analysis includes the new results from the DOM cohort, and the updated results with extended follow-up from the NHS cohort (6). In total, 1,824 cases were included in the previous meta-analysis (10), while 2,296 cases were included in the current meta-analysis. These relatively scarce published data somewhat limit the conclusions that can be drawn and we are unable to exclude a modest association between urinary melatonin and breast cancer risk. Further prospective data are needed but difficult to generate because few large cohort studies have the appropriate baseline urine samples. Other epidemiological approaches, such as Mendelian randomisation analyses if appropriate genetic instruments can be identified, may be informative.

To evaluate potential reverse causation bias, we restricted the meta-analysis to five studies that excluded cases diagnosed soon after baseline and observed similar null findings. Although the p-value for heterogeneity was not statistically significant, the amount of heterogeneity quantified in I^2 was moderate (53%). Some differences in the study designs may explain the moderate heterogeneity in this sensitivity analysis. There were differences in the numbers of years that were excluded in the sensitivity analyses for each individual study (i.e. 4-6 years), and the ORs appeared to be closer to the null hypothesis for studies with a longer period of exclusion. To allow for better examination of whether time to diagnosis is relevant to the associations of melatonin with risk of breast cancer, future collaborative reanalyses using individual-level data from published prospective studies are required (26). Also, while the ORDET study for postmenopausal women collected 12-hour overnight urine samples, the rest of the studies collected first-morning urine voids, but we would not expect a large difference in the measurements as most production of melatonin occurs during the biological night. Moreover, there was reduced power to detect or refute an association in the

analyses that excluded cases diagnosed in early follow-up. Hence, the results in the analyses that excluded cases diagnosed in early follow-up would be more prone to more extreme values owing to sampling variation.

Possible associations of demographic and lifestyle factors with endogenous melatonin levels remain unclear. Unexpectedly, the proportion of nulliparous women was higher among controls than cases, but this could be due to chance. Among the control participants in the DOM study, we found an inverse association of aMT6s levels with age consistent with previous studies (3, 27). We also observed a higher aMT6s levels in parous than nulliparous women, which has also been found in a previous Nurses' Health Study analysis (28), though not in another similar analysis (3). It is not clear why melatonin levels should be higher in parous women and this may be a chance finding. We found no evidence for an association of melatonin levels with other reproductive factors. However, we did not find any significant association of aMT6s levels with body mass index (27, 28) or smoking status (28), although such associations have been reported in some previous studies.

Putative mechanisms linking melatonin and breast cancer risk involve steroid hormones, and hence the melatonin-breast cancer relationship may differ between premenopausal and postmenopausal women potentially via interaction with ovarian hormones (9). In the current meta-analysis, we did not find significant heterogeneity by menopausal status, but there was limited power for this comparison, and insufficient data in the included studies for us to evaluate possible heterogeneity by tumour receptor status.

Our use of first morning urinary samples is a valid and reliable surrogate for nocturnal melatonin production (29). Previous studies demonstrated high correlation of aMT6s levels between two days of measurements (30), and reported good reproducibility of first morning urinary melatonin levels over a 3-year period in premenopausal women (31) and over a 5-year period in postmenopausal women (32). Such reproducibility is comparable to that of other plasma hormones, such as oestradiol and oestrone over a 2 to 3-year period (33).

There were some limitations of the analyses in the DOM study and the meta-analyses. In the DOM study, we were unable to adjust for alcohol intake, personal history of benign breast disease, use of beta-blockers, calcium channel blockers, or psychotropic medications (27), or

shift or night work status (31), as those data were not available. However, we note that in a subsequent study of women for a breast cancer screening project in the same region, the prevalence of alcohol consumption in postmenopausal women was found to be low (34). There is no consistent and strong cross-sectional evidence for the associations of melatonin with alcohol consumption and antidepressant use (27, 28, 35), so it is unlikely that our results would be largely confounded by these factors. In the meta-analyses, the ratios of the highest over the lowest cut-offs for aMT6s varied across studies, ranging from two-fold to nearly four-fold. However, our meta-analysis aimed to compare the top and bottom ends of the distribution of aMT6s in relation to breast cancer risk, which only assumes that the distributions, not the actual concentrations, of hormone levels are similar across studies. Lastly, we cannot exclude the possibility that other parameters of melatonin production, such as the difference between the nocturnal peak level and daytime level, would be associated with breast cancer risk (9).

To conclude, the current prospective evidence does not support a strong association between urinary melatonin levels and breast cancer risk. However, the published observational data remain scarce, limiting the conclusions that can be drawn. Further prospective studies, as well as meta-analyses, together with other epidemiological approaches such as Mendelian randomisation analyses, are required to clarify the association.

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References

1. 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(12):898-905.
2. 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(3):729-37.
3. Wang XS, Tipper S, Appleby PN, Allen NE, Key TJ, Travis RC. First-morning urinary melatonin and breast cancer risk in the Guernsey Study. *Am J Epidemiol.* 2014;179(5):584-93.
4. Sturgeon SR, Doherty A, Reeves KW, Bigelow C, Stanczyk FZ, Ockene JK, et al. Urinary levels of melatonin and risk of postmenopausal breast cancer: women's health initiative observational cohort. *Cancer Epidemiol Biomarkers Prev.* 2014;23(4):629-37.
5. Brown SB, Hankinson SE, Eliassen AH, Reeves KW, Qian J, Arcaro KF, et al. Urinary melatonin concentration and the risk of breast cancer in Nurses' Health Study II. *Am J Epidemiol.* 2015;181(3):155-62.
6. Devore EE, Warner ET, Eliassen AH, Brown SB, Beck AH, Hankinson SE, et al. Urinary Melatonin in Relation to Postmenopausal Breast Cancer Risk According to Melatonin 1 Receptor Status. *Cancer Epidemiol Biomarkers Prev.* 2017;26(3):413-9.
7. Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. *J Natl Cancer Inst.* 2005;97(14):1084-7.
8. Schernhammer ES, Hankinson SE. Urinary melatonin levels and postmenopausal breast cancer risk in the nurses' health study cohort. *Cancer Epidemiol Biomarkers Prev.* 2009;18(1):74-9.
9. Travis RC, Allen DS, Fentiman IS, Key TJ. Melatonin and breast cancer: a prospective study. *J Natl Cancer Inst.* 2004;96(6):475-82.
10. Xu J, Huang L, Sun GP. Urinary 6-sulfatoxymelatonin level and breast cancer risk: systematic review and meta-analysis. *Sci Rep.* 2017;7(1):5353.
11. Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of shift-work, painting, and fire-fighting. *Lancet Oncol.* 2007;8(12):1065-6.
12. IARC Monographs Vol 124 group. Carcinogenicity of night shift work. *Lancet Oncol.* 2019;20(8):1058-9.
13. Nooshinfar E, Safaroghli-Azar A, Bashash D, Akbari ME. Melatonin, an inhibitory agent in breast cancer. *Breast Cancer.* 2017;24(1):42-51.
14. Travis RC, Key TJ. Oestrogen exposure and breast cancer risk. *Breast Cancer Res.* 2003;5(5):239-47.
15. Cook MR, Graham C, Kavet R, Stevens RG, Davis S, Kheifets L. Morning urinary assessment of nocturnal melatonin secretion in older women. *J Pineal Res.* 2000;28(1):41-7.
16. Graham C, Cook MR, Kavet R, Sastre A, Smith DK. Prediction of nocturnal plasma melatonin from morning urinary measures. *J Pineal Res.* 1998;24(4):230-8.
17. de Waard F, Collette HJ, Rombach JJ, Baanders-van Halewijn EA, Honing C. The DOM project for the early detection of breast cancer, Utrecht, The Netherlands. *J Chronic Dis.* 1984;37(1):1-44.
18. Elias SG, Onland-Moret NC, Peeters PH, Rinaldi S, Kaaks R, Grobbee DE, et al. Urinary endogenous sex hormone levels in postmenopausal women after caloric restriction in young adulthood. *Br J Cancer.* 2004;90(1):115-7.
19. Onland-Moret NC, van Gils CH, Roest M, Grobbee DE, Peeters PH. The estrogen receptor alpha gene and breast cancer risk (The Netherlands). *Cancer Causes Control.* 2005;16(10):1195-202.
20. van Noord PA, Dubas JS, Dorland M, Boersma H, te Velde E. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril.* 1997;68(1):95-102.
21. Emaus MJ. Etiology and early detection of breast cancer : Biomarkers, lifestyle and mammographic density [Dissertation]. Utrecht, the Netherlands: Utrecht University 2015.
22. Onland-Moret NC, Kaaks R, van Noord PAH, Rinaldi S, Key T, Grobbee DE, et al. Urinary endogenous sex hormone levels and the risk of postmenopausal breast cancer. *Br J Cancer.* 2003;88(9):1394-9.

23. 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(4):891-6.
24. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses 2009 [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
25. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. *J Stat Softw*. 2010;36(3):48.
26. The Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous Sex Hormones and Breast Cancer in Postmenopausal Women: Reanalysis of Nine Prospective Studies. *J Natl Cancer Inst*. 2002;94(8):606-16.
27. Davis S, Kaune WT, Mirick DK, Chen C, Stevens RG. Residential magnetic fields, light-at-night, and nocturnal urinary 6-sulfatoxymelatonin concentration in women. *Am J Epidemiol*. 2001;154(7):591-600.
28. Schernhammer ES, Kroenke CH, Dowsett M, Folkard E, Hankinson SE. Urinary 6-sulfatoxymelatonin levels and their correlations with lifestyle factors and steroid hormone levels. *J Pineal Res*. 2006;40(2):116-24.
29. Arendt J, Bojkowski C, Franey C, Wright J, Marks V. Immunoassay of 6-hydroxymelatonin sulfate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. *J Clin Endocrinol Metab*. 1985;60(6):1166-73.
30. Levallois P, Dumont M, Touitou Y, Gingras S, Masse B, Gauvin D, et al. Effects of electric and magnetic fields from high-power lines on female urinary excretion of 6-sulfatoxymelatonin. *Am J Epidemiol*. 2001;154(7):601-9.
31. 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(6):936-43.
32. 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(8):806-8.
33. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. *Cancer Epidemiol Biomarkers Prev*. 1995;4(6):649-54.
34. Onland-Moret NC, Peeters PHM, van der Schouw YT, Grobbee DE, van Gils CH. Alcohol and Endogenous Sex Steroid Levels in Postmenopausal Women: A Cross-Sectional Study. *J Clin Endocrinol Metab*. 2005;90(3):1414-9.
35. Stevens RG, Davis S, Mirick DK, Kheifets L, Kaune W. Alcohol consumption and urinary concentration of 6-sulfatoxymelatonin in healthy women. *Epidemiology*. 2000;11(6):660-5.

Table 1. Baseline characteristics of women at recruitment by case-control status in the DOM study

Characteristics, mean (SD) or number (%) unless specified	Controls (N=274)	Cases (N=274)
Age at baseline (years)	58.5 (3.6)	58.6 (3.6)
Body mass index (kg/m ²)	26.5 (4.3)	26.7 (4.2)
Family history of breast cancer (%)	26 (9%)	45 (16%)
Nulliparous (%)	67 (24%)	50 (18%)
Age at first child (years) (among parous women)	28 (5)	27 (4)
Number of children (among parous women)	3.2 (1.9)	3.0 (1.6)
Age at menarche (years)		
11-12	51 (19%)	43 (16%)
13+	92 (34%)	64 (23%)
Not recorded	131 (48%)	167 (61%)
Age at menopause (years)	50 (4)	50 (4)
Ever use of oral contraceptives (%)	7 (3%)	13 (5%)
Ever use of HRT (%)	6 (2%)	7 (3%)
Past or current smoker (%)	65 (24%)	68 (25%)

HRT: hormone replacement therapy. N: number. SD: standard deviation.

Table 2. Associations between creatinine-adjusted 6-sulfatoxymelatonin levels (natural logarithmic values) and selected characteristics of control participants in the DOM study, after adjustment for age at urine collection and melatonin and creatinine assay batch, where appropriate

Characteristics	No. of controls	% change per unit	95% CI		P for trend
Age at baseline (years)	274	-3.3	-6.0,	-0.7	0.01
Body mass index (kg/m ²)	274	-1.8	-4.0,	0.5	0.1
Age at menarche (years)	143	-5.0	-12.5,	2.4	0.2
Age at menopause (years)	274	1.6	-0.9,	4.0	0.2
Number of children among parous women	207	3.8	-1.6,	9.2	0.2
Age at first child among parous women (years)	207	-1.4	-3.5,	0.7	0.2
	No. of controls	Geometric mean, ng/mg creatinine	95% CI		P for heterogeneity
Family history of breast cancer					
No	248	30.6	27.8,	33.8	0.8
Yes	26	29.5	21.7,	40.1	
Season of urine collection					
Winter (January-March)	91	27.5	20.2,	37.6	0.8
Spring (April-June)	67	28.8	20.2,	41.0	
Summer (July-September)	78	35.3	23.9,	52.1	
Autumn (October-December)	38	32.3	22.2,	47.0	
Parity					
Nulliparous	67	25.1	20.8,	30.4	0.02
Parous	207	32.5	29.3,	36.2	
Ever use of oral contraceptives					
Never user	267	30.6	27.9,	33.6	NA
Ever user	7	29.2	16.0,	53.2	
Ever use of HRT					
Never user	268	30.4	27.7,	33.3	NA
Ever user	6	39.4	20.9,	74.0	
Ever smoker					
Never smoker	209	30.1	27.1,	33.5	0.6
Current or past smoker	65	32.0	26.4,	38.8	

Tests for trend of continuous characteristics in relation to aMT6s levels were assessed by Wald's test from linear regression, and tests for heterogeneity for categorical characteristics

in relation to aMT6s levels were assessed by the global F test from analyses of covariance. NA: test for heterogeneity was not performed owing to the low number of participants in one of the comparison groups.

Table 3. Median creatinine-adjusted 6-sulfatoxymelatonin and odds ratios for breast cancer by third of creatinine-adjusted 6-sulfatoxymelatonin in 274 women who developed breast cancer and 274 matched control participants from the DOM study

	Third of creatinine-adjusted 6-sulfatoxymelatonin		
	Lowest (<22.6 ng/mg)	Middle (22.6-46.2 ng/mg)	Highest (>46.2 ng/mg)
Median 6-sulfatoxymelatonin* (IQR)			
Cases	13.6 (8.6-18.3)	32.7 (27.9-36.4)	70.7 (56.6-106.7)
Controls	12.9 (7.9-17.0)	34.2 (27.7-40.0)	68.5 (55.6-100.2)
OR (95% CI) for breast cancer			
Age-adjusted	1.00 (-)	0.67 (0.45-1.01)	0.69 (0.44-1.08)
Multivariable-adjusted ^ψ	1.00 (-)	0.70 (0.45-1.09)	0.72 (0.44-1.19)
Multivariable-adjusted, follow-up ≥4 years [±]	1.00 (-)	0.62 (0.37-1.03)	0.67 (0.38-1.19)
Multivariable-adjusted, follow-up ≥4 years [#]	1.00 (-)	0.61 (0.37-1.00)	0.67 (0.38-1.18)

CI: confidence interval; IQR: interquartile range; OR: odds ratio.

* Creatinine-adjusted 6-sulfatoxymelatonin

^ψ Adjusted for age at recruitment, body mass index, family history of breast cancer, age at menarche, age at menopause, parity and age of first birth, ever use of oral contraceptives (ever, never), ever use of hormone replacement therapy, and smoking status.

[±] Based on based on 204 matched case-control sets and with thirds of creatinine-adjusted 6-sulfatoxymelatonin defined using cut-points from the main analysis.

[#] Based on based on 204 matched case-control sets and with thirds defined using cut-points based on tertiles in controls from match sets with cases diagnosed ≥4 years after blood collection. Cut-points for the middle and the highest thirds were 24.2 and 46.9 ng/mg creatinine, respectively.

Figure 1. Meta-analysis of seven prospective studies on risk of breast cancer for the highest versus the lowest level of urinary 6-sulfatoxymelatonin

Relative risks (RR) of the individual studies were presented as squares with lines corresponding to the 95% confidence intervals. Sizes of the squares were inversely proportional to the variance of the study-specific logarithmic relative risks. The diamond represents the overall RR.

P for heterogeneity=0.2, $I^2=26$.

Figure 2. Sensitivity analysis of the meta-analysis of risk of breast cancer for the highest versus the lowest level of urinary 6-sulfatoxymelatonin in five prospective studies in which breast cancer cases diagnosed within early periods of follow-up were excluded

Relative risks (RR) from the individual studies were presented as squares with lines corresponding to the 95% confidence intervals. Sizes of the squares were inversely proportional to the variance of the study-specific logarithmic relative risks. The diamond represents the overall RR. P for heterogeneity=0.08, $I^2=53\%$