Circadian Disruption, Mammographic Density and Risk of Breast Cancer

CIRCADIAN DISRUPTION, MAMMOGRAPHIC DENSITY AND RISK OF BREAST CANCER

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ABSTRACT

Humans are synchronized to the 24-hour day by the light-dark cycle of the environment. Through alteration of the suprachiasmatic nucleus (SCN), the brain's circadian pacemaker, exposure to light at night (LAN) influences the functions in the body that operate with circadian regularity, including the endocrine, immune and digestive systems.[1] The SCN also signals to the pineal gland to modulate production of melatonin, a hormone that has established antimitotic and antiproliferative properties, and has been shown to regulate estrogen and other hormones important in breast cancer etiology.[1-3]

People who work occupational night shifts are exposed to LAN and thereby experience circadian disruption, including delayed melatonin onset and reduction in peak nightly production.[4-6] In 2007, the International Agency for Research on Cancer (IARC) at the World Health Organization (WHO) declared shift work that involves circadian disruption to be "probably carcinogenic to humans" (group 2A).[7] The IARC working group cited strong experimental evidence from animals and supportive but limited human evidence from epidemiologic studies.

This dissertation investigates several relationships on the pathway from rotating night shift work exposure to breast cancer, through mammographic breast density. Mammographic density, or the proportion of fibroglandular tissue in a woman's breast as viewed on a mammogram, is the strongest risk factor for breast cancer, and has been reported as associated

with a 4-6 fold increased risk of breast cancer.[8-10] It is therefore, a reasonable intermediate endpoint for breast cancer.

The analyses in this dissertation use data from two large longitudinal cohorts of female registered nurses in the United States, the Nurse's Health Study and Nurse's Health Study II, and are presented in a series of three papers. In the first paper, the prospective and long-term association of rotating night shift work and breast cancer is assessed with 24 years of follow-up, allowing for some analysis of the timing of exposure and tumor subtypes. In the second paper, the prospective relationship of rotating night shift work and mammographic density, as measured from screening mammograms, is evaluated. In the third paper, first morning void urinary 6-sulfatoxymelatonin, the main metabolite of melatonin excreted in urine, serves as a biomarker of circadian disruption, and is evaluated in relation to mammographic density in a cross-sectional analysis.

Overall, this dissertation work provides evidence in favor of an association between long-term rotating night shift work and breast cancer, and suggests that long durations of shift work early in a nurse's career may be of particular importance. Such shift work may occur in a time period, between puberty and breast involution due to childbirth or aging, during which breast tissue is vulnerable to carcinogenic influences. Rotating night shift work and a single measure of urinary melatonin did not appear to be related to mammographic breast density, suggesting that if rotating night shift work raises a woman's risk of breast cancer, it is unlikely to do so through influence on mammographic density.

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ROTATING NIGHT SHIFT WORK AND RISK OF BREAST CANCER IN THE NURSES' HEALTH STUDIES

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ABSTRACT

Background. In 2007, the International Agency for Research on Cancer (IARC) declared shift work to be probably carcinogenic to humans, citing earlier results from the Nurses' Health Study (NHS and NHS2) cohorts. We updated these findings with twice the follow-up time.

Methods. We prospectively evaluated the association between rotating night shift work and breast cancer risk among 78,516 NHS and 114,559 NHS2 participants using Cox proportional hazards models. All statistical tests were two-sided.

Results. Over 24 years of follow-up, 9,541 incident invasive breast malignancies occurred. Compared to women who never worked rotating night shifts, women in NHS with 30+ years of rotating night shift work at baseline had no increased risk of breast cancer (HR=0.95, 95%CI 0.77-1.17; P_{trend}=0.63). In NHS2, breast cancer risk was significantly increased for women with 20+ years of rotating night shift work at baseline (HR=2.15, 95%CI 1.23-3.73; P_{trend}=0.23) and marginally increased for women with 20+ years of cumulative rotating night shift work (using updated exposure information) (HR =1.40, 95%CI 1.00-1.97; P_{trend}=0.74). These associations did not differ significantly by menopausal status at cancer diagnosis or hormone receptor status of tumors.

Conclusions. Results from our updated analyses of rotating night shift work and breast cancer risk are consistent with long-term rotating night shift work being associated with an increased risk of breast cancer. The addition of follow-up time in NHS, which occurred primarily post retirement, eliminated a previously observed increase in risk in these women and suggests that their heightened risk may wane with time.

INTRODUCTION

Breast cancer is the most common cancer among women worldwide.[1] The noticeably higher prevalence in industrialized nations compared with developing countries suggests that environmental aspects of modern society may play an important role in breast cancer etiology.[2] Disruption of the circadian system with exposure to light during the environmental nighttime hours as with occupational night shift work schedules has been hypothesized to influence carcinogenesis through suppression of melatonin, modulation of sex hormones, or altered expression of peripheral clock genes.[3-6] Supporting epidemiologic studies as well as strong mechanistic data from animal studies led the International Agency for Research on Cancer (IARC) to classify night shift work that involves circadian disruption as probably carcinogenic to humans (group 2A) in 2007.[7]

Since the IARC report, five meta-analyses have been published in an effort to summarize the growing literature on the association between night shift work and breast cancer risk, with varying approaches and conclusions. He et al, Wang et al, and Jia et al found moderate increased risk of breast cancer with night shift work, reporting pooled estimates in the range of 1.19-1.20.[8-10] The overall estimate from Kamdar et al was similar in magnitude but was marginally significant.[11] Based only on case-control studies, Ijaz et al reported a 9% increased risk of breast cancer for every 5 years of night shift work[12] and He et al reported a 16% increased risk of breast cancer for every 10 years of shift work.[8] Each of the meta-analyses cited significant heterogeneity across studies, with differing results by type and quality of study. For all, there was insufficient evidence from cohort studies alone to draw a conclusion about the relationship of shift work and breast cancer risk.

Among the three cohort studies published since the IARC decision, two found statistically significant positive associations[13, 14] and one found no evidence of an association[15]. However, they were limited by their small sample sizes (Knutsson et al N=4036, Akerstedt et al N=13,656) or short follow-up time (Pronk et al less than 5 yrs for self-reported shift work exposure).

The Nurses' Health Study (NHS) and Nurses' Health Study II (NHS2) were among the few cohort study analyses with prospectively collected shift work exposure that informed the 2007 IARC decision.[16, 17] With double the follow-up time and twice as many breast cancer cases, we are now also able to investigate timing of risk and as well as breast cancer tumor markers.

METHODS

The NHS was established in 1976 when 121,701 female registered nurses, ages 30-55, returned a mailed questionnaire with detailed information about their lifestyles, occupational and environmental exposures, medication use, and medical conditions. The NHS2 was established in 1989 when 116,430 female registered nurses, ages 25-42, returned a similar questionnaire. Participants in both cohorts have provided updated information biennially thereafter, and cumulative follow-up in the cohorts is >90%. Both studies are currently ongoing. The Institutional Review Board of Brigham & Women's Hospital (Boston, MA) approved both studies, and all participants provided informed consent through the return of the initial questionnaire.

Exposure assessment

Rotating night shift work duration was assessed through self-reported answers to the following question: "What is the total number of years during which you worked rotating night shifts (at least 3 nights/month in addition to days/evenings in that month)?" in 1988 for NHS and in 1989 for NHS2. In NHS2, a cumulative shift work measure was determined by adding baseline history to subsequently updated shift work information, collected in 1991, 1993, 1997, 2001, 2005 and for a subset or women with email addresses who were sent an online questionnaire in 2007 (N=35,418, 34% of participants active in 2007).

Each question that followed a gap in exposure assessment was asked in such a way as to allow for determination of months of shift work accumulated in each prior two-year cycle. In addition, the 2001 questionnaire asked about shift work in the period 1995-1997. Answers were very similar to those given on the 1997 questionnaire (Pearson's $r=0.53$, $p<0.0001$), indicating that recall of shift work information to fill in gaps was reasonably comparable to real-time collected information. If no shift work information was available for a given cycle, the value from the previous cycle was used to fill in the missing information. If the information was also missing in the previous cycle, participants were excluded from analyses for that cycle and subsequent cycles until or if information was again provided (i.e. they contributed person-time only as long as exposure status was captured). Of those asked about current shift work exposure in 2007, only 8% were still working rotating night shifts. Therefore, for 2009 and subsequent cycles when shift work duration was not assessed, zero shift work was assumed.

Outcome assessment

Breast cancer cases were identified as having occurred during the period June 1, 1988 to June 1, 2012 (NHS) and June 1, 1989 to June 1, 2013 (NHS2). Nurses who reported breast cancer were asked for permission to review their medical records, and breast cancer was

confirmed through review of these records. When medical records were unavailable, breast cancer subjects were included in the analysis if they were corroborated by a phone interview or written confirmation from the subject. Approximately two-thirds of the deaths among cohort members were reported to us by next of kin or the postal system in response to follow-up questionnaires. In addition, we searched the National Death Index to identify deaths due to breast cancer among the non-respondents from each two-year questionnaire. Only confirmed invasive breast cancers (i.e. excluding breast cancer in situ) were used in these analyses.

For secondary analyses of breast cancer by hormone receptor status, estrogen receptor (ER) and progesterone receptor (PR) status were determined by immunohistochemical staining of tumor tissue. The breast cancer tissue collection, tissue microarray (TMA) construction, and staining and reading for tumor markers has been described in detail elsewhere.[18] When TMA results were unavailable, medical record documentation of ERPR status was used instead. ER and PR status was not available for 14% of the cancers in NHS and 7% of the cancers in NHS2

Study population for analysis

At baseline (1988 in NHS and 1989 in NHS2), there were 103,415 participants in NHS, and 116,430 women in NHS2. Of these, participants with prior cancers except non-melanoma skin cancer (NHS: 7,957 (8%); NHS2: 1,050 (1%)) and those who did not answer the initial shift work history question (NHS: 16,942 (16%); NHS2: 581 (<1%)) were excluded. The remaining datasets for analysis comprised 78,516 women, ages 42-67, in NHS and 114,559 women, ages 24-42, in NHS2.

Covariate assessment

The following covariates were collected by questionnaire and were considered for inclusion in all multivariable-adjusted models as potential confounders or breast cancer risk factors in both cohorts, unless otherwise noted: height, body mass index (BMI), BMI at age 18, childhood body size (average of age 5 and age 10 diagrams), adolescent body size (average of age 10 and age 20 diagrams), age at menarche, oral contraceptive use, age at first birth, parity, breastfeeding duration, menopausal status, type of menopause (natural or surgical), age at menopause, menopausal hormone therapy (MHT) use and duration of types of MHT, first degree family history of breast cancer, personal history of benign breast disease, smoking status and frequency, alcohol consumption, nurse's highest education level (NHS only), husband's highest education level, and mammography use.

As several covariates have common reference categories, combination variables were created to allow for multiple factors to be used in the same model. Specifically, age at first birth and parity were combined and categorized as nulliparous, age <25 yrs and 1-2 children, age <25 yrs and 3+ children, age 25-29 and 1-2 children, age 25-29 and 3+ children, age 30+ and 1-2 children, and age 30+ and 3+ children in NHS. In NHS2, a younger cohort at baseline, broader categories were needed: nulliparous, parous and age <25 yrs, parous and age 25-29, and parous and age 30+. In both cohorts, menopausal status, type of menopause and age at menopause were combined and categorized as premenopausal, natural menopause age <45 yrs, natural menopause age 45+ yrs, surgical menopause age <45 yrs, surgical menopause age 45+ yrs. See Table 2 footnotes for specific categorizations of other covariates.

All variables except for height and duration of MHT by type were included in multivariable models as categorical variables with missing indicators. Less than 1% of participants were missing information on height and were excluded. Those with missing duration

of MHT by type were given the value of 0 months of MHT. BMI was carried forward for one questionnaire cycle to fill in some missing BMI (NHS: 9% missing reduced to 3% after carrying forward; NHS2: 14% reduced to 7%).

Statistical analyses

Cox proportional hazards models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) over the entire follow-up period. As shift work exposure assessment differed by cohort (i.e. not updated in NHS; updated in NHS2), models are presented separately for each cohort. Women were categorized according to the duration of rotating night shift work (NHS: never, 1-14 years, 15-29 years, 30+ years; NHS2: never, 1-9 years, 10-19 years, 20+ years). All models were simultaneously adjusted for age in months and time period in two-year intervals. Participants were censored at the time of breast cancer diagnosis, diagnosis of other cancer (except non-melanoma skin cancer) or death, whichever came first.

Multivariable models were adjusted for breast cancer risk factors and possible confounders of the shift work and breast cancer association. Each covariate was added into the age-adjusted model individually to see if the exposure-outcome associations changed appreciably. All covariates were included in the final multivariable-adjusted model because they either changed the estimate (i.e. they were confounders) or were associated with the outcome and thereby improved precision. Childhood body size and adolescent body size were highly correlated, so only adolescent body size was used as it was more strongly related to the outcome. See Table 2 footnotes for the complete list of covariates included in the multivariable models.

We performed tests for trend with continuous exposure measures using the midpoint of shift work duration categories and truncating the highest category. All p-values are two-sided

and values less than 0.05 were considered statistically significant. SAS software, version 9.3 (SAS Institute, Cary, North Carolina, United States) was used for all statistical analyses.

Secondary analyses

As similar main analyses with approximately half the follow-up time were previously published,[16, 17] we stratified by follow-up time period to separate early vs. late effects of rotating night shift work on breast cancer risk (i.e. ≤10 and >10 years of follow-up). To investigate the relationship of breast cancer risk with recency of night shift work exposure, we also ran models using an exposure variable separating never, current and past shift work, with categories for different times since stopping shift work, in the full dataset as well as a reduced dataset restricted to ever shift workers. Since updated shift work information was needed, this analysis was only possible in NHS2. Women were deemed to have stopped shift work at the last cycle with reported shift work information, regardless of whether there were prior cycles with no reported shift work.

We also ran models stratified by menopausal status and breast cancer hormonal receptor status of tumors (ER+PR+, ER+PR-, ER-PR-), as these attributes of breast cancer cases may inform etiologic interpretation of results. ER-PR+ tumor status was considered to be an artifact of reading[19] and was not included as a subtype. Cases of other subtypes and those missing ERPR subtype were treated as censored events in this competing risks analysis. Wald tests for interaction were used for analyses stratified by follow-up and menopausal status. The Likelihood Ratio Test was used to test for heterogeneity among the results by ER and PR status.

In NHS2, updated exposure information allowed us to separate shift work duration by time accrued pre- and postmenopausally. We ran models to assess the relationship of

premenopausal shift work and postmenopausal shift work and breast cancer, excluding 2731 (2%) participants who were postmenopausal at baseline as we were unable to attribute reported shift work duration to either the pre- or postmenopausal period. Both measures of shift work were treated as continuous variables and included in the models together to determine the associations independent of the other measure.

All multivariable models were adjusted for mammogram in the past 2 years (yes, no), as it predicts breast cancer diagnosis. To further account for possible bias due to mammography screening (i.e. shift workers may be less likely to seek screening and therefore be less likely to be diagnosed with breast cancer), we also performed a secondary analysis using inverse probability weighting by predicted mammography use.[20]

RESULTS

The participants in the NHS and NHS2 cohorts included in our analyses showed several general patterns in the distribution of baseline characteristics between cohorts and across categories of shift work (see Table 1). Participants in the NHS sample were roughly 20 years older than those in NHS2, and those in the highest shift work category were approximately 6 years older than those with no shift work exposure in both cohorts. In addition, in both cohorts, women at baseline with the highest level of shift work (30+ years in NHS, 20+ years in NHS2) were heavier, more likely to have had menarche before age 12, more likely to be current smokers with more pack-years of smoking, but with lower consumption of alcohol, compared with never shift workers. They also had a lower percentage with benign breast disease, although this could be due to their lower mammography use.

Table 1. Age-adjusted baseline characteristics by categories of rotating night shift work duration (NHS: 1988, N=78,516; NHS2: 1989, N=114,559)

	NHS				NHS2			
Characteristic	**Never n=31,746**	**1-14 years n=40,966**	**15-29 years n=4,424**	**30+ years n=1,380**	**Never n=43,529**	**1-9 years n=65,783**	**10-19 years n=5,085**	**20+ years n=162**
Age	54.3 (7.2)	54.7 (7.1)	56.1 (6.9)	60.4 (4.6)	34.8 (4.7)	34.6 (4.7)	37.2(3.4)	41.0 (2.4)
Height, inches	64.5 (2.4)	64.5 (2.4)	64.4 (2.5)	64.5 (2.5)	64.9 (2.6)	64.9 (2.6)	64.9 (2.7)	63.9 (2.9)
BMI, kg/m^2	25.3 (4.8)	25.6 (4.9)	27.0 (5.5)	26.6 (5.2)	23.9 (4.9)	24.1 (5.1)	25.3 (5.9)	24.8 (5.8)
BMI at age 18, kg/m^2	21.2 (2.9)	21.3 (3.0)	21.9 (3.4)	21.9 (3.7)	21.2 (3.2)	21.3 (3.4)	22.0 (4.1)	21.3 (4.2)
Childhood body size [a]	2.4 (1.3)	2.4 (1.3)	2.4 (1.4)	2.3 (1.4)	2.6 (1.2)	2.6 (1.2)	2.7 (1.3)	2.6 (1.3)
Adolescent body size [a]	2.7 (1.2)	2.7 (1.2)	2.7 (1.3)	2.7 (1.3)	2.9 (1.1)	2.9 (1.1)	3.0 (1.2)	2.7 (1.2)
Menarche before age 12	22	23	24	30	24	25	29	35
Ever oral contraceptive use	49	49	46	44	83	83	83	57
Nulliparous	5	6	6	6	28	32	36	42
Number of children [b]	3.2 (1.5)	3.1 (1.5)	3.2 (1.6)	3.2 (1.6)	2.1 (0.9)	2.0 (0.9)	2.1 (0.9)	2.0 (0.7)
Age at first birth [b]	24.9 (3.2)	25.3 (3.4)	24.9 (3.5)	25.3 (3.1)	25.2 (4.0)	25.7 (4.1)	25.3 (4.1)	23.0 (3.5)
Ever breastfed [b]	47	49	47	43	48	46	39	32
Postmenopausal	67	68	70	86	2	2	3	4
Age at menopause [c]	48.8 (4.8)	48.7 (4.8)	48.3 (4.7)	48.4 (4.3)	37.7 (4.3)	37.5 (4.7)	37.4 (3.6)	40.4 (0.8)
Menopause due to surgery [c]	41	42	44	40	93	92	96	88
Current menopausal hormone therapy use [c]	35	35	29	29	83	79	84	82
First-degree family history of breast cancer	11	11	11	12	6	6	5	2
History of benign breast disease	37	38	34	30	28	29	27	17
Current smoker	17	19	25	25	12	13	19	23
Pack-years smoked [d]	23.1 (19.5)	23.2 (19.4)	26.1 (20.0)	26.2 (20.0)	11.4 (8.2)	11.3 (8.2)	11.8 (8.3)	12.3 (7.6)
Alcohol consumption, grams/day	6.1 (10.6)	6.3 (10.7)	5.3 (10.5)	5.5 (9.7)	3.0 (6.0)	3.2 (6.1)	2.9 (6.1)	1.3 (4.4)
Physical activity, MET-hours/week	14.6 (20.8)	16.0 (21.9)	16.1 (21.7)	19.3 (28.3)	22.7 (34.2)	26.0 (37.9)	32.8 (48.4)	25.7 (56.2)
Nurse's education level bachelor's or higher [e]	31	30	24	22	--	--	--	--
Husband's education level college or higher [f]	55	56	42	49	80	83	80	90
Ever had mammogram	77	76	70	72	38	37	34	29

Values are means (SD) or percentages and are standardized to the age distribution of the study population.

a Body size recalled using pictures of body outlines, numbered 1-9, leanest to fattest (NHS: 1988, NHS2: 1989)

b Among parous women only.

c Among postmenopausal women only.
d Among smokers only.
e Nurse's own education level. (NHS only: 1992)
f Among married or widowed women only. (NHS: 1992; NHS2: 1999)

Women were much more likely to be nulliparous in NHS2 (28-42%), compared with NHS (5-6%). Women with the longest shift work history were more likely to be nulliparous in NHS2, although, among parous, their age at first birth was lower than it was for the comparable group in NHS. Ever oral contraceptive and current MHT was higher in NHS2 compared with NHS.

In NHS, the women with the highest duration of shift work were less likely to have attained education levels above bachelor's degrees and less likely to have had husbands with education level above college. In NHS2, we did not have a measure of SES until spousal education attainment was collected in 1999 (10 years post baseline). Using this measure, the highest shift work group in NHS2 at baseline had the highest spousal education attainment. However, it should be noted that by the approximate midpoint of follow-up in 1999, the highest shift work group had lower spousal education attainment, similar to NHS at baseline (data not shown).

During 24 years of follow-up, we documented 9541 total invasive breast cancers (5971 in NHS and 3570 in NHS2), with a median time to breast cancer event of 13 years in NHS and 14 years in NHS2. NHS2 cumulative shift work analyses included 3188 breast cancers, due to skipping of cycles with missing updated shift work information as previously described.

In NHS, we observed no association between baseline duration of rotating night shift work and breast cancer risk in age-adjusted models, with never shift workers as the reference group ($HR_{1\text{-}14\ yrs\ vs\ 0\ yrs}$ =1.03, 95% CI 0.98-1.09; $HR_{15\text{-}29\ yrs\ vs\ 0\ yrs}$ =1.02, 95% CI 0.91-1.14; $HR_{30+\ yrs\ vs\ 0\ yrs}$ =0.92, 95% CI 0.75-1.13; p_{trend}=0.89). Adjustment for possible confounders and breast cancer risk factors resulted in minimal change to these null results (MV-$HR_{1\text{-}14yrs}$=1.01, 95% CI

Table 2. Associations of duration of rotating night shift work and invasive breast cancer during 24 years of follow-up (NHS: 1988-2012; NHS2: 1989-2013)

	No. of cases	Person-years	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95% CI) [b]
NHS rotating night shift work history				
Never	2382	640,594	Ref	Ref
1-14 yrs	3162	817,778	1.03 (0.98-1.09)	1.01 (0.96-1.07)
15-29 yrs	331	84,887	1.02 (0.91-1.14)	1.06 (0.94-1.19)
30+ yrs	96	25,178	0.92 (0.75-1.13)	0.95 (0.77-1.17)
	5971	1,568,438	$p_{trend} = 0.89$	$p_{trend} = 0.63$
NHS2 1989 baseline rotating night shift work history (early career)				
Never	1318	978,847	Ref	Ref
1-9 yrs	2071	1,475,921	1.06 (0.99-1.13)	1.05 (0.98-1.13)
10-19 yrs	168	112,752	0.94 (0.80-1.10)	1.00 (0.85-1.17)
20+ yrs	13	3,335	1.83 (1.05-3.17)	2.15 (1.23-3.73)
	3570	2,570,855	$p_{trend} = 0.58$	$p_{trend} = 0.23$
NHS2 cumulative rotating night shift work (updated) [a]				
Never	950	675,209	Ref	Ref
1-9 yrs	2002	1,384,743	1.03 (0.96-1.12)	1.04 (0.96-1.12)
10-19 yrs	201	140,868	0.90 (0.77-1.05)	0.94 (0.81-1.10)
20+ yrs	35	13,705	1.29 (0.92-1.81)	1.40 (1.00-1.97)
	3188	2,214,524	$p_{trend} = 0.73$	$p_{trend} = 0.74$

a For NHS2, analyses using updated duration of shift work excluded participants during the cycles in which they were missing shift work exposure information, resulting in fewer cases and person-years, compared to analyses using history of shift work reported at baseline in 1989.
b Multivariable-adjusted models include the following covariates: age (months), height (continuous in inches), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30+ kg/m2), BMI at age 18 (<18.5, 18.5-24.9, 25.0-29.9, 30+ kg/m2), adolescent body size (average of age 10 and age 20 diagrams: 1, 1.5-2, 2.5-3, 3.5-4, 4.5+), age at menarche (<12 yrs, 12-13 yrs, 14+ yrs), age at first birth and parity combined (NHS: nulliparous, age <25 yrs 1-2 children, age <25 yrs 3+ children, age 25-29 yrs 1-2 children, age 25-29 yrs 3+ children, age 30+ yrs 1-2 children, age 30+ yrs 3+ children; NHS2: nulliparous, parous age <25 yrs, parous age 25-29 yrs, parous age 30+ yrs), breastfeeding (NHS: none, 1-11 months, 12+ months; NHS2: none, 1-12 months, >12 months), type of menopause and age at menopause combined (premenopausal, post natural age <45, post natural age 45+, post surgery age <45, post surgery age 45+), menopausal hormone therapy (never, past, current), duration of estrogen alone MHT (continuous in months), duration of estrogen and progesterone MHT (continuous in months), first-degree family history of breast cancer (yes, no), history of benign breast disease (yes, no), alcohol consumption (0, 0.1-14, 14.1-28, >28g/day), physical activity (<=8, 8.1-16, 16.1-24, >24 MET-hrs/week), and current mammography use (yes, no). All categorical covariates were included in models with missing indicators.

0.96-1.07; MV-$HR_{15\text{-}29\ yrs\ vs\ 0\ yrs}$ =1.06, 95% CI 0.94-1.19; MV-$HR_{30+\ yrs\ vs\ 0\ yrs}$ =0.95, 95% CI 0.77-1.17; p_{trend}=0.63). (See Table 2)

By contrast, in NHS2, 20+ years of rotating night shift work at baseline was associated with a significantly increased risk of breast cancer, compared with baseline never shift work, in both the age-adjusted model ($HR_{base20+\ yrs\ vs\ 0\ yrs}$ =1.83, 95% CI 1.05-3.17, p_{trend}=0.58) and the multivariable-adjusted model (MV-$HR_{base20+\ yrs\ vs\ 0\ yrs}$ =2.15, 95% CI 1.23-3.73, p_{trend}=0.23). We observed no association between shorter durations of shift work at baseline and breast cancer risk. Women with cumulative rotating night shift work exposure of 20+ years had a marginally significant increased risk of breast cancer, compared to women who never worked rotating night shifts (age-adjusted model $HR_{cum20+\ yrs\ vs\ 0\ yrs}$ =1.29, 95% CI 0.92-1.81; MV-adjusted model $HR_{cum20+\ yrs\ vs\ 0\ yrs}$ =1.40, 95% CI 1.00-1.97, p_{trend}=0.74). Results were null for the other durations of shift work. (See Table 2)

Stratification by follow-up period in both cohorts and both measures of shift work in NHS2 showed a general pattern of increased risk with the highest level of shift work duration during the first 10 years of follow-up, which was not apparent in the remainder of the full follow-up. In NHS, the trend across categories in the first 10 years was statistically significant (p_{trend}=0.04), and the HR for 30+ years of shift work was non-significantly elevated (MV-$HR_{30+\ yrs\ vs\ 0\ yrs}$=1.26, 95% CI 0.97-1.64). In the last 14 years of follow-up, the HR was inverse (MV-HR_{30+yrs}=0.68, 95% CI 0.49-0.95), with $p_{interaction}$=0.03. In NHS2, the HRs for baseline 20+ yrs as well as cumulative 20+ years were significantly positive in the first 10 years of follow-up (MV-$HR_{base\ 20+\ yrs\ vs\ 0\ yrs}$=2.35, 95% CI 1.04-5.31 and MV-$HR_{cum\ 20+\ yrs\ vs\ 0\ yrs}$=2.13, 95% CI 1.19-3.81, respectively), and non-significantly positive with lower estimates in the last 14 years of follow-up (MV-$HR_{base\ 20+\ yrs\ vs\ 0\ yrs}$ =1.95, 95% CI 0.92-4.15 and MV-$HR_{cum\ 20+\ yrs\ vs\ 0\ yrs}$=1.19, 95% CI:

0.78-1.81, respectively). Interactions with follow-up period were not significant in NHS2. (See Table 3)

We observed no significant associations with breast cancer risk for current or past shift work with different times since stopping working night shifts, compared with never shift work. When restricted to women who ever worked rotating night shifts, we noted a significant trend for increasing risk of breast cancer with greater time since stopping shift work (P_{trend}=0.04; Table 4).

Using the same categories of rotating night shift work as in the main analyses, we were only able to stratify by menopausal status in NHS2 because of the small number of premenopausal cases in the highest level of shift work exposure in NHS. Baseline 20+ years of shift work was significantly associated with postmenopausal breast cancer (MV-$HR_{20+\ yrs\ vs\ 0\ yrs,\ postmeno}$=3.24, 95% CI 1.68-6.25), although this level of shift work had few cases (n=10), and the interaction between shift work and menopausal status was not significant ($p_{interaction}$=0.17). The cumulative shift work and breast cancer results were null and did not differ by menopausal status ($p_{interaction}$ =0.22). In addition, neither measure of shift work duration accrued pre/postmenopausally was associated with breast cancer in multivariable-models, adjusting for the other measure (MV-HR_{preSW}=1.00, 95% CI 0.99-1.01; MV-HR_{postSW}=0.98, 95% CI 0.90-1.06).

The associations of shift work and breast cancer did not differ by ER and PR status of the breast cancer in both cohorts across the full follow-up period (NHS $p_{heterogeneity}$=0.18; NHS2 baseline $p_{heterogeneity}$=0.48; NHS2 cumulative $p_{heterogeneity}$=0.70), although small sample sizes in the highest shift work categories limit interpretability (See Table 5). Restricting to ER+PR+ tumors only, the association of cumulative rotating shift work and breast cancer in NHS2 was strengthened (MV-$HR_{cum\ 20+\ yrs\ vs\ 0\ yrs}$=1.62, 95% CI 1.07-2.45), when compared with the main

Table 3. Associations of duration of rotating night shift work and invasive breast cancer, stratified by follow-up period, during 24 years of follow-up (NHS: 1988-2012; NHS2: 1989-2013)

	Follow-up <=10 years				Follow-up >10 years				
	No. of cases	Person-years	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95% CI) [b]	No. of cases	Person-years	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95% CI) [b]	MV $p_{interaction}$
NHS rotating night shift work history									
Never	977	298,701	Ref	Ref	1405	336,729	Ref	Ref	
1-14 yrs	1415	383,622	1.11 (1.02-1.21)	1.09 (1.00-1.18)	1747	427,392	0.97 (0.91-1.05)	0.96 (0.89-1.03)	
15-29 yrs	146	40,739	1.03 (0.86-1.23)	1.07 (0.90-1.28)	185	43,381	1.01 (0.87-1.18)	1.05 (0.90-1.23)	
30+ yrs	60	12,537	1.23 (0.95-1.60)	1.26 (0.97-1.64)	36	12,418	0.65 (0.47-0.91)	0.68 (0.49-0.95)	
	2598	735,599	p_{trend} = 0.08	p_{trend} = 0.04	3373	819,920	p_{trend} = 0.15	p_{trend} = 0.25	p_{int} = 0.03
NHS2 1989 baseline rotating night shift work history (early career)									
Never	416	412,724	Ref	Ref	902	553,730	Ref	Ref	
1-9 yrs	637	622,782	1.03 (0.91-1.17)	1.02 (0.90-1.15)	1434	833,620	1.07 (0.98-1.16)	1.07 (0.98-1.16)	
10-19 yrs	57	47,867	0.94 (0.71-1.24)	0.96 (0.73-1.27)	111	63,327	0.94 (0.77-1.14)	1.01 (0.83-1.24)	
20+ yrs	6	1,491	2.13 (0.95-4.80)	2.35 (1.04-5.31)	7	1,801	1.63 (0.77-3.45)	1.95 (0.92-4.15)	
	1116	1,084,864	p_{trend} = 0.76	p_{trend} = 0.71	2454	1,452,478	p_{trend} = 0.65	p_{trend} = 0.24	p_{int} = 0.85
NHS2 cumulative rotating night shift work (updated) [a]									
Never	341	321,600	Ref	Ref	609	346,804	Ref	Ref	
1-9 yrs	621	602,095	0.98 (0.86-1.12)	0.97 (0.85-1.11)	1381	767,303	1.06 (0.96-1.16)	1.07 (0.97-1.18)	
10-19 yrs	60	50,481	0.92 (0.70-1.21)	0.94 (0.71-1.23)	141	88,801	0.90 (0.74-1.07)	0.95 (0.79-1.14)	
20+ yrs	12	2,956	1.99 (1.11-3.56)	2.13 (1.19-3.81)	23	10,637	1.10 (0.72-1.66)	1.19 (0.78-1.81)	
	1034	977,132	p_{trend} = 0.83	p_{trend} = 0.75	2154	1,213,546	p_{trend} = 0.58	p_{trend} = 0.89	p_{int} = 0.73

a For NHS2, analyses using updated duration of shift work excluded participants during the cycles in which they were missing shift work exposure information, resulting in fewer cases and person-years, compared to analyses using history of shift work reported at baseline in 1989.

b Multivariable-adjusted models. See Table 2 footnotes for list of covariates.

Table 4. Associations of time since stopping rotating night shift work and invasive breast cancer during 24 years of follow-up in NHS2 only (1989-2013)

	No. of cases	Person-years	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95% CI) [a]
NHS2 time since stopping rotating night shift work among all				
Never shift work	1060	786,772	Ref	Ref
Current shift work	478	467,992	0.97 (0.87-1.09)	0.96 (0.84-1.09)
Past, <=8 years since stopping shift work	798	606,237	1.01 (0.92-1.11)	1.01 (0.91-1.13)
Past, 9-16 years since stopping shift work	907	476,794	1.08 (0.98-1.19)	1.10 (0.99-1.22)
Past, >16 years since stopping shift work	327	233,060	1.09 (0.94-1.27)	1.10 (0.95-1.28)
	3570	2,570,855		
NHS2 time since stopping rotating night shift work, restricted to ever rotating night shift workers only				
Current shift work (i.e. 0 years since stopping)	478	467,992	Ref	Ref
Past, <=8 years since stopping shift work	798	606,237	1.03 (0.92-1.17)	1.05 (0.93-1.19)
Past, 9-16 years since stopping shift work	907	476,794	1.11 (0.97-1.27)	1.14 (0.99-1.31)
Past, >16 years since stopping shift work	327	233,060	1.17 (0.96-1.43)	1.19 (0.97-1.47)
	2510	1,784,083	Ptrend=0.06	Ptrend=0.04

a Multivariable-adjusted models are adjusted for the covariates listed in Table 2 footnotes, and are additionally adjusted for duration of rotating night shift work (continuous in months).

Table 5. Associations of duration of rotating night shift work and estrogen receptor (ER) and progesterone receptor (PR) status [a] of invasive breast cancer tumors during 24 years of follow-up (NHS: 1988-2012; NHS2: 1989-2013)

	ER+PR+			ER+PR-			ER-PR-			
	No. of cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95% CI) [c]	No. of cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95% CI) [c]	No. of cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95% CI) [c]	MV $p_{heterogeneity}$
NHS rotating night shift work history										
Never	1390	Ref	Ref	319	Ref	Ref	327	Ref	Ref	
1-14 yrs	1879	1.05 (0.98-1.13)	1.03 (0.96-1.10)	414	1.00 (0.86-1.16)	0.98 (0.85-1.14)	398	0.95 (0.82-1.10)	0.94 (0.81-1.09)	
15-29 yrs	199	1.05 (0.90-1.22)	1.11 (0.95-1.29)	34	0.76 (0.53-1.08)	0.82 (0.57-1.17)	45	1.01 (0.74-1.38)	1.06 (0.77-1.45)	
30+ yrs	54	0.92 (0.70-1.21)	0.96 (0.73-1.27)	11	0.74 (0.40-1.35)	0.77 (0.42-1.40)	8	0.59 (0.29-1.20)	0.63 (0.31-1.27)	
	3522	p_{trend} = 0.56	p_{trend} = 0.33	778	p_{trend} = 0.12	p_{trend} = 0.21	778	p_{trend} = 0.34	p_{trend} = 0.47	p_{het}= 0.18
NHS2 1989 baseline rotating night shift work history (early career)										
Never	708	Ref	Ref	112	Ref	Ref	200	Ref	Ref	
1-9 yrs	1166	1.11 (1.01-1.22)	1.11 (1.01-1.22)	201	1.21 (0.96-1.52)	1.21 (0.96-1.52)	268	0.90 (0.75-1.08)	0.92 (0.76-1.11)	
10-19 yrs	87	0.90 (0.72-1.13)	0.97 (0.78-1.22)	16	1.03 (0.61-1.74)	1.14 (0.67-1.93)	23	0.88 (0.57-1.35)	0.95 (0.61-1.47)	
20+ yrs	5	1.33 (0.55-3.22)	1.58 (0.65-3.83)	1	1.29 (0.18-9.35)	1.58 (0.21-11.72)	2	2.08 (0.51-8.47)	2.15 (0.52-8.92)	
	1966	p_{trend} = 0.67	p_{trend} = 0.31	330	p_{trend} = 0.41	p_{trend} = 0.25	493	p_{trend} = 0.44	p_{trend} = 0.70	p_{het}= 0.48
NHS2 cumulative rotating night shift work (updated) [b]										
Never	539	Ref	Ref	81	Ref	Ref	146	Ref	Ref	
1-9 yrs	1152	1.04 (0.94-1.15)	1.04 (0.94-1.16)	204	1.24 (0.96-1.61)	1.25 (0.97-1.62)	269	0.90 (0.73-1.10)	0.92 (0.75-1.12)	
10-19 yrs	105	0.81 (0.66-1.00)	0.85 (0.69-1.05)	20	1.09 (0.67-1.78)	1.20 (0.73-1.96)	42	1.22 (0.86-1.72)	1.29 (0.91-1.82)	
20+ yrs	24	1.50 (1.00-2.27)	1.62 (1.07-2.45)	2	0.79 (0.19-3.24)	0.89 (0.22-3.64)	2	0.50 (0.12-2.02)	0.53 (0.13-2.14)	
	1820	p_{trend} = 0.53	p_{trend} = 0.89	307	p_{trend} = 0.60	p_{trend} = 0.36	459	p_{trend} = 0.74	p_{trend} = 0.54	p_{het}= 0.70

a ERPR status was not available for 14% of the cancers in NHS and 7% of the cancers in NHS2. ER-PR+ tumor status was considered to be an artifact of reading[19] and was not included as a subtype.

b For NHS2, analyses using updated duration of shift work excluded participants during the cycles in which they were missing shift work exposure information, resulting in fewer cases and person-years, compared to analyses using history of shift work reported at baseline in 1989.

c Multivariable-adjusted models. See Table 2 footnotes for list of covariates.

result in Table 2. Combining the highest two categories of shift work for both cohorts to better balance number of women in each exposure category showed resulted in null findings with no significant heterogeneity by ER/PR status (data not shown).

The results from secondary analyses using inverse probability weighting for mammographic screening were not substantially different from the main results using traditional model adjustment for current mammography use. Reduced sample sizes were available for the IPW models because no weights could be determined if mammography use information was missing, so comparisons were made between models using traditional adjustment and IPW weighting utilizing the same smaller dataset. In both cohorts, the multivariable-adjusted hazard ratios were similar to our unweighted results (NHS unweighted $HR_{30+\ yrs\ vs\ 0\ yrs}$ =0.97 vs. weighted $HR_{30+\ yrs\ vs\ 0\ yrs}$ =1.00; NHS2 unweighted $HR_{base20+\ yrs\ vs\ 0\ yrs}$ =2.60 vs. weighted $HR_{base20+\ yrs\ vs\ 0\ yrs}$ =2.55 and unweighted $HR_{cum20+\ yrs\ vs\ 0\ yrs}$ =1.41 vs. weighted $HR_{cum20+\ yrs\ vs\ 0\ yrs}$ =1.51), indicating minimal bias due to differential screening practices among shift workers.

DISCUSSION

We saw no association between rotating night shift work and breast cancer incidence over the full 24 years of follow-up in the NHS cohort. The women included in this analysis were 42-67 years old at baseline in 1988, when shift work history was recorded. Current rotating night shift work (yes/no) was asked of the cohort 8 years later in 1996, and only 3% were still working rotating night shifts at that time. We seem to have captured primarily post-retirement time with the expansion of follow-up and likely very little additional shift work was accumulated. This may in part explain the lack of an association we observed in NHS with the additional 14 years of follow-up.

In NHS2, the younger age of the cohort as well as updated exposure information throughout follow-up allowed us to assess breast cancer risk with more recent shift work exposure. We found a strong positive association with breast cancer among the women who had accumulated 20+ years of rotating night shift work early in their careers, in their 20's and 30's. Those participants also contributed to the 20+ year shift work category in the cumulative shift work measure, but were mixed with women who had different patterns of shift work accumulation after baseline, likely attenuating this association. Nonetheless, the cumulative measure of shift work was consistent with a marginally significant increased risk of breast cancer.

To explore this further, we conducted analyses restricting to ever shift workers, and observed a significant trend with longer time since stopping shift work being associated with greater breast cancer risk in these women. In 2009, women in NHS2 were asked about their primary work schedule during the age ranges of 20-25, 26-35, 36-45 and 46+. For the person-time attributed to the >16 years of time since stopping shift work category, 94% reported being rotating night shift workers before age 35 (compared with 67% for current, 82% for <=8 years since stopping, and 89% for 9-16 years since stopping shift work). Hence, greater time since stopping shift work may be a marker for shift work performed at young adult ages.

We explored the associations separately for the first 10 years of follow-up and the remaining 14 years of follow-up, to understand the long-term findings in the context of our previously published shorter-term associations.[16, 17] In both cohorts, and for both measures of shift work in NHS2, we saw that breast cancer risk associated with night shift work was higher in the earlier versus later portion of follow-up. The estimates were higher in NHS2, where the shift work performance was likely closer in proximity to breast cancer risk than in NHS. We

investigated the suggestive inverse finding in the later part of follow-up for NHS as possibly reflecting a healthy worker effect, but did not see any evidence of differential dropping out of the analysis by shift work category, and therefore believe it to be due to chance.

To our knowledge, no other studies have specifically explored timing or proximity of shift work with breast cancer risk. However, duration of shift work may serve as a proxy for recency of exposure. Data from the Current Population Survey in the US[21] suggests that a large proportion of people who work night shifts do so to accommodate schooling and childcare needs, presumably at young ages. Other work from our group[22] suggests that most nurses in our cohorts who engage in shift work do so before age 25, possibly during training programs. Longer durations of shift work in this population likely include shift work that occurred during training and then continued on, closer to breast cancer diagnosis. In other populations, studies that have found a significant association with duration of shift work, have done so with durations of at least 15 years.[23-25]

Further, timing of shift work with respect to breast tissue development may be critical. In our analyses, the strongest associations with breast cancer risk were for those women who worked 20+ years on rotating night shifts early in their careers as young adults. The early-career time in these nurses may be within a window of major breast tissue change – the period between onset of puberty and breast involution due to childbirth (postlactational) or aging (lobular) - and therefore vulnerable to cancer risk factors. In a recent Spanish study, Papantoniou et al saw a slightly higher risk of breast cancer among women exposed to night shift work prior to first full-term pregnancy compared to those exposed after first full-term pregnancy.[26] Additional analyses in datasets that allow for separation of shift work exposure with respect to such early-career events are warranted.

In addition, as circulating estradiol levels have been reported to be higher in night shift workers compared to day shift workers,[27] we evaluated the shift work and breast cancer association by presence of estrogen and progesterone receptors in the tumor tissue. Small numbers in the highest categories of shift work duration limited determination of statistically significant heterogeneity. However, NHS2 results indicated a potentially stronger association with ER+PR+, supporting the hypothesized hormonal pathway for shift work to affect breast cancer risk.

Finally, as night shift workers are less likely to adhere to breast cancer screening guidelines[28] and we noted lower proportion of mammography use with increasing shift work duration in our data (see Table 1), we ran models using inverse probability weights for likelihood of mammography based on factors that have been shown to predict screening behavior.[28] We saw little evidence of bias in our main results due to differential screening practices and it is unlikely that such bias may have distorted an association.

The NHS and NHS2 cohorts provide rich data for examining the association of rotating night shift work and breast cancer, but also have several notable limitations. Rotating night shift work for a given month was defined as 3 or more night shifts on a rotating schedule in addition to other day/evening shifts in that month; in other words, a nurse with 20 years of night shifts, but not on a rotating shift schedule, would answer ‘none’ to this question. Also, although night shift workers may get more exposure to electric light at night than day shift workers, almost all persons in the modern world are exposed to light at night, at least in the evening. It has been shown from controlled studies in the laboratory that such lighting can delay melatonin onset and duration depending on intensity and wavelength.[29] Thus, our exposure definition itself may not

capture the intensity or pattern of night shift work that is most disruptive, and may have limited our ability to identify a dose-response relationship.

Still, the NHS and NHS2 cohorts are among the largest prospective cohort studies available for quantifying the relationship between rotating night shift work and breast cancer. They are unique in their ability to prospectively measure night shift work as well as most of the lifestyle and reproductive factors that are important for breast cancer development. The studies also include long follow-up and a large number of breast cancer cases to allow exploration of risk patterns over time as well as some separation of effects for subtypes of breast cancer.

The updated long-term findings in the NHS and NHS2 cohorts have important implications for future IARC evaluations of the shift work and breast cancer association. Our results may serve to put the literature into the context of short-term vs long-term effects, and suggest that there may be a period of increased risk, that wanes with time.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ROTATING NIGHT SHIFT WORK AND MAMMOGRAPHIC BREAST DENSITY IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN IN THE NURSES' HEALTH STUDIES

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ABSTRACT

Introduction. Mammographic density is a strong risk factor for breast cancer and is therefore considered to be a good intermediate marker of risk. Shift work that involves circadian disruption has been deemed a probable carcinogen by the International Agency for Research on Cancer (IARC) for its reported association with increased risk of breast cancer. However, to our knowledge, only one prior study has examined the association of shift work and mammographic density.

Methods. We conducted a prospective analysis among women in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS2) who provided information on their rotating night shift work history and screening mammograms from which we were able to determine percent density (%), absolute dense area (cm^2) and absolute non-dense area (cm^2). Multivariable linear regression models were fit for each density measure separately, and were stratified a priori by menopausal status at the time of mammogram (N_{pre}=1,906, N_{post}=1,860). Differences in mean density measures were reported for durations of 1-9 years, 10-19 years and 20+ years of rotating night shift work, and for current shift work, compared to never shift work.

Results. Duration of rotating night shift work cumulative through time of mammogram was not associated with a statistically significant difference in mean mammographic density, compared to those with no rotating night shift work experience, in premenopausal women ($\beta_{pct,\ 1\text{-}9\ yrs\ vs\ 0\ yrs}$=-0.42, 95% CI: -2.00,1.17; $\beta_{,\ 10\text{-}19\ yrs\ vs\ 0\ yrs}$=0.02, 95% CI: -3.09,3.13; $\beta_{pct,\ 20+\ yrs\ vs\ 0\ yrs}$=-1.37, 95% CI: -7.20,4.47; p_{trend}=0.73) or in postmenopausal women ($\beta_{pct,\ 1\text{-}9\ yrs\ vs\ 0\ yrs}$=-0.09, 95% CI: -1.51,1.32; $\beta_{pct,\ 10\text{-}19\ yrs\ vs\ 0\ yrs}$=1.45, 95% CI: -1.22,4.12; $\beta_{pct,\ 20+\ yrs\ vs\ 0\ yrs}$=1.73, 95% CI: -2.13,5.60; p_{trend}=0.22). Current rotating night shift work was associated with a non-significant increase in mean percent density, compared with never shift workers in premenopausal women ($\beta_{pct,\ current\ vs}$

$_{never}$=2.22, 95% CI: -0.97,5.41) and postmenopausal women ($\beta_{pct,\ current\ vs\ never}$=1.40, 95% CI: -4.76,7.57).

Conclusions. While long duration of rotating night shift work has been associated with increased risk of breast cancer in our population, we saw no evidence that it acts through influence on mammographic breast density.

INTRODUCTION

High mammographic breast density, or the proportion of radiologically dense breast tissue on a woman's mammogram, is the strongest known risk factor for breast cancer with relative risks in the range of 4-6, comparing very dense breasts to very fatty breasts.[1-3] Mammographic density is considered to be a good intermediate endpoint for studies of prevention and intervention,[4] yet relatively little is understood about its causes and correlates. Several of the studied predictors of mammographic density are hormonally related, suggesting that it may be a marker of cumulative exposure to estrogen.[5-8]

Rotating night shift work has also been shown to be a consistent risk factor for breast cancer.[9-13] A recent updated analysis of the Nurses' Health Study II cohort reported the relative risk for invasive breast cancer to be 1.41 (95% CI:1.00,1.97 for women with 20+ years of rotating night shift work, compared to those with no shift work experience, over 24 years of follow-up (Wegrzyn dissertation paper 1, unpublished). Exposure to light at night as in night shift work is believed to alter circadian patterns, suppress melatonin production, and thereby alter hormonal profiles that play a role in cancer-related pathways. The International Agency for Research on Cancer (IARC) has declared shift work that involves circadian disruption to be "probably carcinogenic to humans" (group 2A).[14]

Several factors have been explored for their potential to predict or affect mammographic density, including exogenous hormone use, circulating endogenous hormone levels, diet and physical activity factors. However, to our knowledge, night shift work has only been explored in one recent study, but may have been limited by small sample size and an exposure measure that may not have adequately captured the disruption of the circadian system.[15]

In this analysis, we prospectively assessed the associations between rotating night shift work and mammographic density within a large dataset with rich information on many important potential confounders and predictors of mammographic density. We hypothesized that longer durations of night shift work would lead to increased mammographic density, thereby providing support for a hormonal pathway in these associations.

METHODS

We used subsets of women participating in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS2) for these analyses. The NHS was established in 1976, among 121,700 female registered nurses in the United States, ages 30-55, to assess risk factors for cardiovascular disease and cancer. The NHS2 was established in 1989, enrolling 116,430 female registered nurses in the United States, ages 25-42, to further assess risk factors for many clinical outcomes in a slightly younger population. Both studies are ongoing longitudinal studies, and participants report detailed information about their lifestyles, occupational and environmental exposures, pharmaceutical intake and medical conditions on biennial questionnaires. Subsets of both cohorts have also provided biological and radiological samples.

The Institutional Review Board of Brigham & Women's Hospital (Boston, MA) approved both studies, and all participants provided informed consent through the return of the initial questionnaire.

Study sample

Mammograms were collected from women within the NHS and NHS2, who were included in ongoing nested case-control studies of breast cancer. Participants in these substudies were asked to provide blood samples at various times (starting in 1989 for NHS and in 1996 for

NHS2) for use in studies of biomarker exposures. Every case was matched to one or two controls based on age, menopausal status, postmenopausal hormone use and fasting status at blood collection and timing of the blood collection with respect to the menstrual cycle. Mammograms were also collected as part of these nested case-control studies of breast cancer and were targeted to be screening mammograms as close as possible to the date of blood collection (before and after), with median time between mammogram date and blood collection date of approximately 9 months in premenopausal women and approximately 19 months in postmenopausal women.

Rotating night shift work information was collected from questionnaires for all participants in both NHS (in 1988) and NHS2 (in 1989 and then updated in 1991, 1993, 1997, 2001, 2005 and 2007). The dataset used in these analyses was restricted to controls from the nested case-control studies (i.e. women free of breast cancer) with mammograms and rotating night shift work information. In NHS, 48 mammograms were excluded (2%) because they occurred prior to the shift work exposure assessment in 1988. The total sample size available for analyses was 4,017 (1906 premenopausal; 1860 postmenopausal; 251 missing menopausal status).

Data Collection and Measurements

Rotating night shift work assessment

Rotating night shift work was assessed through self-reported answers to the following question: "What is the total number of years during which you worked rotating night shifts (at least 3 nights/month in addition to days/evenings that month)?" This question was asked of all participants in the NHS and NHS2 studies, but at different times. In NHS, the question was asked in 1988. Since mammograms were obtained starting in 1989 with a median year of

collection of 1991, the NHS question can be considered to have been collected at roughly the same time, but reflecting cumulative past exposure. In NHS2, the question was asked in 1989 and then updated in 1991, 1993, 1997, 2001, 2005 and 2007 in such a way as to allow for determination of shift work exposure within each 2-year period. If no shift work information was available for a given cycle, the value from one previous cycle was used to fill in the missing information. To represent cumulative night shift work up to the time of the mammogram, we summed the durations collected through the question closest to but prior to the mammogram. The exposure measure of years of rotating night shift work for both cohorts was categorized as never, 1-9 yrs, 10-19 yrs and 20+ yrs.

In NHS2, a current rotating night shift work exposure measure was determined from the information collected for the most recent 2-year period prior to the mammogram date (e.g. For a mammogram date in 2002, the duration of shift work was taken from the 2001 questionnaire, referring to the period of June 1999 – June 2001). A three category variable was used in a secondary analysis, with categories of current, past only and never rotating night shift work, adjusted for cumulative shift work duration (in months) up to the same questionnaire cycle.

Mammographic density assessment

Film mammograms were obtained for approximately 80% of the participants who were queried for mammograms. Other work from our group has shown that the women for whom mammograms were obtained were similar to those for whom mammograms were not obtained with respect to age, BMI, parity, family history and circulating hormone levels.[16, 17]

We used percent density, absolute dense area, and absolute non-dense area as measures of mammographic breast density. These were determined quantitatively using a computer-

assisted method. For all three outcomes, measures from the cranio-caudal mammographic views of both breasts from each subject were averaged. For each image, a trained reader manually set the appropriate gray-scale threshold level defining the edge of the breast. The software package, Cumulus, then calculated the total number of pixels within the entire region of interest and within the regions identified as dense and non-dense. These values were used to calculate the percentage (dense area divided by total area) and absolute areas of the breast area that are dense and non-dense.[17]

The measures of density from all the mammograms for this study were determined by one trained reader, whose within-person intraclass correlation coefficient has been reported as >0.90.[18] The reader was blinded to the case-control status of the mammograms, and a random 10% of mammograms were included in all batches as duplicate quality control samples.

For NHS, mammograms were read in two batches. For NHS2, there were three batches, with evidence of batch-to-batch variability, or drift toward smaller dense area measurements over the course of the three batches. Density measurements for the latter batches were recalibrated to the first batch. Details of the recalibration method are described elsewhere.[16, 19] All models in our analysis used the recalibrated NHS2 measures and were adjusted for a combined cohort and batch indicator variable (NHS batch 1, NHS batch 2, NHS2).

Covariate assessment

The following covariates were pulled from the questionnaire closest to and prior to the mammogram date and were considered for inclusion in all multivariable models as potential confounders or predictors of mammographic density: age, height, BMI, BMI at age 18, childhood body size (average of age 5 and 10 diagrams), adolescent body size (average of age 10

and 20 diagrams), age at menarche, age at first birth, parity, breastfeeding, age at menopause, menopausal hormone therapy (MHT) use, duration of types of MHT, first-degree family history of breast cancer, personal history of benign breast disease, smoking status, alcohol consumption, physical activity, and husband's highest education level.

As age at first birth and parity both contained a common reference, they were combined to allow for the inclusion of both in the same model (nulliparous, age <25 yrs and 1-2 kids, age <25 yrs and 3+ kids, age 25-29 and 1-2 kids, age 25-29 and 3+ kids, age 30+ and 1-2 kids, and age 30+ and 3+ kids). See Table 2 footnotes for specific categorizations of other covariates.

As BMI is highly correlated with mammographic density measures, we excluded participants with missing BMI information (<4%). Categorical variables were included in multivariable models with missing indicators. Missing values of continuous variables were set to the median and missing indicators were included in the models.

Statistical analyses

Linear regression was used to calculate beta estimates, or differences in the mean mammographic density measures, comparing each category of rotating night shift work to the no night shift work category. Models were run separately for each of the three outcome measures, and by menopausal status in the main analyses, as mammographic density distributions are very different among premenopausal and postmenopausal women. As residuals were skewed for the absolute density measures, square root transformations were applied to those outcomes and models were also fit using the transformed versions as the dependent variables. Generalized estimating equations were used to account for the correlation between matched controls, using the repeated statement of the SAS GENMOD procedure.

All models were adjusted for age at mammogram and the cohort and batch combination variable. Two nested multivariable models were run to highlight the change in estimates with adjustment for BMI and then other variables in addition to BMI. Model 2 was adjusted for age, cohort/batch, height, BMI at time of mammogram, BMI at age 18, and adolescent body size. Childhood body size and adolescent body size are highly correlated, so only adolescent body size was used, as it is more strongly related to the outcome. Model 3 was adjusted for the above as well as other predictors of mammographic density or possible confounders of the shift work and mammographic density association. Each covariate was added to the model individually to see if the exposure-outcome associations changed appreciably. All covariates were included in the final multivariable-adjusted model because they either substantially changed the estimate (i.e. they were confounders) or were associated with the outcome. See Table 2 footnotes for the complete list of covariates included in the multivariable models.

Statistical significance in the main analyses was determined by Wald tests. Tests for trend were performed with continuous exposure measures using the midpoint of shift work duration categories and truncating the highest category. P-values were two-sided and values less than 0.05 were considered statistically significant. SAS software, version 9 (SAS Institute, Cary, North Carolina, United States) was used for all statistical analyses.

Secondary analyses

Several secondary analyses were performed. For greater statistical power than our main analyses stratified by menopausal status, we ran models in the full NHS and NHS2 dataset of premenopausal and postmenopausal mammograms (N=4,017), with adjustment for menopausal status at time of mammogram.

To see if recent rotating night shift work was associated with mammographic density, we ran models using a shift work variable with current, past and never categories. This analysis was only possible in NHS2, for which we have updated shift work information. Current shift work was defined as reporting any shift work in the two years prior to the closest questionnaire before mammogram. Past shift work was defined as reporting no shift work on that same questionnaire, but reporting previous shift work. Never shift work was defined as not reporting any shift work up to the time of mammogram. The models were additionally adjusted for cumulative shift work duration (in months) up to the same questionnaire. Models were run stratified by menopausal status at time of mammogram as well as combined with adjustment for menopausal status for greater power.

In addition, as other work from our group has shown that long durations of shift work early in adult life may confer an increased risk of breast cancer (Wegrzyn dissertation paper 1, unpublished) we ran models to assess association of shift work history in 1989 and mammographic density measures (at the start of the NHS2 cohort, when participants were ages 25-42 in 1989). Limited sample size required the categories for shift work duration to be never, 1-9 years, and 10+ years.

RESULTS

Age-adjusted characteristics of the study population at the time of mammogram are presented in Table 1. Those who were in the highest category of rotating night shift work duration up to time of mammogram were generally older, had higher BMI as well as childhood and adolescent body size, were more likely to have had menarche before age 12, were more likely to be current smokers, and were less likely to be current users of menopausal hormone therapy.

Table 1. Age-adjusted characteristics at time of mammogram, by categories of rotating night shift work duration and menopausal status at time of mammogram in a NHS and NHS2 pooled dataset (N premenopausal=1,906; N postmenopausal=1,860)

	Premenopausal at time of mammogram Years of rotating night shift work				Postmenopausal at time of mammogram Years of rotating night shift work			
Characteristic	**None n=659**	**1-9 years n=1,126**	**10-19 years n=102**	**20+ years n=19**	**None n=720**	**1-9 years n=927**	**10-19 years n=150**	**20+ years n=63**
Age	46.4 (4.3)	45.8 (4.4)	45.8 (4.4)	49.1 (3.0)	60.0 (7.3)	59.3 (7.5)	59.9 (7.9)	62.9 (7.3)
Average breast density, %	39.9 (19.7)	39.0 (18.7)	37.4 (17.5)	31.9 (16.6)	25.2 (17.6)	24.8 (16.8)	24.2 (18.3)	23.7 (17.2)
Average dense area, cm2	79.5 (52.7)	82.8 (51.3)	81.5 (47.6)	65.7 (35.5)	46.5 (38.6)	48.1 (38.2)	47.4 (45.1)	51.3 (49.9)
Average nondense area, cm2	129.6 (81.0)	140.5 (82.0)	148.3 (85.9)	154.2 (65.0)	155.4 (90.8)	160.6 (94.1)	159.5 (83.6)	171.1 (89.6)
Height, inches	64.7 (2.4)	65.0 (2.5)	64.9 (2.6)	64.0 (2.1)	64.6 (2.4)	64.6 (2.4)	64.4 (2.3)	65.3 (2.5)
BMI, kg/m2	25.2 (4.9)	25.8 (5.6)	27.1 (6.3)	28.5 (5.7)	25.8 (4.9)	26.2 (5.3)	27.9 (6.0)	26.8 (4.8)
BMI at age 18, kg/m2	21.2 (2.8)	21.2 (2.9)	21.8 (3.4)	21.0 (2.2)	21.1 (2.6)	21.2 (2.7)	21.6 (3.4)	21.7 (3.8)
Childhood body size [a]	2.6 (1.2)	2.6 (1.2)	2.6 (1.2)	3.0 (1.4)	2.4 (1.3)	2.4 (1.3)	2.3 (1.3)	2.3 (1.3)
Adolescent body size [a]	2.9 (1.1)	2.9 (1.1)	3.0 (1.1)	3.2 (0.97)	2.7 (1.1)	2.7 (1.2)	2.7 (1.1)	2.7 (1.3)
Menarche before age 12	25	24	28	73	24	21	22	26
Nulliparous	13	13	23	7	8	9	10	5
Number of children [b]	2.5 (0.9)	2.4 (1.0)	2.4 (1.0)	2.1 (0.7)	3.3 (1.6)	3.2 (1.6)	3.2 (1.5)	3.6 (1.5)
Age at first birth [b]	25.6 (3.9)	26.3 (4.3)	25.6 (4.6)	25.6 (2.4)	24.9 (3.4)	25.3 (3.4)	25.3 (3.9)	24.8 (3.0)
Never breastfed	9	13	7	11	3	4	7	6
Age at menopause [c]	-	-	-	-	48.7 (4.9)	48.4 (5.3)	48.3 (5.0)	48.9 (4.6)
Current menopausal hormone therapy use [c]	-	-	-	-	48	47	40	33
First-degree family history of breast cancer	8	9	7	0	10	14	14	23
History of benign breast disease	50	49	46	64	48	47	51	45
Current smoker	7	8	9	40	8	10	13	9
Alcohol consumption, grams/day	4.1 (7.2)	4.8 (7.8)	4.2 (8.2)	10.7 (13.5)	5.5 (9.3)	5.1 (8.5)	5.1 (9.1)	4.6 (6.9)
Physical activity, MET-hrs/wk	16.0 (18.0)	19.3 (23.9)	22.5 (34.9)	19.1 (11.9)	18.5 (31.6)	17.6 (20.2)	21.1 (27.4)	16.1 (18.1)
Husband's education level college or higher [d]	77	80	70	79	61	62	56	49

Values are means (SD) or percentages and are standardized to the age distribution of the study population.

a Body size recalled using pictures of body outlines, numbered 1-9, leanest to fattest (NHS: 1988, NHS2: 1989)

b Among parous women only.

c Among postmenopausal women only.

d Among married or widowed women only. (NHS: 1992; NHS2: 1999)

Differences in average breast density measures, comparing levels of rotating night shift work to no shift work, were determined in three nested models for three outcomes, percent density, absolute dense area and absolute non-dense area (see Table 2). In the fully adjusted models (Model 3), women with 20+ years of shift work experience, who were premenopausal at time of mammogram, had 1.37 percentage points lower mean percent density (95% CI: -7.20,4.47), 13.20 cm^2 smaller mean dense area (95% CI: -26.43,0.04), and 10.13 cm^2 smaller mean nondense area (95% CI: -34.84,14.58) than those with no shift work experience. Women with 20+ years of shift work duration who were postmenopausal at mammogram had 1.73 percentage points higher mean percent density (95% CI: -2.13,5.60), 3.93 cm^2 larger mean dense area (95% CI: -4.90,12.75), and 6.36 cm^2 smaller mean nondense area (95% CI: -23.73,11.00) than those with no shift work experience. None of the estimates were statistically significant, and no significant trends across categories of shift work were evident. All models were repeated with versions of the exposure with 10+ years and 15+ years of shift work as the highest category, and no significant associations were found (data not shown).

Similar models were fit for square-root transformed versions of all three outcomes. On the square root scale in the fully adjusted models, among women premenopausal at time of mammogram, 20+ years of rotating night shift work was associated with 0.10 square-root percentage points lower mean percent density (95% CI: -0.61,0.40), 0.62 sqrt cm^2 smaller mean dense area (95% CI: -1.38,0.14), and 0.40 sqrt cm^2 smaller mean nondense area (95% CI: -1.47,0.68), compared with no night shift work. For the same models among women postmenopausal at time of mammogram, 20+ years of rotating night shift work was associated with 0.16 square-root percentage points lower mean percent density (95% CI: -0.24,0.56), 0.21 sqrt cm^2 smaller mean dense area (95% CI: -0.36,0.77), and 0.22 sqrt cm^2 smaller mean

Table 2. Difference in average breast density measures [β (95% confidence interval)] associated with duration of rotating night shift work in a NHS and NHS2 pooled dataset, stratified by menopausal status (N premenopausal=1,906; N postmenopausal=1,860)

	Premenopausal at time of mammogram					Postmenopausal at time of mammogram				
	Years of rotating night shift work					Years of rotating night shift work				
	None	1-9 yrs β (95% CI)	10-19 yrs β (95% CI)	20+ yrs β (95% CI)	p_{trend}	None	1-9 yrs β (95% CI)	10-19 yrs β (95% CI)	20+ yrs β (95% CI)	p_{trend}
N	**659**	**1,126**	**102**	**19**		**720**	**927**	**150**	**63**	
Percent density (%)										
Model 1 [a]	Ref	-1.01 (-2.85,0.83)	-3.20 (-6.78,0.38)	-3.61 (-11.71,4.49)	0.05	Ref	-0.43 (-2.07,1.21)	-0.77 (-3.94,2.39)	-0.22 (-4.58,4.14)	0.65
Model 2 [b]	Ref	-0.31 (-1.90,1.29)	-0.00 (-3.10,3.09)	-0.91 (-6.91,5.09)	0.81	Ref	0.10 (-1.37,1.58)	1.85 (-0.98,4.67)	1.19 (-2.51,4.90)	0.21
Model 3 [c]	Ref	-0.42 (-2.00,1.17)	0.02 (-3.09,3.13)	-1.37 (-7.20,4.47)	0.73	Ref	-0.09 (-1.51,1.32)	1.45 (-1.22,4.12)	1.73 (-2.13,5.60)	0.22
Dense area (cm2)										
Model 1	Ref	-1.64 (-6.21,2.93)	-2.67 (-12.03,6.70)	-15.41 (-30.19,-0.64)	0.19	Ref	1.55 (-1.78,4.87)	2.45 (-4.48,9.38)	2.38 (-6.54,11.29)	0.37
Model 2	Ref	-1.33 (-5.91,3.25)	-0.76 (-10.08,8.55)	-13.68 (-27.24,-0.12)	0.37	Ref	1.98 (-1.28,5.24)	4.07 (-2.75,10.89)	3.08 (-5.44,11.59)	0.17
Model 3	Ref	-1.38 (-5.98,3.22)	-0.61 (-9.98,8.76)	-13.20 (-26.43,0.04)	0.39	Ref	1.74 (-1.40,4.88)	3.56 (-2.95,10.08)	3.93 (-4.90,12.75)	0.16
Non-dense area (cm2)										
Model 1	Ref	3.88 (-3.38,11.15)	14.75 (-1.51,31.01)	3.19 (-31.81,38.18)	0.09	Ref	6.51 (-0.99,14.00)	6.84 (-6.41,20.08)	4.70 (-16.86,26.26)	0.25
Model 2	Ref	-0.34 (-5.86,5.18)	-2.16 (-15.07,10.75)	-11.24 (-36.11,13.63)	0.53	Ref	3.18 (-2.80,9.16)	-11.31 (-21.85,-0.77)	-5.84 (-23.51,11.82)	0.11
Model 3	Ref	0.14 (-5.36,5.65)	-2.39 (-15.27,10.48)	-10.13 (-34.84,14.58)	0.57	Ref	3.45 (-2.45,9.36)	-10.39 (-20.79,0.01)	-6.36 (-23.73,11.00)	0.13
Square root percent density (%)										
Model 1	Ref	-0.08 (-0.24,0.08)	-0.24 (-0.56,0.08)	-0.30 (-1.06,0.45)	0.09	Ref	-0.03 (-0.21,0.14)	-0.15 (-0.49,0.18)	-0.06 (-0.53,0.41)	0.42
Model 2	Ref	-0.02 (-0.15,0.12)	0.05 (-0.22,0.32)	-0.07 (-0.58,0.44)	0.95	Ref	0.03 (-0.13,0.18)	0.14 (-0.16,0.43)	0.14 (-0.16,0.43)	0.33
Model 3	Ref	-0.03 (-0.16,0.11)	0.05 (-0.22,0.32)	-0.10 (-0.61,0.40)	0.98	Ref	0.01 (-0.14,0.15)	0.10 (-0.18,0.37)	0.16 (-0.24,0.56)	0.33
Square root dense area (cm2)										
Model 1	Ref	-0.09 (-0.33,0.15)	-0.11 (-0.62,0.40)	-0.76 (-1.63,0.10)	0.25	Ref	0.11 (-0.11,0.34)	0.02 (-0.44,0.47)	0.06 (-0.52,0.64)	0.77
Model 2	Ref	-0.06 (-0.30,0.17)	0.03 (-0.47,0.53)	-0.65 (-1.41,0.12)	0.54	Ref	0.15 (-0.07,0.37)	0.17 (-0.27,0.61)	0.14 (-0.40,0.67)	0.32
Model 3	Ref	-0.07 (-0.31,0.17)	0.04 (-0.47,0.54)	-0.62 (-1.38,0.14)	0.56	Ref	0.13 (-0.08,0.34)	0.13 (-0.29,0.55)	0.21 (-0.36,0.77)	0.32
Square root non-dense area (cm2)										
Model 1	Ref	0.16 (-0.14,0.47)	0.64 (-0.01,1.28)	0.12 (-1.36,1.60)	0.08	Ref	0.26 (-0.03,0.56)	0.34 (-0.19,0.87)	0.23 (-0.19,0.87)	0.17
Model 2	Ref	-0.01 (-0.24,0.22)	-0.06 (-0.56,0.44)	-0.49 (-1.58,0.60)	0.57	Ref	0.13 (-0.10,0.36)	-0.37 (-0.78,0.04)	-0.18 (-0.89,0.53)	0.20
Model 3	Ref	0.01 (-0.22,0.24)	-0.07 (-0.57,0.43)	-0.40 (-1.47,0.68)	0.64	Ref	0.15 (-0.08,0.38)	-0.33 (-0.73,0.08)	-0.22 (-0.92,0.49)	0.23

a Model 1 is adjusted for age (continuous in months) and cohort and batch combined (NHS batch 1, NHS batch 2, NHS2)
b Model 2 is adjusted for the above and the following: height (continuous in inches), BMI (continuous in kg/m2), BMI at age 18 (continuous in kg/m2), and adolescent body size (average of age 10 and age 20 diagrams).
c Model 3 is adjusted for the above and the following: age at first birth and parity combined (nulliparous, age <25 yrs 1-2 kids, age <25 yrs 3+ kids, age 25-29 yrs 1-2 kids, age 25-29 yrs 3+ kids, age 30+ yrs 1-2 kids, age 30+ yrs 3+ kids), first-degree family history of breast cancer (yes, no), history of benign breast disease (yes, no), alcohol consumption (0, 0.1-5, >5 g/day). Postmenopausal models are additionally adjusted for menopausal hormone therapy use (current, past, never).

nondense area (95% CI: -0.92,0.49), compared with no night shift work. None of the estimates were statistically significant, and no significant trends across categories of shift work were evident. (See Table 2)

Secondary analyses also did not reveal any significant associations. In multivariable models combining premenopausal and postmenopausal mammograms, and adjusting for menopausal status at mammogram, rotating night shift work duration through time of mammogram was not associated with a significant change in mean percent density, dense area or non-dense area, on the original scale (β_{pct}=-0.32, 95% CI: -3.49,2.84, p_{trend}=0.79; β_{dens}=-2.86, 95% CI: -9.87,4.16 p_{trend}=0.96; $\beta_{nondens}$=-4.65, 95% CI: -18.53,9.23, p_{trend}=0.13) and on the square-root scale ($\beta_{sqrtpct}$=-0.07, 95% CI: -0.40,0.26, p_{trend}=0.90; $\beta_{sqrtdens}$=-0.22, 95% CI: -0.67,0.24, p_{trend}=0.85; $\beta_{sqrtnondens}$=-0.15, 95% CI: -0.72,0.43, p_{trend}=0.25). (See Table 3).

In NHS2, current rotating night shift work, or shift work during the two years prior to the questionnaire before mammogram, was also not associated with any of the outcome measures, compared with never shift work (For percent density, premenopausal $\beta_{current\ vs\ never}$=2.22, 95% CI: -0.97,5.41; postmenopausal $\beta_{current\ vs\ never}$=1.40, 95% CI: -4.76,7.57) (See Table 4). This analysis was repeated in a dataset combining premenopausal and postmenopausal women for great power, and similar results were obtained (For percent density, $\beta_{current\ vs\ never}$=1.14, 95% CI: -1.65,3.93) (See Table 5).

In NHS2, rotating night shift work reported at the start of the cohort (1989), when participants were in the age range 25-42, 10+ years of young adult shift work was also not associated with a significant difference in mean outcome measures, compared to those with 0 years (For percent density, premenopausal $\beta_{10+\ yrs\ vs\ 0\ yrs}$=-0.34, 95% CI: -4.30,3.61, p_{trend}=0.31; postmenopausal $\beta_{10+\ yrs\ vs\ 0\ yrs}$ =1.35, 95% CI: -4.83,7.53, p_{trend}=0.87).

Table 3. Difference in average breast density measures [β (95% confidence interval)] associated with duration of rotating night shift work in a NHS and NHS2 pooled dataset, adjusted for menopausal status (N=4,017)[a]

	Years of rotating night shift work				
	None	1-9 yrs	10-19 yrs	20+ yrs	
		β (95% CI)	β (95% CI)	β (95% CI)	p_{trend}
N	**1467**	**2,187**	**271**	**92**	
Percent density (%)					
Model 1 [b]	Ref	-1.03 (-2.23,0.16)	-1.77 (-4.10,0.55)	-1.93 (-5.74,1.87)	0.06
Model 2 [b]	Ref	-0.38 (-1.43,0.68)	1.08 (-0.98,3.14)	-0.36 (-3.48,2.77)	0.66
Model 3 [b]	Ref	-0.46 (-1.50,0.58)	0.85 (-1.15,2.84)	-0.32 (-3.49,2.84)	0.79
Dense area (cm2)					
Model 1	Ref	-0.45 (-3.18,2.27)	0.24 (-5.23,5.71)	-3.79 (11.15,3.56)	0.62
Model 2	Ref	-0.04 (-2.74,2.65)	2.10 (-3.33,7.53)	-2.87 (-9.83,4.09)	0.87
Model 3	Ref	-0.18 (-2.85,2.49)	1.73 (-3.62,7.07)	-2.86 (-9.87,4.16)	0.96
Non-dense area (cm2)					
Model 1	Ref	5.15 (0.17,10.12)	9.11 (-0.78,19.00)	5.53 (-11.78,22.83)	0.05
Model 2	Ref	1.12 (-2.80,5.05)	-7.98 (-15.77,-0.18)	-4.29 (-18.28,9.70)	0.12
Model 3	Ref	1.31 (-2.60,5.22)	-7.65 (-15.39,0.10)	-4.65 (-18.53,9.23)	0.13
Square root percent density (%)					
Model 1	Ref	-0.09 (-0.20,0.02)	-0.19 (-0.42,0.04)	-0.23 (-0.63,0.17)	0.04
Model 2	Ref	-0.02 (-0.12,0.07)	0.09 (-0.11,0.29)	-0.07 (-0.40,0.25)	0.80
Model 3	Ref	-0.03 (-0.13,0.07)	0.07 (-0.12,0.26)	-0.07 (-0.40,0.26)	0.90
Square root dense area (cm2)					
Model 1	Ref	-0.03 (-0.19,0.13)	-0.06 (-0.39,0.27)	-0.30 (-0.79,0.19)	0.34
Model 2	Ref	0.00 (-0.15,0.16)	0.09 (-0.23,0.41)	-0.22 (-0.67,0.22)	0.93
Model 3	Ref	-0.00 (-0.16,0.15)	0.07 (-0.25,0.38)	-0.22 (-0.67,0.24)	0.85
Square root non-dense area (cm2)					
Model 1	Ref	0.22 (0.02,0.42)	0.42 (0.02,0.82)	0.26 (-0.45,0.98)	0.03
Model 2	Ref	0.06 (-0.10,0.21)	-0.27 (-0.58,0.04)	-0.13 (-0.71,0.44)	0.02
Model 3	Ref	0.07 (-0.09,0.22)	-0.25 (-0.56,0.05)	-0.15 (-0.72,0.43)	0.25

a 251 participants with missing menopausal status were included in this combined dataset with a missing indicator variable.

b Models 1, 2 and 3 are adjusted for the same covariates as listed in the Table 2 footnotes, with additional adjustment for menopausal status (pre/post/missing) in all models.

Table 4. Difference in average breast density measures [β (95% confidence interval)] associated with current (in the last 2 years), past and never rotating night shift work in NHS2 only, stratified by menopausal status (N premenopausal=1,313; N postmenopausal=338)

	Premenopausal at time of mammogram			Postmenopausal at time of mammogram		
	Rotating night shift work [a]			Rotating night shift work [a]		
	Never	Past only β (95% CI)	Current (in last 2 yrs) β (95% CI)	Never	Past only β (95% CI)	Current (in last 2 yrs) β (95% CI)
N	**395**	**753**	**165**	**107**	**193**	**38**
Percent density (%)						
Model 1 [b]	Ref	-0.47 (-2.91,1.96)	-0.78 (-2.91,1.96)	Ref	1.08 (-3.58,5.74)	0.22 (-7.06,7.50)
Model 2 [b]	Ref	-0.57 (-2.65,1.52)	2.10 (-1.17,5.37)	Ref	-0.39 (-4.59,3.82)	0.99 (-5.30,7.27)
Model 3 [b]	Ref	-0.58 (-2.63,1.48)	2.22 (-0.97,5.41)	Ref	-0.85 (-4.88,3.17)	1.40 (-4.76,7.57)
Dense area (cm2)						
Model 1	Ref	-1.84 (-8.88,5.20)	9.86 (-2.22,21.94)	Ref	1.36 (-11.08,13.81)	-0.09 (-20.71,20.52)
Model 2	Ref	-1.99 (-8.96,4.98)	10.86 (-1.16,22.87)	Ref	-0.02 (-12.38,12.34)	1.95 (-18.10,22.00)
Model 3	Ref	-2.19 (-9.08,4.69)	10.30 (-1.54,22.15)	Ref	-0.23 (-11.94,11.48)	4.00 (-15.60,23.59)
Non-dense area (cm2)						
Model 1	Ref	-2.19 (-12.33,7.96)	7.86 (-9.33,25.06)	Ref	1.22 (-20.37,22.80)	7.23 (-28.70,43.16)
Model 2	Ref	-2.01 (-9.72,5.71)	0.57 (-11.19,12.34)	Ref	11.57 (-5.00,28.14)	5.78 (-20.77,32.33)
Model 3	Ref	-2.02 (-9.70,5.65)	-0.38 (-12.09,11.32)	Ref	13.19 (-4.00,30.38)	3.54 (-23.97,31.04)
Square root percent density (%)						
Model 1	Ref	-0.02 (-0.23,0.19)	0.08 (-0.25,0.41)	Ref	0.15 (-0.26,0.57)	0.09 (-0.59,0.76)
Model 2	Ref	-0.03 (-0.20,0.15)	0.19 (-0.08,0.47)	Ref	0.01 (-0.36,0.38)	0.17 (-0.41,0.74)
Model 3	Ref	-0.03 (-0.20,0.14)	0.20 (-0.07,0.47)	Ref	-0.03 (-0.39,0.32)	0.19 (-0.37,0.75)
Square root dense area (cm2)						
Model 1	Ref	-0.09 (-0.45,0.26)	0.49 (-0.10,1.08)	Ref	0.21 (-0.47,0.88)	0.21 (-0.93,1.34)
Model 2	Ref	-0.10 (-0.45,0.25)	0.55 (-0.03,1.13)	Ref	0.12 (-0.54,0.79)	0.34 (-0.75,1.42)
Model 3	Ref	-0.11 (-0.45,0.23)	0.53 (-0.04,1.10)	Ref	0.09 (-0.55,0.73)	0.42 (-0.65,1.48)
Square root non-dense area (cm2)						
Model 1	Ref	-0.06 (-0.47,0.34)	0.33 (-0.34,1.01)	Ref	0.01 (-0.81,0.83)	0.25 (-1.07,1.57)
Model 2	Ref	-0.05 (-0.37,0.26)	0.05 (-0.42,0.51)	Ref	0.40 (-0.24,1.03)	0.17 (-0.81,1.16)
Model 3	Ref	-0.06 (-0.37,0.25)	-0.00 (-0.46,0.46)	Ref	0.46 (-0.19,1.11)	0.06 (-0.98,1.10)

a Current rotating night shift work is defined as reporting any shift work in the 2 years prior to the closest questionnaire before mammogram. Past only is defined as reporting no shift work on that same questionnaire, but reporting previous shift work. Never shift workers did not report any shift work up to the time of mammogram.

b Models 1, 2 and 3 are adjusted for the same covariates as listed in the Table 2 footnotes, with additional adjustment for duration of shift work (months) in all models.

Table 5. Difference in average breast density measures [β (95% confidence interval)] associated with current (in the last 2 years), past and never rotating night shift work in NHS2 only, adjusted for menopausal status (N=1,732)[a]

	Rotating night shift work [b]		
	Never	Past only β (95% CI)	Current (in last 2 yrs) β (95% CI)
N	**533**	**987**	**212**
Percent density (%)			
Model 1 [c]	Ref	-0.39 (-2.47,1.70)	0.15 (-3.20,3.50)
Model 2 [c]	Ref	-0.68 (-2.49,1.14)	0.94 (-1.96,3.83)
Model 3 [c]	Ref	-0.76 (-2.55,1.03)	1.14 (-1.65,3.93)
Dense area (cm2)			
Model 1	Ref	-1.65 (-7.66,4.35)	5.63 (-4.65,15.91)
Model 2	Ref	-1.93(-7.87,4.00)	6.25 (-3.96,16.45)
Model 3	Ref	-2.56 (-8.44,3.32)	5.55 (-4.23,15.33)
Non-dense area (cm2)			
Model 1	Ref	-0.50 (-9.35,8.35)	7.72 (-7.24,22.68)
Model 2	Ref	0.93 (-5.86,7.72)	3.20 (-7.40,13.80)
Model 3	Ref	1.18 (-5.60,7.95)	2.25 (-8.32,12.81)
Square root percent density (%)			
Model 1	Ref	-0.01 (-0.19,0.17)	0.04 (-0.25,0.33)
Model 2	Ref	-0.03 (-0.19,0.12)	0.11 (-0.14,0.36)
Model 3	Ref	-0.04 (-0.19,0.11)	0.12 (-0.12,0.36)
Square root dense area (cm2)			
Model 1	Ref	-0.06 (-0.37,0.25)	0.32 (-0.19,0.84)
Model 2	Ref	-0.08 (-0.38,0.22)	0.36 (-0.14,0.87)
Model 3	Ref	-0.11 (-0.41,0.19)	0.34 (-0.15,0.82)
Square root non-dense area (cm2)			
Model 1	Ref	-0.02 (-0.37,0.33)	0.32 (-0.26,0.90)
Model 2	Ref	0.04 (-0.23,0.31)	0.14 (-0.27,0.56)
Model 3	Ref	0.04 (-0.23,0.31)	0.09 (-0.32,0.50)

a 81 participants with missing menopausal status were included in this combined dataset with a missing indicator variable.
b Current rotating night shift work is defined as reporting any shift work in the 2 years prior to the closest questionnaire before mammogram. Past only is defined as reporting no shift work on that same questionnaire, but reporting previous shift work. Never shift workers did not report any shift work up to the time of mammogram.

c Models 1, 2 and 3 are adjusted for the same covariates as listed in the Table 2 footnotes, with additional adjustment for duration of shift work (months) and menopausal status (pre/post/missing) in all models.

DISCUSSION

Overall, we did not see evidence of an association between years of rotating night shift work and mammographic density, in premenopausal or postmenopausal women. Our results are consistent with the one published study of night shift work and mammographic density that showed no association between night shift work and mammographic density.[15]

We saw the greatest changes in the estimates between Models 1 and 2, highlighting the importance of BMI as a confounder of the associations. Although we additionally adjusted for BMI at age 18 and adolescent body size, it is still possible that residual confounding by BMI may have contributed to our lack of statistically significant results.

We also did not see significant associations between shift work and mammographic density with a measure of shift work close to the time of mammogram (current/past/never). However, the estimates were positive for percent density and may not have reached statistical significance due to small sample size. Current shift work was defined as any shift work reported in the two years prior to the most recent questionnaire before mammogram, so it may have reflected shift work performed as far as 4-6 years prior to the mammogram (depending on whether missing shift work was carried forward for one cycle), and any short-term impact to breast tissue may have been missed. We attempted to look at current shift work separating 1-14 months and 15+ months in the last two years in an effort to isolate those who underwent more circadian disruption in the time most proximal to mammogram, but did not have a large enough sample size to see important differences.

In addition, we may have been unable to capture the timing of shift work that is most relevant for breast tissue changes that result in elevated density. We were underpowered to look

at those with consistent shift work early in their adulthood (20+ years in their 20s and 30s), the group that saw a markedly increased risk of breast cancer in the NHS2 (Wegrzyn dissertation paper 1, unpublished). Further, our shift work measure itself is limited in that it uses a single definition of rotating night shift work (3 or more night shifts in a month with other day/evening shifts) and may not capture the frequency or severity of circadian disruption that could most affect mammographic density.

Finally, some research shows that night shift workers are less likely to adhere to screening guidelines and get mammograms at the recommended times,[20] and the probability of getting a mammogram was inversely correlated with BMI in our data.[21] Thus, it's possible that the population for which we were able to obtain mammograms is somehow less at risk for high mammographic density, resulting in selection bias. However, other work from our group has shown that the women for whom mammograms were obtained were similar to those for whom mammograms were not obtained with respect to age, BMI, parity, family history and circulating hormone levels.[16, 17]

In conclusion, in our study population of nurses from the NHS and NHS II cohorts, we found no evidence for an association between rotating night shift work and mammographic density. It is unlikely that previously observed associations between rotating night shift work and breast cancer risk act through influence on mammographic breast density.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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URINARY 6-SULFATOXYMELATONIN AND MAMMOGRAPHIC BREAST DENSITY IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN IN THE NURSES' HEALTH STUDY II

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ABSTRACT

Introduction. Mammographic density is a strong risk factor for breast cancer and is considered a good intermediate marker of breast cancer risk. Melatonin is a hormone that has anti-carcinogenic function and is hypothesized to play a role in the reported increased risk for breast cancer among long-term rotating night shift workers. To our knowledge, only two studies have investigated the association of melatonin and mammographic density

Methods. We conducted a cross-sectional analysis among women in the Nurses' Health Study II (NHS2) who provided a first morning void urine sample and screening mammogram within a few years of each other. Levels of urinary 6-sulfatoxymelatonin, the main metabolite of melatonin, were determined by ELISA, and were standardized by creatinine concentration from the same sample. Percent density (%), absolute dense area (cm^2) and absolute non-dense area (cm^2) were determined from film mammograms using a computer-assisted method. Multivariable linear regression models were fit for each density measure separately, and were stratified by menopausal status a priori (N_{pre}=480, N_{post}=73). Differences in mean density measures were reported by quartiles and tertiles of aMT6s in premenopausal women and postmenopausal women, respectively, adjusted for potential confounders and major predictors of mammographic density.

Results. Concentrations of creatinine-adjusted aMT6s were not associated with a statistically significant difference in mean mammographic density, absolute dense area or nondense area, in premenopausal women ($\beta_{pct,\ Q4\ vs\ Q1}$= -0.52, 95% CI: -4.65,3.61; p=0.92; $\beta_{dens,\ Q4\ vs\ Q1}$= -5.27, 95% CI: -18.44,7.89; p=0.36; $\beta_{nondens,\ Q4\ vs\ Q1}$= -2.17, 95% CI: -18.87,14.53; p=0.56) or in postmenopausal women ($\beta_{pct,\ T3\ vs\ T1}$= 0.38, 95% CI: -5.49,6.26; p=0.62; $\beta_{dens,\ T3\ vs\ T1}$= 10.34, 95% CI: -7.33,28.01; p=0.11; $\beta_{nondens,\ T3\ vs\ T1}$= 23.76, 95% CI: -18.42,65.94; p=0.10), comparing the

highest quantile to the lowest quantile. Results were unchanged when restricted to those who were non-smokers, non-users of antidepressant medication, and those who did not work night shifts within 2 weeks prior to urine collection. Interaction by BMI was not significant (p_{pct}=0.98; p_{dens}=0.28; $p_{nondens}$=0.86)

Conclusions. We saw no evidence of an association between a single measure of urinary melatonin and mammographic density, among premenopausal or postmenopausal women.

INTRODUCTION

Women with a large proportion of fibroglandular breast tissue, or high mammographic density, have a markedly higher risk of breast cancer, compared to women with fattier breast tissue. With 4-6 fold increased risk, high mammographic breast density is the strongest known risk factor for breast cancer.[1-3] It may be a marker of cumulative exposure to estrogen, and may therefore serve as a good intermediate endpoint for studies of hormonally-related exposures.[4-8]

Melatonin is a hormone that serves as a marker of the circadian clocks of the body. Its release is stimulated by darkness and suppressed by light. Melatonin has been hypothesized to a play a role in cancer-related pathways through its regulation of gonadal function, modulation of the immune system, and through direct anti-proliferative effects on tumors.[9, 10] The reported association of occupational night shift work and breast cancer is thought to work through disruption of the circadian patterns of melatonin production and subsequent regulation of sex hormones.[11]

To our knowledge, melatonin has been explored for its potential to predict or affect mammographic density in only two published studies. Peplonska et al reported no association between melatonin concentration collected in spot morning urine samples and percent density and absolute dense area.[12] However, they also previously reported no association with rotating night shift work and this measure of urinary melatonin, suggesting that the circadian disruption that may affect breast cancer risk was not captured by the melatonin measurement.[13] Nagata et al found a significantly positive association between melatonin concentration measured from first-void morning samples and mammographic density in premenopausal women (n=175), and

no association in postmenopausal women (n=123), but may have been limited by small sample size.[14]

In this study, we conducted a cross-sectional analysis of urinary melatonin concentration from first morning void samples and mammographic density in women free of breast cancer in the Nurses' Health Study II cohort. We hypothesized that higher levels of urinary melatonin would be associated with lower percent density and absolute dense area, and higher absolute non-dense area.

METHODS

The Nurses' Health Study II (NHS2) provided the data for these analyses. The NHS2 cohort was established in 1989, when 116,430 female registered nurses in the United States, ages 25-42, returned a questionnaire reporting detailed information about their lifestyles, occupational and environmental exposures, medication use and medical conditions. The NHS2 is currently ongoing and participants update this information biennially. Follow-up in this cohort is >90%.

The Institutional Review Board of Brigham & Women's Hospital (Boston, MA) approved the NHS2, and all participants provided informed consent through the return of the initial questionnaire.

Study sample

Within the NHS2, a subcohort of women provided blood and urine samples in the period 1996-1999. Within the group that provided these samples, a nested case-control study of breast cancer was formed, with one or two controls matched to cases based on age, menopausal status, postmenopausal hormone use, fasting status at blood collection and timing of the blood collection with respect to the menstrual cycle. Mammograms were collected for the case-control

study and were targeted to be screening mammograms close to the biomarker sample dates (before and after). Only urine samples and mammograms from controls (i.e. women free of breast cancer) were used for the present cross-sectional analyses (N=553), with median time between urine collection date and mammogram date of approximately 10 months in premenopausal women and approximately 6 months in postmenopausal women.

Data Collection and Measurements

Urinary melatonin metabolite measurement

Urine samples were collected without preservatives and were shipped to our laboratory overnight on ice. Ninety-three percent of samples were received within 26 hours of collection. The stability of urinary aMT6s when processing is delayed for 24-48 hours has been previously shown to be reasonable.[15] Since receipt, the samples have been stored in continuously monitored liquid nitrogen freezers in the vapor phase (<=-130 deg C).

The primary metabolite of melatonin, 6-sulfatoxymelatonin (aMT6s), was measured in the urine samples as an estimate of circulating melatonin levels. Samples were assayed in three batches. In 2001, the Endocrine Core Laboratory of Dr. M. Wilson (Yerkes National Primate Research Center, Emory University, Atlanta, Georgia) measured urinary aMT6s using a competitive enzyme-linked immunosorbent assay (ALPCO Diagnostics, Windham, New Hampshire) and urinary creatinine using a modified Jaffe method. In 2003/2005 and 2007, the laboratory of Dr. Vincent Ricchiuti (now the Carroll Laboratory, Brigham and Women's Hospital, Boston, Massachusetts) measured urinary aMT6s using a commercially available enzyme-linked immunosorbent assay (IBL International GmbH, Hamburg, Germany) and

urinary creatinine using the COBAS Integra 400 assay (Roche Diagnostics, Indianapolis, Indiana).

Urinary aMT6s was divided by urinary creatinine to account for differences in concentration due to volume of the sample. aMT6s levels in this analysis are expressed as ng/mg creatinine. Replicate quality control samples (10% of samples) were included in each batch. As reported previously, within-batch coefficients of variation ranged from 2.4% to 13.9% for melatonin and from 1.2% to 9.2% for creatinine.[16]

The three batches of measurements exhibited substantial drift, and were recalibrated using samples obtained from each of the batches (45 total, 15 from each batch that represented high, medium and low levels of aMT6s) and assayed at the Carroll Laboratory in 2013. Details of the drift recalibration are described elsewhere.[16] Briefly, linear regression was used for each batch, regressing the 2013 rerun values on the original laboratory values, and the estimates were used to predict recalibrated values for participants in that batch.

Mammographic density assessment

Film mammograms were obtained for approximately 80% of the participants who were queried for mammograms. The mammograms were targeted to be screening mammograms, taken as close to the timing of the biospecimen collection as possible, as described above. Other work from our group has shown that the women for whom mammograms were obtained were similar to those for whom mammograms were not obtained with respect to age, BMI, parity, family history and circulating hormone levels.[17]

We used percent density, absolute dense area, and absolute non-dense area as measures of mammographic breast density. These were determined quantitatively using a computer-

assisted method. For all three outcomes, measures from the cranio-caudal mammographic views of both breasts from each subject were averaged. For each image, a trained reader manually set the appropriate gray-scale threshold level defining the edge of the breast. The software package, Cumulus, then calculated the total number of pixels within the entire region of interest and within the regions identified as dense and non-dense. These values were used to calculate the percentage (dense area divided by total area) and absolute areas of the breast area that are dense and non-dense.[18]

The measures of density from all the mammograms for this study were determined by one trained reader, whose within-person intraclass correlation coefficient has been reported as >0.90.[19] The reader was blinded to the case-control status of the mammograms, and a random 10% of mammograms were included in all batches as duplicate quality control samples.

The mammograms were read in three batches, with evidence of drift toward smaller dense area measurements over the course of the three batches. Density measurements for the latter batches were recalibrated to the first batch. Details of the recalibration project are described elsewhere.[17, 20] Briefly, multivariable linear regression was used to estimate the effect of batch on density measurements and the coefficient for batch was added to the raw measurements in the second and third batches. These recalibrated mammographic density measures were used in our analyses without further adjustment for batch.

Covariate assessment

Covariates were pulled from the questionnaire accompanying urine collection kits or the main NHS2 questionnaire closest to and prior to the urine sample date. The following covariates were considered for inclusion in all multivariable models as potential confounders or predictors

of mammographic density: age, height, BMI, BMI at age 18, childhood body size (average of age 5 and 10 diagrams), adolescent body size (average of age 10 and 20 diagrams), age at menarche, age at first birth, parity, breastfeeding, age at menopause, menopausal hormone therapy (MHT) use, duration of types of MHT, first-degree family history of breast cancer, personal history of benign breast disease, smoking status and pack-years, alcohol consumption, physical activity, husband's highest education level, and cumulative rotating night shift work. Cumulative rotating night shift work was determined by summing rotating night shift work history collected in 1989 (0-20+ years of 3 or more night shifts per month in months that also included day/evening shifts) and updated rotating night shift work duration up to the questionnaire prior to urine sample. Season of urine collection, first morning void sample (yes, no), antidepressant medication use (yes, no) and night shift work within 2 weeks prior to urine collection were obtained from the questionnaire that accompanied the kits and were used in secondary analyses.

Categorical variables were included in multivariable models with missing indicators. Missing values of continuous variables were set to the median and missing indicators were included in the models. Less than 5% missing was noted for all variables.

Statistical analyses

Linear regression was used to calculate beta estimates, or differences in the mean mammographic density measures, comparing the highest category to the lowest category of creatinine-adjusted urinary aMT6s. Models were run for each of the three mammographic density outcomes, separately by menopausal status. Quartiles of adjusted aMT6s concentration were used as the exposure measure for premenopausal women (N=480) and tertiles were used for postmenopausal women (N=73). As residuals were skewed, square root transformations were

applied to all three outcomes and models were fit using the transformed versions as the dependent variables. Generalized estimating equations were used to account for the correlation between matched controls, using the repeated statement of the SAS GENMOD procedure.

Three nested multivariable models were run to highlight the change in estimates with adjustment for BMI and then other variables in addition to BMI. Model 1 was adjusted for age, calendar season of urine collection and cumulative duration of rotating night shift work. Model 2 was adjusted for the above as well as BMI at time of urine collection and BMI at age 18. Model 3 was adjusted for the above as well as other predictors of mammographic density or possible confounders of the shift work and mammographic density association. Each covariate was added to the model individually to see if the exposure-outcome associations changed appreciably. All covariates were included in the final multivariable-adjusted model because they either changed the estimate (i.e. they were confounders) or were associated with the outcome. See Table 2 footnotes for the complete list of covariates included in the multivariable models.

Statistical significance in the analyses was determined by Wald tests. Tests for trend were performed with continuous exposure measures using the midpoint of the aMT6s categories. P-values were two-sided and values less than 0.05 were considered statistically significant. SAS software, version 9 (SAS Institute, Cary, North Carolina, United States) was used for all statistical analyses.

Secondary analyses

Since BMI is an important determinant of mammographic density and is also highly correlated with urinary melatonin concentration,[21] we investigated it as a potential effect modifier in our analyses. We ran models stratified by BMI level (<25 kg/m2 or under/normal

weight, and 25+ kg/m2 or overweight/obese) in premenopausal women. Limited sample size did not allow for the same stratification to be performed in postmenopausal women.

We also ran separate models restricting to non-smokers, non-users of antidepressant medications at time of urine collection, and those who provided first morning void samples, to ensure that our results were unaffected by these factors that can affect melatonin measurements. We also restricted to women who did not report any night shift work within the 2 weeks prior to urine collection in premenopausal women (none of the postmenopausal women reported such shift work), as night shifts have been shown to acutely reduce melatonin production.[22]

RESULTS

Age-adjusted characteristics of the study population at the time of urine collection are presented in Table 1, stratified by menopausal status, as all analyses were conducted separately by menopausal status at urine collection and mammogram. Those in the highest quantiles of creatinine-adjusted urinary aMT6s had lower BMI and were less likely to be current smokers, compared to those in the lowest quantiles. They were also less likely to have worked night shifts in the prior two weeks before collection (among premenopausal women) and had worked fewer cumulative months of rotating night shifts up to that time.

Differences in average breast density measures, comparing the highest quantiles of creatinine-adjusted urinary aMT6s to the lowest, were determined in three nested models for three outcomes, percent density, absolute dense area and absolute non-dense area (see Table 2). In the fully adjusted models (Model 3), women in the highest quartile of aMT6s, who were premenopausal at time of mammogram, had 0.52 percentage points lower mean percent density (95% CI: -4.65,3.61; p_{trend}=0.92), 5.27 cm^2 lower mean absolute dense area (95% CI:

Table 1. Age-adjusted characteristics at time of urine collection, by quantiles of urinary 6-sulfatoxymelatonin (aMT6S) concentration [a] and menopausal status in NHS2 (N premenopausal=480; N postmenopausal=73)

Characteristic	Premenopausal [b] Quartiles of aMT6S				Postmenopausal [b] Tertiles of aMT6S		
	Q1 n=120	Q2 n=120	Q3 n=120	Q4 n=120	T1 n=24	T2 n=25	T3 n=24
Average urinary aMT6S concentration	18.0 (6.3)	36.7 (4.6)	53.1 (5.6)	87.4 (22.7)	15.3 (6.6)	34.4 (6.3)	79.3 (30.8)
Age at urine collection	44.5 (4.1)	43.6 (3.9)	43.0 (4.5)	43.7 (4.0)	48.8 (2.3)	49.3 (1.6)	48.4 (2.8)
Age at mammogram	45.7 (4.1)	44.5 (3.9)	44.3 (4.1)	44.7 (3.8)	50.5 (3.0)	50.1 (2.8)	48.9 (2.5)
Average breast density, %	41.5 (18.9)	42.6 (21.4)	41.9 (20.0)	46.0 (18.9)	26.1 (20.6)	28.2 (17.0)	32.2 (15.1)
Average dense area, cm2	98.0 (55.8)	104.0 (60.3)	91.2 (46.1)	99.8 (48.1)	63.7 (44.2)	62.3 (39.2)	83.3 (43.1)
Average nondense area, cm2	152.5 (87.3)	152.1 (84.7)	149.2 (89.5)	130.0 (73.1)	229.5 (127.1)	172.2 (78.6)	198.2 (103.0)
Height, inches	65.1 (2.6)	65.2 (2.5)	64.8 (2.8)	64.8 (2.4)	64.2 (1.7)	65.0 (2.6)	64.7 (2.2)
BMI, kg/m2	26.0 (5.6)	25.4 (6.1)	25.4 (5.4)	23.7 (5.1)	31.3 (9.8)	27.1 (5.7)	27.6 (6.1)
BMI at age 18, kg/m2	21.1 (2.9)	21.0 (2.7)	21.4 (3.2)	20.5 (2.5)	25.6 (3.0)	21.8 (3.8)	20.4 (2.3)
Childhood body size [c]	2.6 (1.2)	2.6 (1.1)	2.6 (1.3)	2.7 (1.1)	2.6 (1.1)	2.7 (1.3)	2.9 (1.5)
Adolescent body size [c]	2.8 (1.1)	2.9 (1.0)	2.9 (1.1)	3.0 (1.1)	3.0 (1.2)	2.9 (1.1)	3.1 (1.2)
Menarche before age 12	21	22	22	23	29	20	36
Nulliparous	16	17	21	17	38	20	7
Number of children [d]	2.3 (0.8)	2.3 (1.0)	2.4 (0.8)	2.4 (0.8)	2.3 (0.9)	2.0 (0.9)	2.0 (1.1)
Age at first birth [d]	27.1 (4.5)	26.7 (4.9)	26.3 (4.3)	26.2 (4.5)	24.8 (4.3)	23.9 (5.1)	25.2 (5.1)
Never breastfed	18	25	15	16	27	32	22
Age at menopause [e]	-	-	-	-	42.4 (5.1)	43.4 (3.2)	43.3 (3.9)
Current menopausal hormone therapy use [e]	-	-	-	-	71	79	89
First-degree family history of breast cancer	7	10	12	10	8	5	20
History of benign breast disease	49	56	49	52	54	61	63
Current smoker	6	6	2	2	20	8	9
Alcohol consumption, grams/day	3.6 (5.3)	4.5 (6.8)	2.9 (5.7)	3.7 (6.3)	3.9 (5.7)	3.0 (4.7)	2.5 (4.4)
Physical activity, MET-hrs/wk	18.2 (21.4)	21.6 (23.0)	20.6 (24.7)	20.2 (22.3)	14.4 (16.7)	14.4 (14.18)	10.0 (11.3)
Husband's education college or higher [f]	84	82	85	86	83	78	83
Any night shifts in last 2 weeks	12	13	8	10	0	0	0
Cumulative months of rotating night shift work [g]	35.8 (38.3)	32.3 (37.6)	31.7 (48.0)	28.7 (39.9)	47.3 (68.7)	49.2 (57.7)	33.3 (43.4)
First morning urine sample	77	92	94	97	95	95	97

Antidepressant use at time of urine collection	12	11	13	11	17	17	27
Season of urine collection [h]							
February-April	35	30	27	24	34	16	23
May-July	25	22	17	28	33	24	23
August-October	23	24	26	23	5	12	27
November-January	16	23	30	24	29	48	28

Values are means (SD) or percentages and are standardized to the age distribution of the study population, except for aMT6S, age at urine collection and age at mammogram.

a Urinary 6-sulfatoxymelatonin (aMT6S) concentration is divided by urinary creatinine from the same sample to adjust for volume differences.

b Premenopausal at time of urine collection and premenopausal at time of mammogram. Postmenopausal at time of urine collection and postmenopausal at time of mammogram.

c Body size recalled using pictures of body outlines, numbered 1-9, leanest to fattest (NHS: 1988, NHS2: 1989)

d Among parous women only.

e Among postmenopausal women only.

f Among married or widowed women only. (NHS: 1992; NHS2: 1999)

g Total months of rotating night shift work (3+ night shifts in months with day/evening shifts) through the questionnaire closest to and prior to urine collection.

h Season of urine collection based on duration of daylight (May-July highest; Nov-Jan lowest).

Table 2. Difference in average breast density measures [β (95% confidence interval)] associated with urinary 6-sulfatoxymelatonin (aMT6S) concentration [a] in NHS2, stratified by menopausal status (N premenopausal=480; N postmenopausal=73)

	Premenopausal [b]					Postmenopausal [b]			
	Quartiles of aMT6S					Tertiles of aMT6S			
	Q1	Q2 β (95% CI)	Q3 β (95% CI)	Q4 β (95% CI)	p_{trend}	T1	T2 β (95% CI)	T3 β (95% CI)	p_{trend}
N	**120**	**120**	**120**	**120**		**24**	**25**	**24**	
Percent density (%)									
Model 1 [c]	Ref	0.68 (-4.63,5.98)	-0.59 (-5.57,4.38)	3.69 (-1.22,8.60)	0.10	Ref	0.51 (-9.00,10.03)	7.97 (-1.59,17.53)	0.05
Model 2 [d]	Ref	-0.29 (-4.63,4.05)	-1.20 (-5.44,3.04)	-0.34 (-4.45,3.77)	0.93	Ref	-5.15 (-13.53,3.24)	2.43 (-5.77,10.63)	0.26
Model 3 [e]	Ref	-0.68 (-5.02,3.67)	-1.50 (-5.68,2.68)	-0.52 (-4.65,3.61)	0.92	Ref	-3.07 (-11.47,5.33)	0.38 (-5.49,6.26)	0.62
Dense area (cm2)									
Model 1	Ref	4.26 (-11.16,19.68)	-9.68 (-22.77,3.40)	-0.28 (-13.64,13.08)	0.88	Ref	-1.43 (-22.83,19.97)	28.85 (4.92,52.77)	0.01
Model 2	Ref	3.09 (-11.57,17.75)	-10.24 (-23.23,2.75)	-4.76 (-18.05,8.54)	0.38	Ref	-11.39 (-31.95,9.17)	21.69 (-2.22,45.60)	0.02
Model 3	Ref	1.79 (-12.43,16.01)	-11.10 (-23.82,1.63)	-5.27 (-18.44,7.89)	0.36	Ref	-10.02 (-30.76,10.72)	10.34 (-7.33,28.01)	0.11
Non-dense area (cm2)									
Model 1	Ref	0.82 (-22.26,23.90)	-1.57 (-24.25,21.10)	-20.85 (-42.24,0.54)	0.02	Ref	-54.64 (-115.21,5.94)	-35.74 (-95.55,24.07)	0.42
Model 2	Ref	5.07 (-13.48,23.61)	1.24 (-17.27,19.75)	-2.10 (-18.75,14.54)	0.57	Ref	-16.47 (-60.18,27.24)	8.26 (-34.26,50.79)	0.46
Model 3	Ref	5.31 (-13.11,23.73)	0.64 (-17.53,18.81)	-2.17 (-18.87,14.53)	0.56	Ref	-22.85 (-64.30,18.59)	23.76 (-18.42,65.94)	0.10
Square root percent density (%)									
Model 1	Ref	0.02 (-0.44,0.48)	-0.07 (-0.51,0.387)	0.32 (-0.10,0.74)	0.07	Ref	0.27 (-0.72,1.26)	1.05 (0.08,2.02)	0.02
Model 2	Ref	-0.07 (-0.44,0.30)	-0.12 (-0.49,0.25)	-0.03 (-0.38,0.32)	0.98	Ref	-0.32 (-1.13,0.49)	0.38 (-0.40,1.17)	0.16
Model 3	Ref	-0.09 (-0.46,0.28)	-0.14 (-0.50,0.22)	-0.04 (-0.39,0.31)	0.99	Ref	-0.16 (-0.99,0.67)	0.24 (-0.37,0.84)	0.30
Square root dense area (cm2)									
Model 1	Ref	0.19 (-0.57,0.95)	-0.42 (-1.09,0.25)	0.09 (-0.58,0.76)	0.82	Ref	-0.00 (-1.38,1.38)	1.91 (0.52,3.31)	0.00
Model 2	Ref	0.11 (-0.59,0.82)	-0.46 (-1.11,0.20)	-0.19 (-0.85,0.46)	0.48	Ref	-0.65 (-1.91,0.60)	1.26 (-0.08,2.60)	0.01
Model 3	Ref	0.06 (-0.63,0.75)	-0.50 (-1.15,0.14)	-0.22 (-0.87,0.43)	0.46	Ref	-0.60 (-1.89,0.70)	0.82 (-0.23,1.87)	0.05
Square root non-dense area (cm2)									
Model 1	Ref	0.02 (-0.88,0.92)	-0.14 (-1.05,0.77)	-0.83 (-1.71,0.05)	0.03	Ref	-1.59 (-3.67,0.50)	-1.01 (-3.01,1.00)	0.50
Model 2	Ref	0.19 (-0.53,0.92)	-0.02 (-0.76,0.71)	-0.06 (-0.75,0.63)	0.68	Ref	-0.33 (-1.91,1.24)	0.53 (-0.92,1.99)	0.29
Model 3	Ref	0.21 (-0.52,0.94)	-0.03 (-0.76,0.70)	-0.05 (-0.75,0.64)	0.68	Ref	-0.67 (-2.15,0.80)	0.78 (-0.58,2.15)	0.09

a Urinary 6-sulfatoxymelatonin (aMT6S) concentration is divided by urinary creatinine from the same sample to adjust for volume differences.
b Premenopausal at time of urine collection and premenopausal at time of mammogram. Postmenopausal at time of urine collection and postmenopausal at time of mammogram.
c Model 1 is adjusted for age (continuous in months), cumulative shift work duration (continuous in months), season of urine collection (Feb-Apr, May-Jul, Aug-Oct, Nov-Jan)

d Model 2 is adjusted for the above and the following: BMI (continuous in kg/m2) and BMI at age 18 (continuous in kg/m2).
e Model 3 is adjusted for the above and the following: age at menarche (<12, 12-13, 14+), age at first birth and parity combined (premenopausal: nulliparous, age <25 yrs 1-2 kids, age <25 yrs 3+ kids, age 25-29 yrs 1-2 kids, age 25-29 yrs 3+ kids, age 30+ yrs 1-2 kids, age 30+ yrs 3+ kids; postmenopausal: nulliparous, parous age <25 yrs, parous age 25-29 yrs, parous age 30+ yrs) and history of benign breast disease (yes, no). Postmenopausal models are additionally adjusted for menopausal hormone therapy use (current, past, never).

-18.44,7.89; p_{trend}=0.36) and 2.17 cm^2 lower mean absolute nondense area (95%CI: -18.87,14.53; p_{trend}=0.56), compared with the women in the lowest quartile of aMT6s. Women in the highest tertile of aMT6s, who were postmenopausal at mammogram, had 0.38 percentage points higher mean percent density (95% CI: -5.49,6.26; p_{trend}=0.62), 10.34 cm2 higher absolute dense area (95% CI: -7.33,28.01; p_{trend}=0.11), and 23.76 cm2 lower absolute nondense area (95% CI: -18.42,65.94; p_{trend}=0.10) than those in the lowest tertile. None of the estimates were statistically significant, and no significant trends across quantiles of aMT6s were evident.

Square-root transformed measures were fit with the same model covariates. On the square root scale in the fully adjusted models, among women premenopausal at time of mammogram, the highest quartile of aMT6s was associated with 0.04 square-root percentage points lower mean percent density (95%CI: -0.39,0.31; p_{trend}=0.99), 0.22 sqrt cm^2 smaller dense area (95% CI: -0.87,0.43; p_{trend}=0.46) and 0.05 sqrt cm^2 smaller nondense area (95% CI: -0.75, 0.64; p_{trend}=0.68), compared with those in the lowest quartile. Among women postmenopausal at time of mammogram, the highest tertile of aMT6s was associated with 0.24 square-root percentage points higher mean percent density (95%CI: -0.37,0.84; p_{trend}=0.30), 0.82 sqrt cm^2 largerer dense area (95% CI: -0.23,1.87; p_{trend}=0.05) and 0.78 sqrt cm^2 smaller nondense area (95% CI: -0.58, 2.15; p_{trend}=0.09), compared with those in the lowest tertile.

Secondary analyses did not reveal any significant associations. In multivariable models among premenopausal women with BMI <25 kg/m2, the highest quartile of aMT6s was associated with 3.03 percentage points higher percent density, compared with those in the lowest quartile (95% CI: -2.34,8.41; p_{trend}=0.43). Among premenopausal women with BMI 25+ kg/m2, the highest quartile of aMT6s was associated with 4.14 percentage points lower percent density, compared with those in the lowest quartile (95% CI: -11.07,2.79; p_{trend}=0.55). The term for

interaction by BMI level was not significant for all outcomes (p values ranged 0.28-0.99). (See Table 3)

Fully adjusted models were also run separately for datasets restricted to those who were non-smokers at the time of urine collection (premenopausal n=462, postmenopausal n=64), those who provided first morning void samples (premenopausal n=438, postmenopausal n=70), those who were not taking antidepressant medications at time of urine collection (premenopausal n=424, postmenopausal n=59), and those who did not report night shift work within two weeks prior to urine collection (premenopausal n=444, no postmenopausal women reported such night shift work). All analyses yielded results similar to the main results in Table 2 (For non-smokers, $\beta_{pct,\ Q4\ vs\ Q1\ premeno}$= -0.68, 95% CI: -4.89,3.53, p=0.98, $\beta_{pct,\ Q4\ vs\ Q1\ postmeno}$= 3.94, 95% CI: -2.53,10.41, p=0.06; For first morning void only, $\beta_{pct,\ Q4\ vs\ Q1\ premeno}$= -0.55, 95% CI: -4.87,3.76, p=0.94, $\beta_{pct,\ Q4\ vs\ Q1\ postmeno}$= 0.60, 95% CI: -6.49,7.70, p=0.52; For non-antidepressant medication use, $\beta_{pct,\ Q4\ vs\ Q1\ premeno}$= -0.98, 95% CI: -3.28,5.24, p=0.76, $\beta_{pct,\ Q4\ vs\ Q1\ postmeno}$= 1.97, 95% CI: -5.61,9.56, p=0.33; For no night shifts in two weeks, $\beta_{pct,\ Q4\ vs\ Q1\ premeno}$= -0.38, 95% CI: -4.70,3.94, p=0.92).

DISCUSSION

We did not see evidence of an association between levels of creatinine-adjusted urinary melatonin (aMT6s) concentration and mammographic density, in our cross-sectional analysis of premenopausal and postmenopausal women in the NHS2. In general, the fully adjusted estimates were negative for percent density (the hypothesized direction) but no clear patterns or statistically significant trends emerged to suggest a consistent relationship.

Table 3. Difference in average breast density measures [β (95% confidence interval)] associated with urinary 6-sulfatoxymelatonin (aMT6S) concentration [a] in NHS2, stratified by body mass index, among PREMENOPAUSAL [b] women only (N BMI<25 =296; N BMI 25+ =184)

	BMI <25					BMI 25+					
	Quartiles of aMT6S					Quartiles of aMT6S					
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4		
		β (95% CI)	β (95% CI)	β (95% CI)	p_{trend}		β (95% CI)	β (95% CI)	β (95% CI)	p_{trend}	$p_{interaction}$
N	**60**	**75**	**73**	**88**		**60**	**45**	**47**	**32**		
Percent density (%)											
Model 1 [c]	Ref	2.70 (-3.14,8.54)	0.30 (-5.27,5.87)	2.79 (-2.90,8.47)	0.45	Ref	-5.57 (-13.49,2.34)	-3.76 (-10.96,3.44)	-3.70 (-10.88,3.48)	0.49	
Model 2 [d]	Ref	3.06 (-2.51,8.63)	0.53 (-4.74,5.80)	2.76 (-2.70,8.22)	0.49	Ref	-5.29 (-13.02,2.44)	-2.55 (-9.52,4.43)	-3.41 (-10.37,3.55)	0.51	
Model 3 [e]	Ref	3.24 (-2.36,8.84)	0.40 (-4.86,5.66)	3.03 (-2.34,8.41)	0.43	Ref	-6.66 (-13.77,0.44)	-4.26 (-10.99,2.46)	-4.14 (-11.07,2.79)	0.55	0.98
Dense area (cm2)											
Model 1	Ref	16.00 (-2.17,34.16)	-6.86 (-22.72,9.01)	2.68 (-13.47,18.83)	0.75	Ref	-14.97 (-38.67,8.73)	-12.79 (-33.87,8.29)	-10.40 (-33.51,12.71)	0.51	
Model 2	Ref	16.19 (-1.95,34.33)	-6.69 (-22.52,9.13)	2.70 (-13.41,18.81)	0.74	Ref	-14.32 (-37.65,10.63)	-9.87 (-30.37,10.63)	-9.87 (-32.84,13.10)	0.53	
Model 3	Ref	16.44 (-1.26,34.14)	-6.41 (-22.30,9.47)	3.80 (-12.08,19.69)	0.86	Ref	-18.01 (-39.69,3.67)	-12.85 (-32.78,7.09)	-11.46 (-35.19,12.27)	0.53	0.28
Non-dense area (cm2)											
Model 1	Ref	-4.09 (-24.53,16.36)	-6.40 (-27.11,14.30)	-13.26 (-33.48,6.96)	0.16	Ref	24.33 (-14.25,62.91)	19.14 (-15.28,53.57)	17.39 (-15.21,49.99)	0.54	
Model 2	Ref	-6.09 (-25.42,13.24)	-7.69 (-27.24,11.86)	-13.57 (-33.02,5.87)	0.18	Ref	22.05 (-15.31,59.42)	13.65 (-20.93,48.22)	16.16 (-14.89,47.21)	0.54	
Model 3	Ref	-6.74 (-27.20,13.72)	-11.45 (-31.40,8.50)	-18.12 (-37.86,1.61)	0.05	Ref	35.31 (-19.02,89.63)	46.75 (-29.40,122.9)	83.65 (8.26,159.05)	0.05	0.86
Square root percent density (%)											
Model 1	Ref	0.21 (-0.24,0.66)	0.03 (-0.41,0.46)	0.22 (-0.21,0.65)	0.43	Ref	-0.54 (-1.27,0.20)	-0.35 (-1.04,0.34)	-0.23 (-0.95,0.49)	0.75	
Model 2	Ref	0.24 (-0.19,0.66)	0.04 (-0.37,0.45)	0.22 (-0.20,0.63)	0.47	Ref	-0.51 (-1.22,0.20)	-0.22 (-0.89,0.45)	-0.20 (-0.90,0.51)	0.79	
Model 3	Ref	0.26 (-0.16,0.69)	0.04 (-0.37,0.45)	0.24 (-0.16,0.65)	0.41	Ref	-0.62 (-1.28,-0.04)	-0.38 (-1.03,0.27)	-0.19 (-0.87,0.48)	0.93	0.99
Square root dense area (cm2)											
Model 1	Ref	0.74 (-0.10,1.57)	-0.29 (-1.05,0.46)	0.16 (-0.60,0.92)	0.83	Ref	-0.78 (-2.00,0.45)	-0.59 (-1.73,0.55)	-0.35 (-1.63,0.92)	0.75	
Model 2	Ref	0.75 (-0.08,1.59)	-0.28 (-1.04,0.47)	0.16 (-0.60,0.92)	0.82	Ref	-0.75 (-1.94,0.45)	-0.41 (-1.51,0.70)	-0.31 (-1.58,0.96)	0.79	
Model 3	Ref	0.77 (-0.05,1.59)	-0.28 (-1.03,0.48)	0.21 (-0.54,0.96)	0.93	Ref	-0.93 (-2.04,-0.18)	-0.60 (-1.67,0.47)	-0.32 (-1.58,0.95)	0.85	0.32
Square root non-dense area (cm2)											
Model 1	Ref	-0.09 (-1.03,0.86)	-0.30 (-1.28,0.67)	-0.56 (-1.51,0.38)	0.19	Ref	0.80 (-0.54,2.14)	0.59 (-0.64,1.82)	0.68 (-0.46,1.81)	0.39	
Model 2	Ref	-0.17 (-1.07,0.72)	-0.35 (-1.27,0.57)	-0.57 (-1.47,0.34)	0.21	Ref	0.74 (-0.57,2.04)	0.39 (-0.83,1.62)	0.63 (-0.45,1.71)	0.39	
Model 3	Ref	-0.19 (-1.14,0.75)	-0.53 (-1.46,0.40)	-0.79 (-1.72,0.14)	0.05	Ref	1.33 (-0.67,3.33)	1.82 (-0.91,4.54)	3.76 (0.95,6.57)	0.27	0.41

a Urinary 6-sulfatoxymelatonin (aMT6S) concentration is divided by urinary creatinine from the same sample to adjust for volume differences.
b Premenopausal at time of urine collection and premenopausal at time of mammogram.
c Model 1 is adjusted for age (continuous in months), cumulative shift work duration (continuous in months), season of urine collection (Feb-Apr, May-Jul, Aug-Oct, Nov-Jan)
d Model 2 is adjusted for the above and BMI at age 18 (continuous in kg/m2).
e Model 3 is adjusted for the above and the following: age at menarche (<12, 12-13, 14+), age at first birth and parity combined (BMI <25: nulliparous, age <25 yrs 1-2 kids, age <25 yrs 3+ kids, age 25-29 yrs 1-2 kids, age 25-29 yrs 3+ kids, age 30+ yrs 1-2 kids, age 30+ yrs 3+ kids; BMI 25+: nulliparous, parous age <25 yrs, parous age 25+) and history of benign breast disease (yes, no).

Mammographic density is highly correlated with BMI, with heavier women having more fatty tissue and lower mammographic density.[23] Urinary melatonin also appears to be strongly and inversely related to BMI, in early life[24] as well as later adult life[21]. Although we attempted to remove bias due to confounding by BMI through additional adjustment for BMI at age 18 and childhood and adolescent body size, it is possible that our estimates were still subject to residual positive confounding, resulting in upwardly biased estimates (i.e. higher melatonin levels appearing less protective than they are). Further, higher BMI is associated with greater excretion of creatinine in urine, which is commonly used to adjust for urinary volume differences between samples. This would also serve to further depress adjusted melatonin values in those with higher BMI.

In this same cohort, our measure of urinary melatonin was not associated with breast cancer in a recent nested case-control study, which reported a non-significant inverse association between the highest quartile of melatonin compared with the lowest ($OR_{Q4\ vs\ Q1}$ = 0.91, 95% CI: 0.64,1.28, p_{trend}=0.38), among 600 cases and 786 controls.[16] However, long duration of rotating night shift work in the same cohort was associated with a significantly increased risk of breast cancer, when shift work measures from young adulthood ($HR_{20+\ yrs\ vs\ 0\ yrs}$=2.15, 95%CI 1.23-3.73) as well as updated throughout 24 years of follow-up ($HR_{20+\ yrs\ vs\ 0\ yrs}$=1.40, 95%CI 1.00-1.97) (Wegrzyn dissertation paper 1, unpublished). Therefore, it is possible that our single measure of melatonin may not have captured the circadian disruption that leads to breast tissue changes relevant to the development of breast cancer.

Overall, our results are consistent with the two published papers that investigated urinary melatonin and mammographic density and reported no significant associations.[12, 14]

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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