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NUTRITIONAL, HORMONAL, AND PSYCHOLOGICAL RISK FACTORS FOR BREAST CANCER

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NUTRITIONAL, HORMONAL, AND PSYCHOLOGICAL RISK FACTORS FOR BREAST CANCER

A Dissertation Presented

by

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ABSTRACT

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RISK FACTORS FOR BREAST CANCER

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Over the course of a lifetime, one in eight women will develop breast cancer. To date, 30-40% of breast cancer cases can be attributed to established risk factors, which supports the need for identification of additional modifiable risk factors. Therefore, we conducted three epidemiologic studies to examine the associations between nutritional, hormonal, and psychological risk factors and breast cancer risk.

In our first study, we examined the relationship between urinary melatonin levels and the risk of breast cancer in a nested-case control study within the Nurses' Health Study II. While limited in some respects, experimental and epidemiologic evidence support the potential relationship between melatonin as a protective factor against breast cancer risk. In this updated analysis of our previously published results with longer follow-up time and a greater sample size, adding cases that occurred more distant from urine collection, we did not observe a significant association between urinary melatonin levels and breast cancer risk.

In our second study, we investigated the associations between dairy consumption (overall and by specific dairy products) and circulating hormone levels in the Nurses' Health Study. In this study, we observed a significant positive association between low-fat dairy intake and plasma levels of IGF-I that persisted after adjustment for IGFBP-3, and a significant decrease in SHBG;

however, no significant associations emerged between dairy intake and prolactin or sex steroid hormone levels.

In our third study, we examined the associations between depressive symptoms, antidepressant (AD) use, and breast cancer risk in the Women's Health Initiative Observational Study. Depressive symptoms and AD use were associated with a significant increased risk of total breast cancer; however, no association was observed with invasive breast cancer. Depressive symptoms and AD use were each associated with a non-significant increased risk of in situ breast cancer. However, this association appeared due largely to a positive association with in situ breast cancer that was based, at least in part, on increased mammographic screening among exposed women.

In summary, these studies add to the growing literature of potentially modifiable risk factors and their relationships with breast cancer risk.

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CHAPTER 1

URINARY MELATONIN AND THE RISK OF BREAST CANCER IN THE NURSES' HEALTH STUDY II

Abstract

Experimental and epidemiologic data support melatonin's protective role in breast cancer etiology, yet studies in premenopausal women are scarce. In a case-control study nested within the Nurses' Health Study II cohort, we measured the concentration of melatonin's major urinary metabolite, 6-sulfatoxymelatonin (aMT6s) among 600 predominantly premenopausal women with incident breast cancer and 786 matched controls. Using multivariable conditional logistic regression models, we computed odds ratios (OR) and 95% confidence intervals (CI). Melatonin levels were not significantly associated with the risk of total breast cancer (top (Q4) vs. bottom (Q1) quartile of aMT6s, OR=0.91, 95% CI: 0.64, 1.28, P_{trend}=0.38) or subtypes including invasive, in situ, or ER+/PR+ breast cancer. Findings did not vary by body mass index, smoking status, menopausal status, or time between urine collection and diagnosis (all $P_{interaction} > 0.12$). For example, the OR of total breast cancer among women with ≤5 years between urine collection and diagnosis was 0.74 (Q4 vs. Q1, 95% CI: 0.45, 1.20, P_{trend}=0.09), and 1.20 with >5 years (Q4 vs. Q1, 95% CI: 0.72, 1.98, P_{trend}=0.70). Our data do not support an overall association between urinary melatonin levels and breast cancer risk.

Introduction

Recent meta-analyses of epidemiologic studies on shift work and breast cancer risk have reported that women who work night shifts have a 19-51% increased risk of breast cancer (1-3), and decreased melatonin production due to light exposure is a potential biological mechanism underlying this relationship. Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring

hormone produced primarily by the pineal gland, the synthesis and release of which is stimulated by darkness and suppressed by light, with low circulating levels during the day and the highest levels typically observed at night between 2-4 am (4). Melatonin is metabolized through the liver and excreted in the urine, and 6-sulfatoxymelatonin (aMT6s) is the main metabolite of melatonin measured in urine to estimate circulating melatonin levels (4).

Multiple lines of evidence support the potential anti-estrogenic, antioxidant, and anti-proliferative properties of melatonin (4-6). Melatonin may influence estrogen signaling directly at the tissue level through interaction with the estrogen receptor or indirectly via down-regulation of the hypothalamic-pituitary-gonadal axis, resulting in reduced circulating estrogens (7-8). Further, melatonin has been shown to down-regulate aromatase expression, thereby reducing local estrogen production and suppressing tumor growth (8).

In addition to the potential estrogen-mediated pathways, the activation of melatonin receptors (e.g., MT1 G protein-coupled receptor), which bind melatonin, has been shown to inhibit breast tumor initiation, growth, and cell proliferation (9). In an *in vitro* study, the MT1 melatonin receptor was associated with suppressed tumor growth in the MCF-7 breast cancer cell line (10). Further, the antioxidant properties of melatonin may combat oxidative stress by suppressing tumor initiation and promoting apoptosis (11).

Eight prospective epidemiologic studies (12-19) have examined this relationship with conflicting results, due in part to the relatively small sample size and short follow-up time in prior studies as well as the variation in assessment methods of aMT6s levels. We conducted a prospective nested case-control study of urinary aMT6s levels and breast cancer risk in predominantly premenopausal women in the Nurses' Health Study II (NHSII) cohort. This analysis is an extension of our previous report (13) with six additional years of follow-up and triple the sample size.

Methods

Study population

The NHS II is an ongoing prospective cohort of 116,430 female registered nurses who were ages 25-42 years at baseline in 1989. Self-administered questionnaires are completed biennially to update information on lifestyle factors, health behaviors, medical history, and incident disease. Between 1996 and 1999, 29,611 women ages 32-54 years provided blood and urine samples and completed a short questionnaire to record the date and time of urine collection, the number of nights worked in the past two weeks, the participant's current weight, smoking status, and other lifestyle variables. Among these, 18,521 premenopausal women who had not used oral contraceptives, been pregnant or breastfed within the past six months, and had no personal history of cancer provided a single urine sample and two blood samples timed with their menstrual cycle. The remaining 11,090 women (e.g., postmenopausal, using hormonal contraception, or not able to provide timed samples) contributed an untimed sample. Urine samples were collected with no preservatives and shipped overnight on ice to our laboratory. Ninety-three percent of samples were received within 26 hours of collection, and we have previously demonstrated that levels of urinary aMT6s remain stable when processing is delayed for 24-48 hours (20). Samples have been stored in the vapor phase of liquid nitrogen freezers (≤ -130 °C) since collection.

Follow-up of the blood and urine substudy cohort is close to 95%. The Institutional Review Boards of the Brigham and Women's Hospital and the University of Massachusetts Amherst approved this analysis.

Exposure assessment

Nocturnal melatonin secretion was estimated by measuring the concentration of the major urinary metabolite of melatonin, aMT6s, in urine samples (80% first morning urine, 20% randomly timed spot urine). In 2001, urinary aMT6s was assayed by the Endocrine Core

Laboratory of Dr. M. Wilson (Yerkes National Primate Research Center, Emory University, Atlanta, GA) using a competitive enzyme-linked immunosorbent assay (ALPCO, Windham, NH) with a lower detection limit of 0.8 ng/mL. The same laboratory measured urinary creatinine levels using creatinine reagents purchased from Sigma Diagnostics. From 2003 through 2007, urinary melatonin was assayed in the Ricchuiti Laboratory (now the Carroll Laboratory, Boston, MA) using commercially available ELISA kits with a lower detection limit of 0.8 ng/mL (IBL, Hamburg, Germany), and urinary creatinine levels were measured using the COBAS Integra 400 assay (Roche Diagnostics, Indianapolis, IN). For each participant, urinary aMT6s was divided by urinary creatinine to account for differences in urine concentration, resulting in normalized urinary aMT6s expressed as ng/mg creatinine.

Assays were run in a total of three batches: one batch at Emory University (2001), and two batches at Carroll Laboratory (2003/2005 and 2007 samples). As the original data showed considerable differences in absolute levels of aMT6s across batches, we completed a drift recalibration project. A total of 45 urine samples (15 control participants from each cycle: 2001, 2003/2005, and 2007) that represented the low (n=5), medium (n=5), and high (n=5) tertiles of melatonin values per cycle were sent to the Carroll Laboratory in 2013 and assayed as described above. The correlation between the original assay results and the rerun results (samples run in 2013) was >0.90 for all follow-up cycles, indicating the different assays were measuring the same analyte although with differing absolute levels. We used samples from the original batches and the rerun set of 45 samples to statistically account for laboratory drift over time. As described elsewhere (21), we performed linear regression within each batch to regress the rerun values on the original lab values, and the resulting intercept and slope were used to predict recalibrated values for participants in that batch. Using the recalibrated data, we created quartiles of creatinine-adjusted melatonin levels based on the distribution of controls for all analyses.

Masked replicate quality control samples (10% of samples) were included in each batch to assess the coefficient of variation (CV). Within batch CVs in three total batches ranged from

2.4% to 13.9% for melatonin and 1.2% to 9.2% for creatinine. Samples for cases and controls were treated identically, case-control sets were assayed together, and laboratory personnel were masked to the case-control status of all specimens.

Outcome assessment

We identified incident invasive and in situ breast cancer cases by self-report on biennial questionnaires. Deaths were reported by family members, the United States Postal Service, or through the National Death Index. A study physician performed medical record review to confirm breast cancer cases and abstract information on invasiveness and hormone receptor status. If medical record confirmation was not possible, the nurse participant confirmed her diagnosis, and these cases (n=19) were included in this analysis given that 99% of self-reported breast cancer cases in this cohort are confirmed upon medical record review. A total of 600 breast cancer cases were diagnosed after urine collection and before June 1, 2007. As previously reported (13), cases diagnosed with breast cancer by June 2001 (n=192) were matched with two controls, and the present study additionally includes cases diagnosed after June 2001 (n=408) who were matched with one control. All cases were matched with controls by year of birth (± 2 years), menopausal status at urine collection (premenopausal vs. not), month/year (± 2 months) and time of collection (± 2 hours), luteal day of menstrual cycle at urine collection if the sample was timed (± 1 day), fasting status at urine collection (yes, no), and ethnicity (African American, Asian, Caucasian, Hispanic, other).

Statistical analysis

For this study, we selected NHSII participants who were cancer free at urine collection (1996-1999) and diagnosed with breast cancer between urine collection and June 2007 as well as their matched controls. Seven statistical aMT6s outliers were identified on the log scale using the extreme Standardized deviate many-outlier procedure (22) before normalization to creatinine

concentrations, and three case/control sets with missing melatonin or creatinine measurements were excluded, resulting in a total of 1,386 participants who were eligible for this analysis. Urinary aMT6s measurements below the lower limit of the assay (n=10) were set equal to the detection limit as a conservative estimate.

We used conditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (95% CI) in our primary analyses. For subanalyses, we used conditional logistic regression if the stratification variable was a case characteristic (i.e., invasive vs. in situ breast cancer) and sufficient numbers were available (i.e., ER+/PR+ status, time between urine collection and diagnosis). For other subanalyses with limited numbers (i.e., ER+/PR- status, ER-/PR- status, and stratification by smoking, BMI, and menopausal status), we used unconditional logistic regression with adjustment for matching factors to maximize statistical power. Tests for trend were performed using melatonin continuously, and *P* values were calculated using the Wald statistic.

Lifestyle factors and other characteristics were collected on the biennial questionnaire closest to urine collection as well as the questionnaire completed at the time of blood and urine collection. In addition to the matching factors (i.e. simple models), multivariable models were adjusted for age at menarche (≤11, 12, 13, ≥14 years); parity and age at first birth combined (nulliparous, 1–2 children and <25 years at first birth, 1–2 children and 25–29 years at first birth, 1–2 children and ≥30 years at first birth, ≥3 children and <25 years at first birth, ≥3 children and ≥25 years at first birth); age at menopause (premenopausal, ≤45, >45 years); alcohol intake (none, <5, 5-9, ≥10 grams/day); family history of breast cancer (yes, no); history of benign breast disease (yes, no); BMI (kg/m^2, continuous). To explore the differing influence of BMI on pre- and postmenopausal women, we included an interaction term for BMI (<25 kg/m^2, ≥25 kg/m^2) and menopausal status (premenopausal, postmenopausal) in our model; however, this did not significantly impact effect estimates and therefore was not retained in our final models. Additional adjustment for oral contraceptive use (never, past, current), hormone replacement

therapy (never, past, current), physical activity (METs/week), chronotype (morning type, evening type, neither), smoking status (never, past, current), breastfeeding (ever, never), and current antidepressant medication use (yes, no) did not alter effect estimates; thus, these variables were not retained on our final models.

To evaluate whether the association between melatonin levels and breast cancer risk varied across strata of smoking status at urine collection (never smokers, past and current smokers), BMI at urine collection (<25 kg/m^2, ≥ 25 kg/m^2), menopausal status at diagnosis (premenopausal, postmenopausal), and time between urine collection and diagnosis (dichotomized at the median, ≤ 5 years, >5 years), we added an interaction term for each potential effect modifier (multiplying the dichotomous effect modifier by the midpoint of each quartile of melatonin) to our model and used the likelihood ratio test for interaction to determine statistical significance.

All statistical tests were two-sided; $P<0.05$ was used to define statistical significance. Analyses were conducted in SAS version 9.2 (Cary, NC).

Results

Our study comprised 1,386 participants including 600 cases and 786 matched controls. Cases and controls were similar with regard to most breast cancer risk factors, including age at menarche, parity, age at first birth, and BMI (Table 1). However, cases were more likely than controls to have a history of benign breast disease and family history of breast cancer. Participants were predominantly premenopausal at diagnosis (79%), and the urine provided by the majority of women was a first morning sample (80%).

Among the 786 controls, most baseline characteristics including age at menarche, age at first birth, and parity were similar across quartiles of creatinine-adjusted aMT6s (Table 2). Controls in the highest quartile of urinary aMT6s had a lower BMI compared to controls in the

lowest quartile (24 vs. 27 kg/m^2). In addition, compared to those in the lowest quartile of urinary aMT6s, controls in the highest quartile were less likely to be past or current smokers (29.5% vs. 42.5%).

Urinary aMT6s was not associated with the risk of breast cancer overall (Table 3). Compared to women in the bottom quartile of urinary aMT6s concentrations, the multivariable OR for women in the top quartile was 0.91 (95% CI: 0.64, 1.28, P_{trend}=0.38). No significant associations were observed when we examined invasive and in situ tumors separately. For invasive tumors, the OR comparing the top versus bottom quartile of urinary aMT6s levels was 0.94 (95% CI: 0.62, 1.43, P_{trend}=0.52), and for in situ tumors, the comparable OR was 0.96 (95% CI: 0.48, 1.89, P_{trend}=0.67). In secondary analyses, we restricted only to women providing first morning urine and excluded current night shift workers as night shift work may alter first morning urinary aMT6s, and our results were unchanged (data not shown).

In analyses stratified by tumor hormone receptor status, we observed no association between urinary melatonin and ER+/PR+ tumors (n=286 cases, Q4 vs. Q1, OR=0.94, 0.56, 1.58, P_{trend}=0.59). Further, no significant association or trend in risk emerged between urinary melatonin and ER+/PR- breast cancer (n=45 cases, Q4 vs. Q1, OR=1.07, 95% CI: 0.42, 2.72, P_{trend}=0.78) or ER-/PR- breast cancer (n=78 cases, Q4 vs. Q1, OR=0.96, 95% CI: 0.47, 1.97, P_{trend}=0.96).

Next, we evaluated the association between urinary aMT6s and breast cancer risk by duration of follow-up (Table 4). A non-significant 26% reduced risk of breast cancer was observed among women diagnosed in five or less years after urine collection (Q4 vs. Q1, OR=0.74, 95% CI: 0.45, 1.20, P_{trend}=0.09) whereas a non-significant increase in risk was observed in women with greater than five years between urine collection and diagnosis (Q4 vs. Q1, OR=1.20, 95% CI: 0.72, 1.98, P_{trend}=0.70; $P_{interaction}$=0.12). The suggestion of an inverse trend emerged among postmenopausal women (Q4 vs. Q1, OR=0.71, 95% CI: 0.38, 1.34, P_{trend}=0.08), although the interaction by menopausal status at diagnosis was not significant

($P_{interaction}$=0.64). Finally, no significant variation was observed in the association across strata of BMI ($P_{interaction}$=0.33) or smoking status ($P_{interaction}$=0.27).

Discussion

In this prospective study, we did not observe a significant association between urinary melatonin levels and breast cancer risk overall. In our previous report including 147 invasive cases and 291 matched controls of the current expanded data set, women with the highest levels of aMT6s had a 41% reduced risk of invasive breast cancer (Q4 vs. Q1, OR=0.59, 95% CI: 0.36, 0.97) (13). In this updated analysis with longer follow-up time and a greater sample size, adding cases that occurred more distant from urine collection, we observed an attenuation of our previously published results.

In total, eight prospective studies (12-19) have been conducted to date, including two in premenopausal women (13, 16), three in postmenopausal women (14-15, 19), and three in pre- and postmenopausal women combined (12, 17-18). A meta-analysis of five of the eight previously published studies (18) found that women with the highest aMT6s levels had a significant reduced risk of breast cancer overall (Q4 vs. Q1, OR=0.81, 95% CI: 0.66, 0.99), supporting a modest inverse association between urinary melatonin and breast cancer risk based on studies that used first morning or 12-hour urine collection methods (18). Further, a significant inverse association was observed in postmenopausal women (Q4 vs. Q1, OR=0.68, 95% CI: 0.49, 0.92), but no association was reported in premenopausal women (Q4 vs. Q1, OR=1.05, 95% CI: 0.71, 1.54) (18).

Among individual prospective studies in postmenopausal women, a significant 38-44% reduced risk of breast cancer was observed in women with the highest versus lowest quartile of melatonin in two studies (14-15), whereas one study found no association (19). However, in the three studies including pre- and postmenopausal women (127-251 cases), no association between aMT6s levels and breast cancer risk was observed (12, 17-18). Various urine collection methods

were utilized in these studies including 24-hour urine (12), randomly timed spot urine (17), and first morning urine samples (18). Despite the moderate correlation of urinary aMT6s levels between these methods (e.g., first morning urine and 24-hour urine, r=0.66 (18)), methods such as 24-hour urine collection may reduce inter-individual variability and fail to capture the nocturnal melatonin peak (23), resulting in potential nondifferential exposure misclassification and accounting, at least in part, for the null findings observed.

As described earlier, a significant inverse association was observed among predominantly premenopausal participants in our initial report of this relationship in the NHSII (13). While in the only other study in premenopausal women of the ORDET cohort (180 cases), overall, a positive association was observed between melatonin and invasive breast cancer, (Q4 vs. Q1, OR=1.43, 95% CI: 0.83, 2.45) (16); however, this association was attenuated after excluding current nonsmokers (OR=1.00, 95% CI: 0.52, 1.94) (16), which suggests that current smoking may alter metabolization rates of urinary melatonin. This is of interest as cytochrome P450 1A2 (CYP1A2) is the primary enzyme in the metabolism of melatonin to urinary aMT6s, and smoking has been shown to stimulate CYP1A2 activity (24-25). In the present study, we did not observe substantial variation in stratified analyses by smoking status; however, the current smoking rate is much lower in the NHSII cohort (7%) compared to the ORDET cohort (24.5%), which limited our ability to separately explore associations in these subgroups.

Several studies attempted to explore whether preclinical disease may influence melatonin levels in early follow-up cycles or melatonin levels in the more distant past may be more biologically relevant. Overall, five prior prospective studies examined the impact of time between urine collection and diagnosis on the association between urinary aMT6s levels and breast cancer risk (14-16, 18-19). Results were inconclusive with some studies suggesting stronger inverse associations after excluding the first several years of follow-up after urine collection (14, 16), some reporting stronger positive associations for breast cancer cases which occurred closer to urine collection (19), and others suggesting no difference in results dependent on time between

urine collection and breast cancer diagnosis (15, 18). Overall, each of these lagged analyses were limited by relatively modest case numbers, thus chance may be the most likely explanation for the observed inconsistencies. In the present study, with limited power, we too observed a non-significant inverse relationship between melatonin and breast cancer risk in women with five or less years between urine collection and diagnosis (Q4 vs. Q1, OR=0.71, 95% CI: 0.43, 1.17), and a non-significant positive association after more than five years (Q4 vs. Q1, OR=1.20, 95% CI: 0.72, 2.02). Although not significant ($P_{interaction}$=0.12), these differences by follow-up time may still serve as a potential explanation for the discrepant findings between our current updated analyses (overall null) compared to our earlier (13) publication (aMT6s significantly inversely associated with breast cancer risk). In general, to overcome power limitations, a detailed re-evaluation (e.g., a pooled analysis including all prospective studies) with both longer follow-up and greater power would be useful.

In line with prior studies (13-16, 19), we found that the association between urinary aMT6s levels and breast cancer risk does not vary by tumor estrogen receptor (ER) expression. A moderate to strong inverse association has been reported between BMI and urinary melatonin levels (12, 20, 26-27); however this relationship has not been consistently observed (14-18). In the present study, we found no evidence that the melatonin and breast cancer risk relationship varied by BMI or menopausal status (or a combination of the two variables) at diagnosis. Given that few studies have explored the potential for effect modification by BMI and/or menopausal status, confirmation of these findings is warranted.

To our knowledge, this is the largest prospective study to date to examine the relationship between urinary aMT6s levels and breast cancer risk. We were able to account for most known breast cancer risk factors in our analyses including lifestyle and personal characteristics as well as family history of disease. First morning urine measurements of aMT6s normalized to creatinine have been shown to provide reliable estimates of overnight melatonin production (28), and a validated ELISA assay was utilized in this study. Furthermore, we had excellent laboratory CVs,

and we recalibrated levels to account for variability in aMT6s levels across laboratories. While breast cancer diagnoses were self-reported in this study, medical records and pathology reports were used when available to confirm diagnoses, and 99% of self-reported breast cancer cases in this cohort are confirmed upon medical record review. Our study is limited as aMT6s was measured in urine collected only once per participant. However, the intra-class correlation over three years among premenopausal women from the NHSII cohort was high (0.72), which supports a single morning urinary aMT6s measurement as a reasonable marker for long-term melatonin levels (20).

In summary, we did not observe an association between urinary melatonin and breast cancer risk overall in this large nested case-control study. Melatonin may play a role in various phases of carcinogenesis, which may account for the conflicting results observed in prospective studies to date. In addition to a pooled analysis of existing data, further large prospective studies with long follow-up and consistent methods of measuring aMT6s are needed to confirm these findings.

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Table 1.1. Age and age-adjusted baseline characteristics of 600 cases and 786 matched controls in the Nurses' Health Study II

	Cases (n=600)	Controls (n=786)
Urinary aMT6s, ng/mg creatinine	48.9 (31.8)	47.9 (29.6)
Age, years[a]	43.9 (4.2)	44.0 (4.1)
Age at menarche, years	12.4 (1.3)	12.4 (1.4)
BMI (kg/m^2)	25.0 (5.0)	25.7 (5.9)
Alcohol consumption, grams/day	4.2 (7.2)	3.6 (6.3)
Age at first birth, years (among parous women only)	26.7 (4.7)	26.3 (4.6)
Parity (among parous women only)	2.2 (0.9)	2.3 (0.9)
Nulliparous (%)	21.4	19.1
Race, Caucasian[a] (%)	96.5	97.5
History of benign breast disease (%)	27.4	19.2
Family history of breast cancer (%)	16.5	10.1
Menopausal status at urine collection, premenopausal[a] (%)	78.8	79.5

Abbreviations: aMT6s=6-sulfatoxymelatonin; BMI=body mass index.
Values are means (SD) or percentages and are standardized to the age distribution of the study population.
[a]Matching variables include age, race, and menopausal status at urine collection.

Table 1.2. Age and age-adjusted baseline characteristics of 786 control participants by quartile of urinary 6-sulfatoxymelatonin (aMT6s) in the Nurses' Health Study II

	Quartiles of urinary aMT6s levels			
	Q1 (n=197)	**Q2 (n=196)**	**Q3 (n=196)**	**Q4 (n=197)**
Urinary aMT6s, ng/mg creatinine	≤26.6	26.7-42.5	42.6-61.8	≥61.9
Age, years	44.8 (3.8)	44.1 (4.1)	43.2 (4.3)	43.8 (4.2)
Age at menarche, years	12.3 (1.5)	12.5 (1.4)	12.5 (1.3)	12.4 (1.4)
BMI (kg/m^2)	26.9 (6.3)	25.9 (6.5)	25.7 (5.1)	24.0 (4.9)
Alcohol consumption, grams/day	3.5 (5.6)	3.9 (6.4)	2.8 (5.5)	4.3 (7.0)
Age at first birth, years (among parous women only)	26.6 (4.5)	26.3 (4.8)	26.3 (4.7)	26.0 (4.3)
Parity (among parous women only)	2.3 (0.9)	2.3 (1.0)	2.3 (0.9)	2.4 (0.9)
Nulliparous (%)	18.7	20.6	17.8	16.5
Race, Caucasian (%)	94.9	98.9	97.6	98.9
History of benign breast disease (%)	17.6	22.1	18.3	19.3
Family history of breast cancer (%)	8.5	10.6	11.3	10.4
Menopausal status at urine collection, premenopausal (%)	77.2	74.9	84.7	80.0

Abbreviations: aMT6s=6-sulfatoxymelatonin; BMI=body mass index; Q=quartile.
Values are means (SD) or percentages and are standardized to the age distribution of the study population.

Table 1.3. Odds ratios (OR) and 95% confidence intervals of breast cancer risk by quartile of urinary 6-sulfatoxymelatonin (aMT6s) in the Nurses' Health Study II (1997-2007)

		Quartiles of urinary aMT6s levels[a]				
	No. of cases/ controls	**Q1**	**Q2**	**Q3**	**Q4**	***P* for trend**
Urinary aMT6s, ng/mg creatinine		**≤26.6**	**26.7-42.5**	**42.6-61.8**	**≥61.9**	
All breast cancers[b]						
No. of case/control participants	600/786	145/197	170/196	125/196	160/197	
Simple OR		1.00 (referent)	1.13 (0.84, 1.52)	0.81 (0.58, 1.12)	0.98 (0.71, 1.34)	0.63
Multivariable OR[c]		1.00 (referent)	1.02 (0.75, 1.40)	0.79 (0.56, 1.11)	0.91 (0.64, 1.28)	0.38
Invasive cancer						
No. of case/control participants	422/551	103/138	117/144	92/140	110/129	
Multivariable OR[c]		1.00 (referent)	0.92 (0.64, 1.33)	0.82 (0.55, 1.23)	0.94 (0.62, 1.43)	0.52
In situ cancer						
No. of case/control participants	159/193	36/48	51/38	27/47	45/60	
Multivariable OR[c]		1.00 (referent)	1.90 (0.93, 3.91)	0.68 (0.33, 1.42)	0.96 (0.48, 1.89)	0.67
ER+/PR+ cancer						
No. of case/control participants	286/414	73/105	80/98	58/110	75/101	
Multivariable OR[c]		1.00 (referent)	0.98 (0.62, 1.55)	0.67 (0.41, 1.10)	0.94 (0.56, 1.58)	0.59

Abbreviations: aMT6s=6-sulfatoxymelatonin; OR=odds ratio; Q=quartile.

[a]Quartiles are based on the distribution in control subjects.

[b]All breast cancer includes 422 invasive cases and matched controls, 159 in situ cases and matched controls, and 19 self-reported cases and matched controls.

[c]Multivariable conditional logistic regression models, in addition to matching variables, are adjusted for the following breast cancer risk factors: age at menarche (≤11, 12, 13, ≥14 years); parity and age at first birth combined (nulliparous, 1–2 children and <25 years at first birth, 1–2 children and 25–29 years at first birth, 1–2 children and ≥30 years at first birth, ≥3 children and <25 years at first birth, ≥3 children and ≥25 years at first birth); age at menopause (premenopausal, ≤45, >45 years); alcohol intake (none, <5, 5-9, ≥10 grams/day); family history of breast cancer (yes, no); history of benign breast disease (yes, no); body mass index (kg/m^2, continuous).

Table 1.4. Odds ratios (OR) and 95% confidence intervals of breast cancer risk by quartile of urinary 6-sulfatoxymelatonin (aMT6s) in the Nurses' Health Study II (1997-2007)

		Quartiles of urinary aMT6s levels[a]					
	No. of cases/controls	**Q1**	**Q2**	**Q3**	**Q4**	***P* for trend**	***P* for interaction**
Urinary aMT6s, ng/mg creatinine		**≤26.6**	**26.7-42.5**	**42.6-61.8**	**≥61.9**		
Menopausal status at diagnosis[b,c]	**536/710**						0.64
Premenopausal women	355/502	1.00 (ref)	1.26 (0.84, 1.88)	0.86 (0.57, 1.30)	1.07 (0.71, 1.62)	0.85	
Postmenopausal women	181/208	1.00 (ref)	1.03 (0.56, 1.90)	0.81 (0.41, 1.58)	0.71 (0.38, 1.34)	0.08	
Body mass index at urine collection[c]	**600/786**						0.33
<25 kg/m^2	359/444	1.00 (ref)	1.01 (0.65, 1.57)	0.96 (0.61, 1.49)	1.02 (0.67, 1.57)	0.95	
≥25 kg/m^2	241/342	1.00 (ref)	1.25 (0.79, 1.98)	0.61 (0.36, 1.01)	1.09 (0.65, 1.83)	0.82	
Smoking status at urine collection[c]	**600/786**						0.27
Never smoker	388/537	1.00 (ref)	1.02 (0.69, 1.50)	0.68 (0.45, 1.02)	1.09 (0.73, 1.62)	0.95	
Past or current smoker	212/249	1.00 (ref)	1.21 (0.72, 2.04)	1.09 (0.61, 1.93)	0.91 (0.53, 1.58)	0.61	
Time between urine and diagnosis[d]	**600/786**						0.12
≤5 years	261/447	1.00 (ref)	0.93 (0.60, 1.45)	0.71 (0.43, 1.18)	0.74 (0.45, 1.20)	0.09	
>5 years	339/339	1.00 (ref)	1.15 (0.72, 1.82)	0.88 (0.54, 1.44)	1.20 (0.72, 1.98)	0.70	

Abbreviations: aMT6s=6-sulfatoxymelatonin; OR=odds ratio; Q=quartile.

[a]Quartiles are based on the distribution in control subjects.

[b]140 women with missing/dubious menopausal status not included.

[c]For menopausal status, body mass index, and smoking status, multivariable unconditional logistic regression was used with adjustment for matching variables and the following breast cancer risk factors: age at menarche (≤11, 12, 13, ≥14 years); parity and age at first birth combined (nulliparous, 1–2 children and <25 years at first birth, 1–2 children and 25–29 years at first birth, 1–2 children and ≥30 years at first birth, ≥3 children and <25 years at first birth, ≥3 children and ≥25 years at first birth); age at menopause (premenopausal, ≤45, >45 years); alcohol intake (none, <5, 5-9, ≥10 grams/day); family history of breast cancer (yes, no); history of benign breast disease (yes, no); body mass index (kg/m^2, continuous).

[d]For time between urine collection and diagnosis, conditional logistic regression was used with adjustment for matching variables and the breast cancer risk factors listed above.

CHAPTER 2

DAIRY INTAKE AND CIRCULATING HORMONE CONCENTRATIONS IN POSTMENOPAUSAL WOMEN

Abstract

Dairy intake may influence circulating hormone levels, yet few epidemiologic studies have examined this relationship. We assessed the cross-sectional relationship of dairy intake (overall and by specific dairy products) and plasma estrogens, androgens, sex hormone binding globulin (SHBG), prolactin, insulin-like growth factor-I (IGF-I), and insulin-like growth factor binding protein-3 (IGFBP-3) in a large sample of postmenopausal women from the Nurses' Health Study (NHS). Average dairy consumption was measured using a validated FFQ collected in 1986 and 1990, and hormone levels were measured in blood provided from 1989-1990. Adjusted geometric means for each hormone were calculated across categories of dairy consumption and linear trends were evaluated. Greater total dairy consumption was associated with increased IGF-I (≥3 vs. <1 servings/day, 147 vs. 137 ng/ml, *P* trend=0.08) and IGFBP-3 levels (4372 vs. 4201 ng/ml, *P* trend=0.01); however, these relationships were attenuated after mutual adjustment for IGFBP-3 or IGF-I. We observed a significant positive association between low-fat dairy intake and plasma levels of IGF-I (150 vs. 133 ng/ml, $P_{trend=}0.03$) that persisted after adjustment for IGFBP-3 (144 vs. 135 ng/ml, $P_{trend=}0.02$) and a significant decrease in SHBG (≥2 vs. <0.5 servings/day, 51 vs. 56 nmol/l, $P_{trend=}0.04$). No significant associations emerged between total dairy, low-fat dairy, and high-fat dairy and prolactin or sex steroid hormone levels. Overall, we observed a modest association between low-fat dairy and IGF-I and SHBG levels. Further research is needed to evaluate whether these changes in circulating hormone levels associated with low-fat dairy intake translate to an increased risk of hormonally mediated diseases such as breast and endometrial cancer.

Introduction

Dietary exposures including dairy intake have long been of interest to human health. Over the past decade, epidemiologic studies have linked greater dairy consumption with a decreased risk of endometriosis, hip fracture, and colorectal cancer, while a positive association between dairy intake and the risk of breast and endometrial cancers has been suggested but remains unclear (1-6). Dairy has a number of constituents that may be physiologically relevant to disease risk including calcium, vitamin D, potassium, protein, and fat, and specific components of dairy foods have been shown to impact circulating hormone levels in women. For example, in a randomized controlled feeding trial in postmenopausal women, daily supplementation of whey protein, a by-product of cheese processed from cow's milk, increased serum insulin-like growth factor-I (IGF-I) levels by 8% over two years (7). Further, in a recent cross-sectional study, a 19% increase in free IGF-I levels was observed in women with a three serving per day increase in milk (8). In addition, dairy intake may impact the hormonal milieu due to endogenous hormones in dairy products that potentially remain bioactive after ingestion (9-10). Dairy products, especially milk, contain estrogens, androgens, and growth factors that have been detected following processing and pasteurization; however, the biochemistry and metabolism of these dairy-derived hormones in humans is not well understood (11).

While prior studies support the hypothesis that dairy intake may impact circulating hormone levels, few epidemiologic studies have examined this relationship in postmenopausal women. A positive association was reported between greater dairy intake and increased IGF-I levels in an earlier cross-sectional analysis in the Nurses' Health Study (NHS; n=1,037) (12), and higher dairy consumption was associated with increased plasma concentrations of 17β-estradiol (estradiol) in another cross-sectional study (n=766) (13). To better understand the relationship between dairy intake and various hormones of interest, we assessed the cross-sectional relationship of dairy intake (overall and by specific dairy products) and plasma sex steroid

hormones, sex hormone binding globulin (SHBG), prolactin, IGF-I, and insulin-like growth factor binding protein 3 (IGFBP-3) in a large sample of postmenopausal women from the NHS.

Methods

Study population

The NHS cohort was established in 1976, when 121,700 female registered nurses aged 30 to 55 years completed a baseline questionnaire on lifestyle factors, medical history, and disease status. Since baseline, study participants have completed biennial questionnaires to update a range of exposure information as well as to report new disease diagnoses.

From 1989 to 1990, when participants were aged 43 to 70 years, 32,826 women provided a blood sample. Details of the collection are described elsewhere (14). Briefly, participants arranged to have their blood drawn and shipped overnight with an icepack to Channing Laboratory, where each specimen was processed and separated into plasma, red blood cell, and white blood cell components. Samples have been stored in continuously monitored liquid nitrogen freezers (≤ -130 °C) since collection. In a validation study, levels of androstenedione, testosterone, prolactin, free estradiol, and SHBG remained relatively stable with greater between-person coefficients of variation (CVs) than within-person CVs when shipped overnight, as compared to other transport methods with varying time and temperature conditions (15).

Women included in this analysis were controls in one of several case-control studies nested within the NHS cohort with endpoints including breast cancer (16-17), ovarian cancer (18), colon cancer (19), diabetes (unpublished data), myocardial infarction (20), colon polyps (21), rheumatoid arthritis (22), Barrett's esophagus (unpublished data), and ischemic stroke (23). This analysis was restricted to postmenopausal women who did not use postmenopausal hormones (PMH) within three months of blood draw, except for the IGF-I and IGFBP-3 analyses

for which current PMH users also were eligible. The Institutional Review Boards of the Brigham and Women's Hospital and the University of Massachusetts Amherst approved this analysis.

Exposure assessment

Diet was assessed in the cohort via a self-administered semi-quantitative food frequency questionnaire (FFQ), which was administered in 1980, 1984, 1986, and every four years thereafter. Participants were asked, on average, how frequently they consumed a typical portion size of specified foods over the past year, with nine possible frequency responses for each food ranging from never to greater than six times per day. Total dairy was calculated as the sum of the following: skim and low-fat milk, whole milk, yogurt, cottage cheese, sherbet, cream, sour cream, ice cream, cream cheese, and other cheese. The low-fat dairy category was calculated as the sum of skim and low-fat milk, yogurt, cottage cheese, and sherbet, and the high-fat dairy category included whole milk, cream, sour cream, ice cream, cream cheese, and other cheese.

The validity of the original 1980 61-item FFQ was examined in a sample of 173 NHS participants by comparing FFQ responses to the mean intake assessed using four 1-week diet records (24-25). Correlation coefficients between the FFQ and mean intake assessed by diet records were 0.81 for skim and low-fat milk, 0.62 for whole milk, 0.94 for yogurt, 0.73 for ice cream, 0.80 for cottage cheese, and 0.57 for other cheeses (24).

Outcome assessment

Details of the laboratory assay methods used in the NHS blood substudy are described elsewhere (26-28). Briefly, estradiol (12 batches), estrone (6 batches), androstenedione, testosterone (15 batches), and DHEA (12 batches) were assayed by radioimmunoassay following extraction and Celite column chromatography (Quest Diagnostics, NHS follow-up cycles 1990-1998) or liquid chromatography-tandem mass spectrometry (Mayo Medical Laboratories, NHS

follow-up cycles 2000-2010). The correlation between these two assay methods is >0.87. DHEAS (15 batches) was assayed by radioimmunoassay without a prior separation step (Quest Diagnostics). Estrone sulfate (6 batches) was measured by radioimmunoassay of estrone after extraction, enzyme hydrolysis, and column chromatography (Quest Diagnostics; University of Massachusetts Medical Center's Longcope Steroid Radioimmunoassay Laboratory; Mayo Medical Laboratories). SHBG (9 batches) was assayed by chemiluminescent immunoassay and prolactin (8 batches) was measured using a microparticle enzyme immunoassay (University of Massachusetts Medical Center's Longcope Steroid Radioimmunoassay Laboratory; Massachusetts General Hospital's Reproductive Endocrinology Laboratory). The correlation between the two prolactin assay methods was 0.91. IGF-I (12 batches) and IGFBP-3 (12 batches) were assayed by the Pollak Laboratory at McGill University using an ELISA after acid extraction using reagents from Diagnostic Systems Laboratory (Webster, TX). Finally, free estradiol and free testosterone were calculated using the Sodergard method (29). When hormone values were below the limit of detection, we set the value to half the limit (n=1 for DHEA; n=2 for estradiol, DHEAS, and androstenedione; n=3 for testosterone and SHBG, n=5 for estrone sulfate, and n=7 for estrone).

To allow for assessment of assay precision, we randomly distributed masked quality control samples (equivalent to 10% of total samples) with all analytic samples. Within-batch coefficients of variation (CVs) ranged from 2 to 15% for all analytes, except for one batch of estradiol (17%) and one batch of testosterone (18%).

Statistical analysis

We calculated the mean and standard deviation for continuous variables, the frequency and percentage for categorical variables, and the median and truncated range (10-90th percentile) for all hormones of interest. We excluded participants with outlying hormone values (<5 values

per hormone, 27 values overall) based on the generalized extreme studentized deviate many-outlier detection method (30). Women with missing data on dairy intake in the 1986 and 1990 FFQ were also excluded from this analysis. After these exclusions, 3,933 women were eligible for our analyses.

For dairy intake, we averaged each woman's reported dairy intake (in servings per day) in 1986 and 1990 to provide a more stable assessment of dairy consumption in the years closest to blood collection. Generalized linear models were used to calculate least square means for each log-transformed hormone across categories of dairy intake, and the adjusted geometric means were then obtained as the exponential of the adjusted least square means. We calculated the percent difference in geometric means for the highest versus lowest category of dairy intake as $(e^{\beta}-1)*100$, where β is the difference in the adjusted least square means of each log-transformed hormone for the highest versus lowest category of dairy intake. To evaluate linear trends across categories of dairy consumption, we modeled a continuous variable by the midpoint of each category of dairy intake and calculated the Wald test statistic and its p-value. To assess whether the association between dairy intake and IGF-I and IGFBP-3 levels varied by PMH use, we modeled an interaction term between PMH use (never/past vs. current) and a continuous variable defined by the midpoint of each category of dairy intake and calculated the *P* for interaction using the likelihood ratio test. All analyses were conducted in SAS 9.2 software (SAS Institute, Cary, North Carolina). All statistical tests were two-sided; $P < 0.05$ was used to define statistically significance.

Data on lifestyle factors and other characteristics were collected on the biennial questionnaire closest to blood collection as well as the questionnaire completed at the time of blood draw. All models were adjusted for covariates known or suspected to be associated with dairy intake and/or hormone concentrations including: age at blood draw (years, continuous); BMI at blood draw (kg/m^2, continuous); PMH use currently or within three months prior to blood

draw (yes, no); age at menarche (≤11, 12, 13, ≥14 years); parity and age at first birth combined (nulliparous, 1–4 children and ≤24 years at first birth, 1–4 children and 25–29 years at first birth, 1–4 children and ≥30 years at first birth, ≥5 children and ≤24 years at first birth, ≥5 children and ≥25 years at first birth); age at menopause (≤45, 46-50, 51-55, ≥56 years); type of menopause (natural menopause, surgery with 0-1 ovaries removed, surgery with 2 ovaries removed), smoking status (never, former, current), alcohol intake (non-drinker, <1 drink/day, ≥1 drink/day), physical activity (MET-hours/week, quartiles), laboratory batch, blood collection month and year (≤6/1989, 7/1989/1989, 1/1990-6/1990, ≥7/1990), and time of blood collection (7am-noon, 1-8pm, 9pm-6am), fasting status (yes, no). Given that women with current PMH use were eligible for the analysis of IGF-I or IGFBP-3 levels, we additionally considered PMH use as a potential confounder; however, we observed no change in estimates with its inclusion and therefore dropped PMH use from our final models. Additional adjustment for other potential confounders including BMI at age 18, family history of breast cancer, history of benign breast disease, history of breastfeeding, and past oral contraceptive use did not alter effect estimates, and therefore were not retained in our final models. Because results from age and batch adjusted models were similar to the multivariable adjusted models, only the latter are presented here.

Results

At blood collection, the mean age was 61.1 years, and the mean BMI was 25.7 kg/m^2 (Table 1). Most women were parous (93.2%) with a mean age at first birth of 23.3 years. The mean age at menopause was 48.9 years, and 48.7% of women ever used PMH. On average, women reported consuming 2.4 servings/day of total dairy, 1.3 servings/day of low-fat dairy, and 0.8 servings/day of high-fat dairy. Overall, 3,933 women provided information on at least one hormone of interest, and the number of plasma samples available for analysis ranged from 724 participants with androstenedione measurements to 1,916 participants with IGF-I measurements.

While age at menarche, parity, PMH use, and physical activity did not vary by categories of total dairy intake, women reporting the highest level of dairy intake per day had a slightly higher BMI, lower alcohol intake, and were less likely to be a current or past smoker compared to women reporting the lowest dairy intake (Table 2).

Greater total dairy consumption was associated with higher IGF-I (≥3 vs. <1 servings/day, 147 vs. 137 ng/ml, *P* trend=0.08) and IGFBP-3 levels (4372 vs. 4201 ng/ml, *P* trend=0.01); however, these relationships were attenuated after mutual adjustment for IGF-I and IGFBP-3 (Table 3). The total dairy/IGF-I relationship was essentially unchanged after additional adjustment for total fat (≥3 vs. <1 servings/day, 144 vs. 135 ng/ml) or total protein intake (145 vs. 135 ng/ml). As additional adjustment for total fat, total protein, and total energy also did not materially influence any other associations between total dairy and the remaining hormones of interest, these variables were not retained in the final model. In a stratified analysis by PMH use, the relationship between total dairy intake (in quartiles) and IGF-I levels among past or never PMH users (Q4 vs. Q1, 155 vs. 153 ng/ml, *P* trend=0.51) appeared slightly weaker than the relationship among current PMH users (Q4 vs. Q1, 125 vs. 119 ng/ml, *P* trend=0.18; *P* for interaction=0.01).

Overall, the IGF-I association appeared stronger among women with greater consumption of low-fat dairy (≥2 vs. <0.5 servings/day, 150 vs. 133 ng/ml, *P* trend=0.03) versus high-fat dairy (≥2 vs. <0.5 servings/day, 146 vs. 143 ng/ml, *P* trend=0.89). Further, the relationship between low-fat dairy and IGF-I persisted after adjustment for IGFBP-3 (144 vs. 135 ng/ml, *P* trend=0.02). Greater low-fat dairy intake was associated with significantly lower SHBG levels (≥2 vs. <0.5 servings/day, 51 vs. 56 nmol/l, *P* trend=0.04), but SHBG levels remained stable across categories of high-fat dairy (≥2 vs. <0.5 servings/day, 52.2 vs. 52.5 nmol/l, *P* trend=0.96). Overall, no associations were observed between high-fat dairy and circulating hormone levels in this sample.

To determine if a single dairy product or group of individual dairy products were stronger contributors to the observed SHBG and IGF-I associations, we examined the relationship of these hormones by categories of the individual dairy foods (Table 4). Overall, no significant associations emerged between individual dairy products and SHBG and IGF-I; however, hormone levels tended to trend in the expected direction.

Discussion

In this cross-sectional study in postmenopausal women, participants consumed more low-fat dairy (1.3 servings/day) than low-fat dairy products (0.8 servings/day), and we observed significant associations between low-fat dairy intake and plasma levels of SHBG and IGF-I. Total dairy intake was positively associated with IGF-I and IGFBP-3; however, these relationships were attenuated after IGF-I and IGFBP-3 were mutually adjusted. No significant associations emerged between total dairy, low-fat dairy, and high-fat dairy and prolactin or sex steroid hormone levels.

Prior studies have linked greater low-fat dairy intake with a 10-20% increase in circulating IGF-I levels (12, 31-32). In a randomized trial of 254 healthy men and women aged 55-85 years, participants were instructed to consume three servings per day of non-fat or 1% milk or maintain their usual diets which included fewer than 1.5 servings per day of dairy foods over a 12-week period (31). In the milk intake group, serum IGF-I levels increased by 10% ($p<0.001$) compared to the control group. In another randomized controlled feeding trial, daily whey protein supplementation increased serum IGF-I levels in postmenopausal women by 8% (7). Further, in a cross-sectional analysis of dietary correlates of plasma IGF-I levels, including a subset of participants in our current study (n=1,037), a positive association was observed between protein intake and circulating IGF-I levels ($p=0.002$), which was mostly attributable to milk intake (mean IGF-I levels by Q4 vs. Q1 of milk intake, 200 vs. 183 ng/ml, $p=0.01$) (12). Finally,

among male participants aged 40-84 in the Physician's Health Study, dairy consumption, especially low-fat milk, was positively associated with IGF-I (tertile 3 vs. tertile 1, 203 vs. 174 ng/ml, p=0.0003) and IGFBP-3 levels (tertile 3 vs. tertile 1, 3129 vs. 2887 ng/ml, p=0.004) (32). Despite the epidemiologic evidence to support a relationship between dairy intake and increased IGF-I levels, it remains unclear if dairy-derived IGF is absorbed by the human gut or if dairy products alter endogenous production. It has been hypothesized that dietary protein casein, the major protein in milk, protects IGF-I from degradation in the gastrointestinal tract, but only animal studies have explored this to date (33). Further, the experimental evidence is conflicting: one animal study found no change in serum IGF-I levels following exposure to orally administered high doses of recombinant IGF-I (34) whereas other animal studies have detected an increase in circulating IGF-I after radioactively labeled IGF-I in milk was fed to neonatal rats (35) as well as increased growth of specific organs following oral administration of IGF-I (35).

Approximately 47% of estrone intake through diet is attributed to dairy products, and water-soluble estrone sulfate, an estrogen readily detected in milk, may remain bioactive after ingestion (36-37). However, no associations emerged between total dairy intake and estradiol, free estradiol, estrone, or estrone sulfate levels in our study. In the only prior study to date on dairy and sex steroid hormones and in contrast to our study, a positive relationship was observed between dairy consumption and total estradiol (Q4 vs. Q1, 63.93 vs. 55.42, *P* trend=0.02) and free estradiol (Q4 vs. Q1, 0.88 vs. 0.77, *P* trend=0.03) (13). In the study by Brinkman et al., while red meat consumption was associated with SHBG levels, no associations emerged between dairy intake and androgens or SHBG, and total fat and total protein were additionally not associated with estrogens, androgens, or SHBG (13). Further, while both studies used quartiles, the distribution of dairy intake in our sample ranged from <1 serving per day to ≥3 servings per day whereas the sample studied by Brinkman et al. ranged from ≥0 servings per week to ≥48 servings per week or up to approximately 7 servings per day. The differences in the distribution of dairy intake between the two studies may explain, in part, the inconsistencies in study findings.

The association between dairy intake and IGF-I levels appeared somewhat stronger among current PMH users, compared to past or never users. Women who were never or past PMH users had significantly higher circulating IGF-I levels compared to current PMH users and hence may have required a higher dose of IGF-I from dairy products to alter their circulating hormone levels. However, IGF-I levels were not substantially different by high vs. low category of dairy within groups, therefore confirmation of these findings in future studies is warranted.

In our study, women reported the frequency of their dairy intake over the past year, which may attenuate our findings if women over or under-reported their dairy consumption. However, we calculated all variables related to dairy intake using data from FFQs completed in 1986 and 1990, which will reduce random measurement error due to within-person variation. In addition, regularly consumed foods such as milk have reduced within-person week-to-week fluctuations and higher correlation coefficients compared to foods that are eaten infrequently (24). The potential for exposure misclassification is further reduced by the high correlations observed between food frequency questionnaires and daily dietary records among NHS participants (r=0.57-0.94) (24).

This study was limited as hormone levels were measured using a single blood sample per participant. However, the intra-class correlation coefficients for these hormones are high over a 10-year period (ICC=0.50-0.70) (28), suggesting that a single blood sample reflects long-term values. Further, we utilized validated assays for all hormone measurements, and 98% of the blood samples were received within 26 hours of the blood draw, which minimizes the potential for the hormone values to vary due to storage or transport conditions. The cross-sectional design is an additional limitation of our analysis, as we cannot determine whether differences in hormone levels are influenced by dairy intake or whether dietary patterns, including dairy consumption, are somehow influenced by hormone levels. Finally, we cannot rule out chance as a possible explanation of our findings in this study.

The present study has several strengths, including the large, prospective study population with data on multiple hormones of interest, as well as detailed information on dairy intake measured using validated FFQs. Data were available on various potential confounders of interest collected near the time of blood draw, which minimizes the potential for residual confounding in our study. To our knowledge, this is the largest study to date to investigate the relationship between dairy intake and circulating hormone levels in postmenopausal women and the only study to examine associations by high-fat and low-fat dairy categories as well as individual dairy products. Finally, we expanded upon prior literature (13) by including prolactin, IGF-I, and IGFBP-3 in addition to the sex steroid hormones of interest.

Our results provide support for a modest association between low-fat dairy and IGF-I and SHBG levels in postmenopausal women. A large pooled analysis of 17 prospective studies observed a 30% increase in breast cancer risk among postmenopausal women with highest vs. lowest IGF-I levels (38), and another study reported a significant inverse relationship between levels of SHBG and breast cancer risk over 20 years of follow-up (28). Given the relationships we observed between low-fat dairy intake and SHBG and IGF-I levels, further research is warranted to evaluate whether these changes in circulating hormone levels associated with low-fat dairy intake translate to an increased risk of hormonally mediated diseases such as breast and endometrial cancer.

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Table 2.1. Characteristics of postmenopausal participants at blood draw in the Nurses' Health Study

	Mean (SD) or %
Age, years	61.1 (5.1)
Body mass index, kg/m^2	25.7 (4.7)
Age at menarche, years	12.6 (1.4)
Parous, %	93.2
Parity, among parous women	3.2 (1.8)
Age at first birth, years	23.3 (7.7)
Age at menopause, years	48.9 (4.8)
Ever PMH use, %	48.7
Physical activity, MET-h/week	16.7 (23.3)
Alcohol intake, grams/day	5.5 (10.1)
Current or past smoker, %	55.8
Mean total dairy intake, servings/day	2.4 (1.5)
Mean low-fat dairy intake, servings/day	1.3 (1.0)
Mean high-fat dairy intake, servings/day	0.8 (0.8)

	N	Median (10th - 90th percentile)
Estrone, pg/ml	1149	24 (13 – 44)
Estradiol, pg/ml	1245	6 (3 – 13)
Free estradiol, pg/ml	822	0.09 (0.04-0.24)
Estrone sulfate, pg/ml	1232	103 (17-406)
Testosterone, ng/dl	1439	20 (10 – 38)
Free testosterone, ng/dl	1176	0.18 (0.08-0.41)
Androstenedione, ng/dl	724	57 (30 – 109)
DHEA, ug/dl	1082	109 (3 – 362)
DHEAS, ug/dl	1335	83 (29 – 264)
Prolactin, ng/ml	1195	10 (6 – 52)
SHBG, nmol/l	1590	56 (26 – 104)
IGF-I, ng/ml	1916	141 (85 – 228)
IGFBP-3, ng/ml	1912	4333 (3115 – 5876)

Table 2.2. Characteristics of postmenopausal participants at blood draw by frequency of total dairy intake in the Nurses' Health Study

	Frequency of total dairy intake (servings/day)			
	<1	**1–1.9**	**2-2.9**	**≥3**
	(n=605)	**(n=1,341)**	**(n=928)**	**(n=1,059)**
Age, years	60.9 (5.0)	60.9 (5.2)	61.2 (5.2)	61.3 (4.9)
Body mass index, kg/m^2	25.2 (4.1)	25.8 (4.6)	25.6 (4.6)	26.1 (5.0)
Age at menarche, years	12.7 (1.5)	12.5 (1.4)	12.6 (1.5)	12.6 (1.5)
Parous, %	93.5	93.3	94.2	92.5
Parity, among parous women	3.2 (1.7)	3.1 (1.8)	3.3 (1.8)	3.3 (1.9)
Age at first birth, years	23.3 (7.6)	23.1 (8.0)	23.5 (7.5)	23.4 (7.6)
Age at menopause, years	48.7 (4.9)	48.8 (4.7)	49.2 (4.8)	49.1 (4.6)
Ever PMH use, %	48.1	48.8	47.4	49.3
Physical activity, MET-h/week	17.4 (21.6)	15.7 (23.5)	17.3 (27.2)	17.1 (21.3)
Alcohol intake, grams/day	6.7 (12.0)	6.1 (10.8)	4.8 (8.6)	4.7 (9.1)
Current or past smoker, %	58.6	58.1	52.8	54.2

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

Table 2.3. Adjusted geometric mean hormone levels by frequency of dairy intake among postmenopausal women in the Nurses' Health Study (1990)

	N	Frequency of dairy intake (servings/day)				Percent difference[1]	P-trend
Total dairy[2]		**<1**	**1-1.9**	**2-2.9**	**≥3**		
Number of participants		**(118-317)**	**(262-690)**	**(164-485)**	**(180-430)**		
Estrone, pg/ml	1149	24.4	23.4	23.3	24.2	-1.0	0.84
Estradiol, pg/ml	1245	6.2	5.9	6.1	6.3	0.4	0.62
Free estradiol, pg/ml	822	0.09	0.09	0.10	0.09	-0.4	0.74
Estrone sulfate, pg/ml	1232	86.7	81.6	84.9	84.7	-2.4	0.84
Testosterone, ng/dl	1439	20.4	19.4	19.6	20.0	-2.3	0.71
Free testosterone, ng/dl	1176	0.19	0.17	0.19	0.19	-0.4	0.74
Androstenedione, ng/dl	724	56.9	55.2	56.7	58.8	3.2	0.37
DHEA, ng/dl	1082	51.5	51.7	61.4	60.1	14.4	0.14
DHEAS, ug/dl	1335	98.0	81.6	85.9	83.5	-17.4	0.31
Prolactin, ng/ml	1195	13.4	12.4	12.4	12.4	-8.3	0.23
SHBG, nmol/l	1590	54.0	55.9	52.8	51.6	-4.6	0.27
IGF-I, ng/ml	1916	137	136	144	147	6.6	0.08
IGF-I with adj. for IGFBP-3	1912	138	137	144	143	6.0	0.19
IGFBP-3, ng/ml	1912	4201	4258	4299	4372	3.9	0.01
IGFBP-3 with adj. for IGF-I	1912	4208	4276	4217	4257	1.3	0.27

Table 2.3. (cont). Adjusted geometric mean hormone levels by frequency of dairy intake among postmenopausal women in the Nurses' Health Study (1990)

	N	Frequency of dairy intake (servings/day)				Percent difference[1]	P-trend
Low-fat dairy		**<0.5**	**0.5-0.9**	**1-1.9**	**≥2**		
Number of participants		**(174-448)**	**(166-426)**	**(211-628)**	**(173-416)**		
Estrone, pg/ml	1149	23.9	23.1	23.6	24.5	2.8	0.49
Estradiol, pg/ml	1245	5.9	6.0	6.0	6.4	3.7	0.13
Free estradiol, pg/ml	822	0.09	0.09	0.09	0.10	0.6	0.23
Estrone sulfate, pg/ml	1232	78.6	87.8	85.3	84.9	7.5	0.46
Testosterone, ng/dl	1439	19.3	20.2	19.4	20.4	5.0	0.42
Free testosterone, ng/dl	1176	0.17	0.19	0.18	0.20	3.4	0.20
Androstenedione, ng/dl	724	56.5	57.5	54.9	58.3	3.0	0.75
DHEA, ng/dl	1082	54.9	50.6	52.5	67.8	19.0	0.33
DHEAS, ug/dl	1335	96.8	83.7	79.6	84.0	-15.3	0.26
Prolactin, ng/ml	1195	12.6	12.0	13.1	12.5	-0.6	0.77
SHBG, nmol/l	1590	56.0	55.7	52.7	51.0	-9.8	0.04
IGF-I, ng/ml	1916	133	139	141	150	11.0	0.03
IGF-I with adj. for IGFBP-3	1912	135	138	140	144	8.2	0.02
IGFBP-3, ng/ml	1912	4169	4266	4279	4438	6.1	0.05
IGFBP-3 with adj. for IGF-I	1912	4224	4261	4228	4280	2.0	0.35

Table 2.3. (cont). Adjusted geometric mean hormone levels by frequency of dairy intake among postmenopausal women in the Nurses' Health Study (1990)

	N	Frequency of dairy intake (servings/day)				Percent difference[1]	P-trend
High-fat dairy		**<0.5**	**0.5-0.9**	**1-1.9**	**≥2**		
Number of participants		**(240-654)**	**(213-710)**	**(171-398)**	**(101-156)**		
Estrone, pg/ml	1149	24.2	24.0	22.7	24.0	-0.9	0.56
Estradiol, pg/ml	1245	6.2	6.2	5.8	5.9	-4.5	0.19
Free estradiol, pg/ml	822	0.09	0.09	0.08	0.09	0.4	0.74
Estrone sulfate, pg/ml	1232	82.1	87.5	81.8	83.0	1.1	0.85
Testosterone, ng/dl	1439	20.3	20.2	18.7	18.8	-8.1	0.11
Free testosterone, ng/dl	1176	0.19	0.19	0.17	0.18	-0.6	0.33
Androstenedione, ng/dl	724	57.5	55.5	56.1	60.5	5.0	0.44
DHEA, ng/dl	1082	54.4	57.4	60.2	53.4	-1.1	0.98
DHEAS, ug/dl	1335	84.7	82.0	88.2	95.7	11.5	0.15
Prolactin, ng/ml	1195	12.2	13.3	12.1	11.8	-3.7	0.53
SHBG, nmol/l	1590	52.5	54.6	55.1	52.2	-0.2	0.96
IGF-I, ng/ml	1916	143	140	135	146	1.9	0.89
IGF-I with adj. for IGFBP-3	1912	140	140	134	143	1.9	0.89
IGFBP-3, ng/ml	1912	4328	4235	4275	4348	0.5	0.75
IGFBP-3 with adj. for IGF-I	1912	4336	4201	4300	4344	0.2	0.76

[1]Percent difference for highest versus lowest category of dairy intake, calculated using $(e^{\beta}-1)*100$, where β is the difference in the adjusted least square means of each log-transformed hormone for the highest versus lowest category of dairy intake.

[2]Total dairy includes skim milk, whole milk, cream, sour cream, sherbet, ice cream, cream cheese, yogurt, cottage cheese, and other cheese; low-fat dairy includes skim milk, sherbet, yogurt, and cottage cheese; high-fat dairy includes whole milk, cream, sour cream, ice cream, cream cheese, and other cheese.

Adjusted for age at blood (years, continuous), age at menarche (≤11, 12, 13, ≥14 years), BMI at blood draw (kg/m^2, continuous), age at first birth and parity (no children, ≤24 years and 1-4 children, 25-29 years and 1-4 children, ≥30 years and 1-4 children, ≤24 years and 5+ children, ≥25 years and 5+ children), age at menopause (≤45, 46-50, 51-55, >55 years), type of menopause (natural menopause, surgery with no or one ovaries removed, surgery with 2 ovaries removed), smoking status (never, former, current), alcohol intake (non-drinker, <1 drink/day, ≥1 drink/day), physical activity (MET-hours/week in quartiles, ≤3, 4-≤10, 11-≤22, >22), date and year of blood draw (≤6/1989, 7/1989/1989, 1/1990-6/1990, ≥7/1990), time of blood draw (7am-noon, 1-8pm, 9pm-6am), fasting at blood draw (yes, no), and laboratory batch.

Table 2.4. Adjusted geometric mean hormone levels of SHBG and IGF-I by frequency of individual dairy products among postmenopausal women in the Nurses' Health Study (1990)

	Frequency of dairy intake (servings/day)				P-trend
SHBG, nmol/l	**<0.5**	**0.5-0.9**	**1-1.9**	**≥2**	
Skim milk	59.0	57.4	48.7	52.2	0.21
Yogurt	53.9	51.9	52.1	56.6	0.51
Cottage cheese	54.7	53.7	52.5	52.9	0.12
Sherbet	55.3	53.2	55.6	51.8	0.42
Whole milk	54.1	53.4	48.4	53.1	0.60
Cream	54.2	54.8	52.0	53.1	0.36
Sour cream	53.3	54.4	54.5	53.4	0.91
Cream cheese	54.2	52.8	52.0	53.1	0.42
Ice cream	53.7	53.7	54.0	52.9	0.43
Other cheese	52.6	57.2	55.5	53.4	0.96
IGF-I, ng/ml					
Skim milk	135	131	127	143	0.30
Yogurt	138	141	138	147	0.42
Cottage cheese	141	141	138	141	0.27
Sherbet	137	141	141	147	0.94
Whole milk	141	139	119	140	0.62
Cream	142	136	134	142	0.93
Sour cream	141	141	140	136	0.13
Cream cheese	142	136	134	142	0.72
Ice cream	143	140	133	139	0.49
Other cheese	139	138	137	142	0.74

Adjusted for age at blood (years, continuous), age at menarche (≤11, 12, 13, ≥14 years), BMI at blood draw (kg/m^2, continuous), age at first birth and parity (no children, ≤24 years and 1-4 children, 25-29 years and 1-4 children, ≥30 years and 1-4 children, ≤24 years and 5+ children, ≥25 years and 5+ children), age at menopause (≤45, 46-50, 51-55, >55 years), type of menopause (natural menopause, surgery with no or one ovaries removed, surgery with 2 ovaries removed), smoking status (never, former, current), alcohol intake (non-drinker, <1 drink/day, ≥1 drink/day), physical activity (MET-hours/week in quartiles, ≤3, 4-≤10, 11-≤22, >22), date and year of blood draw (≤6/1989, 7/1989/1989, 1/1990-6/1990, ≥7/1990), time of blood draw (7am-noon, 1-8pm, 9pm-6am), fasting at blood draw (yes, no), and laboratory batch.

CHAPTER 3

DEPRESSION, ANTIDEPRESSANT USE, AND BREAST CANCER RISK IN POSTMENOPAUSAL WOMEN IN THE WOMEN'S HEALTH INITIATIVE OBSERVATIONAL STUDY

Abstract

Depression is a serious chronic medical condition that can cause significant emotional and physical impairment, and antidepressant (AD) use has become an increasingly popular treatment option for most types of depression. The relationship between depressive symptoms, AD use, and breast cancer risk remains unclear; therefore, we conducted a prospective cohort study of these relationships among 72,191 postmenopausal women in the Women's Health Initiative Observational Study (WHI-OS). Multivariable-adjusted hazard ratios (HRs) were estimated for the independent and joint effects of depressive symptoms and AD use on breast cancer risk using Cox proportional hazards regression, and multiplicative interactions were evaluated using the likelihood ratio test. When analyzed separately, depressive symptoms (HR=1.09, 95% CI: 1.07-1.12) and AD use (HR=1.12, 95% CI: 1.09-1.16) at baseline were associated with a significant increased risk of total breast cancer; however, no association was observed with invasive breast cancer (HR=0.99, 95% CI: 0.74-1.31; HR=0.98, 95% CI: 0.69-1.38, respectively). Depressive symptoms and AD use at baseline were each associated with a non-significant increased risk of in situ breast cancer, which was attenuated after adjustment for mammography screening. We observed significant variation in the risk of total breast cancer among women with depressive symptoms only (HR=1.09, 95% CI: 1.07-1.12), women with AD use only (HR=1.13, 95% CI: 1.10-1.17), and women with both depressive symptoms and AD use (HR=1.12, 95% CI: 1.07-1.19; *P* for interaction=0.004). Overall, a small but significant association was observed between AD use and breast cancer risk. This association appeared due largely to a positive association with in situ breast cancer that was based, at least in part, on increased mammographic screening

among exposed women. Given the high prevalence of these exposures, the results of this study may provide some reassurance to the millions of women who are depressed and/or use ADs each year.

Introduction

Breast cancer is the most prevalent cancer and second leading cause of cancer mortality among women in the United States (1). Depression and antidepressant (AD) use also are common, with 9-11% of middle-aged women reporting depression (2) and 23% of US women reporting current AD use (3). Depression is a serious chronic medical condition hypothesized to increase breast cancer risk through inflammation and a suppressed immune response characterized by decreased cytotoxic T-cell, natural kill cell, and inflammatory cytokine activity (4-10). Compared to healthy individuals, women with depression have higher cortisol levels (9-13), and *in vitro* evidence suggests that cortisol may increase proliferation of mammary cancer cells and contribute to tumor growth (14-15).

ADs are often taken as part of a long-term treatment regimen for depression, mood disorders, anxiety spectrum conditions, and chronic pain, with approximately 78% of AD prescriptions indicated for treatment of depressive disorders (16-17). Over the last twenty years, the rate of AD use has quadrupled; ADs are now the top prescription drug used by adults aged 18 to 44 years, and selective serotonin reuptake inhibitors (SSRIs) are the most commonly used class of AD medication (3). While ADs have anti-inflammatory properties that may mitigate an effect of depression on breast cancer risk, concern has mounted that AD treatment, specifically SSRIs, may increase circulating prolactin levels and thereby increase breast cancer risk. Prolactin has been shown to stimulate cellular proliferation, differentiation, and angiogenesis (18-19), and prior prospective studies have linked prolactin with increased pre- and postmenopausal breast cancer risk (20-21).

Based on the epidemiologic evidence to date, the relationship between depression, AD use, and breast cancer is relatively unclear. In two prospective studies with 10 or more years of follow-up (n=2,107-3,177), women with depression had either a borderline significant or significant 2 to 4 times increased risk of breast cancer compared to healthy women (22-23), while other prospective studies with shorter follow-up have observed no association (24-26). Similarly, two prospective studies found that women using ADs, specifically SSRIs, had a significant 39-53% increased risk of breast cancer (27-28), whereas several case-control studies have reported no association (29-33).

While prior epidemiologic studies support a potential relationship between depression, AD use, and increased breast cancer risk, the data are inconsistent. Therefore, we examined the associations between depression, AD use, and breast cancer among postmenopausal women in the Women's Health Initiative Observational Study (WHI-OS). This study is the first to comprehensively evaluate the independent and joint effects of depression and AD use on breast cancer risk using a prospective design in a large cohort of women with up to 17 years of follow-up. Further, data in the WHI-OS were collected when the prevalence of both depression and AD use were increasing, and the large number of women in the WHI-OS with these exposures is a significant strength over prior studies.

Methods

Study population

The WHI-OS recruited a total of 93,676 women in 40 clinical centers throughout the United States from October 1, 1993 to December 31, 1998. Eligible women were between the ages of 50-79 years old, postmenopausal at enrollment, and intended to reside in the area for at least three years. Details about the WHI-OS study design, recruitment, and data collection methods have been previously published (34-35). Briefly, participants provided written informed

consent and completed baseline questionnaires regarding demographic and lifestyle factors, medical history, and current medication use. During follow-up, participants completed mailed questionnaires each year and attended clinic visits every three years. Human subjects review committees at each participating institution reviewed and approved the study, and the Institutional Review Board of the University of Massachusetts Amherst approved this analysis.

Exposure assessment

Depressive symptoms were assessed at baseline using the Burnam eight-item scale for depressive disorders (36), which consists of six items from the Center for Epidemiologic Studies Depression Scale (CES-D) regarding the frequency of depressive symptoms during the past week and two items from the National Institute of Mental Health's Diagnostic Interview Schedule (DIS) regarding symptoms over the past one to two years. Specifically, participants were asked to indicate how often they felt depressed, had restless sleep, enjoyed life, had crying spells, felt sad, or felt that people disliked them over the past week, scored from 0 "rarely or none of the time (<1 day)" to 3 "most or all of the time (5-7 days)." In addition, participants indicated if they experienced two or more weeks in the past year during which they felt sad, blue, depressed, or lost pleasure in things they usually cared about or enjoyed, or two or more years in their life during which they felt depressed or sad most days, including if they felt depressed or sad much of the time in the past year. These items were scored as 0 "no" or 1 "yes."

Using each participant's Burnam scale responses, we applied a logistic regression algorithm to calculate a score between 0 and 0.99, with higher scores indicative of greater depressive symptoms (36). Consistent with prior studies in the WHI (37-39), the continuous Burnam score was categorized into two groups using the standard cut point of 0.06 to separately classify women experiencing depressive symptoms consistent with disorders such as major depression and dysthymia from women without these symptoms. In a reliability study of WHI

participants, the Burnam scale was found to have 74% sensitivity and 87% specificity when compared to a clinical diagnosis of depression (40).

Participants were asked to bring all current medications to their baseline interview and year three clinic visit. For medications used regularly (i.e. for more than two weeks), clinic interviewers entered the medication names and dose directly from containers into a database that assigned drug codes using Medi-Span software (First DataBank Inc., San Bruno, CA). For our primary analysis, women were categorized as AD users or non-AD users at baseline. For secondary analyses, we additionally considered AD use by drug class (e.g., SSRIs, tricyclic antidepressants (TCAs)) and evaluated the impact of consistency of AD use (e.g., never AD use, AD use at baseline, AD use at year three, AD use at baseline and year three) on breast cancer risk.

Outcome assessment

Details of breast cancer outcomes, including adjudication procedures, in the WHI-OS have been described elsewhere (41). Briefly, participants self-reported incident breast cancer diagnoses on annual questionnaires. Breast cancer diagnoses were adjudicated by physicians who confirmed the self-reported outcomes using relevant medical records and pathology reports, and breast cancer outcomes were then centrally adjudicated by the WHI's Clinical Coordinating Center. Only adjudicated breast cancer cases were included in this analysis, and we will examine relationships by total, invasive, in situ, ER+, and ER- breast cancer subtypes.

Statistical analysis

Women were excluded from this analysis if they reported a history of cancer except for non-melanoma skin cancer at baseline (n=11,726), if they were missing information on the Burnam depression scale (n=2,546), or if they had missing data on one or more of the

confounders included in our multivariable model (n=7,213), resulting in a final sample of 72,191 women.

We categorized participants by depressive symptom status (<0.06, ≥0.06 Burnam score) and AD use (AD user, non-AD user) at baseline to examine the distribution of covariates using t-tests and chi square tests. To visually depict the breast cancer experience of study participants stratified by depressive symptom status and AD use, we used Kaplan-Meier curves and the log rank test to examine the distribution of survival times. Women in this analysis were followed from enrollment through September 2010; participants contributed person-time to the analysis until diagnosis of breast cancer, death, loss to follow-up, or the end of the study/administrative censor date defined as the participant's last available visit, whichever happened first.

We used multivariable Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals of the association between depressive symptoms, AD use, and time to breast cancer diagnosis with adjustment for confounders. We separately examined the relationship between depression, AD use, and the risk of total breast cancer, invasive breast cancer, in situ breast cancer, ER+ breast cancer, and ER- breast cancer. In the subgroup analyses by breast cancer outcome, women who developed other types of breast cancer were censored at the time of their diagnosis (i.e., for the ER+ subgroup analysis, ER- women were censored at the time of diagnosis) per the assumption that the risks of different types of breast cancer are independent. Further, we created a time-varying covariate to capture mammogram screening during follow-up, including only mammograms prior to diagnosis, to explore the impact of screening on the relationship between depressive symptoms, AD use, and in situ breast cancer.

We conducted a stratified analysis to evaluate whether the risk of total breast cancer, invasive breast cancer, or in situ breast cancer varied by strata of AD use (AD user, non-AD user) and depressive symptom status (depressive symptoms, no depressive symptoms). To test the overall statistical significance of the interaction, we included an interaction term in our multivariable model and utilized the likelihood ratio test to compare the full and reduced models.

In addition, dummy variables were used in our multivariable model to examine the association for each level of the interaction.

Finally, we evaluated the association between the drug classes used most commonly in this cohort (i.e., SSRIs, TCAs) and breast cancer risk. Given that some women used SSRIs and TCAs at the same time, we adjusted for TCA use in the SSRI analysis and vice versa, and we explored these relationships among women with and without depressive symptoms at baseline. We additionally examined the effect of consistency of AD use as well as the consistency of SSRI and TCA use and breast cancer risk by strata of depressive symptom status at baseline.

Potential confounders were identified using prior literature and subject area knowledge regarding variables associated with depression, AD use, and/or breast cancer; any covariate that that changed the estimate for the exposure by greater than 10% was retained in the final multivariable model. All models were adjusted for the following variables collected at baseline: age (years, continuous); BMI (kg/m^2, continuous); alcohol use (0, 0.1-<2, 2-<4, 4-<7, ≥7 servings/week), smoking status (never, past, current); physical activity (MET-hours/week, continuous); parity (nulliparous, 1 child, 2 children, ≥3 children); age at first birth (never had a term pregnancy, <20 years, 20-29 years, ≥30 years); breastfeeding (ever, never); oophorectomy (ever, never); PMH use (never, past, current); age at menopause (years, continuous); and race (American Indian or Alaskan Native, Asian or Pacific Islander, Black or African American, Hispanic/Latino, White not of Hispanic origin, Other). Further, to examine the independent relationships of depressive symptoms and AD use with breast cancer risk, we adjusted depression analyses for AD use and vice versa.

All analyses were performed in SAS version 9.2 software (SAS Institute, Cary, North Carolina). All statistical tests were two-sided; $p<0.05$ was used to define statistical significance.

Results

Characteristics of WHI-OS participants by depressive symptom status and AD use at baseline are presented in Table 1. At baseline, 8,021 women (11.1%) were experiencing depressive symptoms and 5,282 women (7.3%) were current AD users. Overall, women with depressive symptoms were younger at enrollment, slightly heavier, less physically active, and more likely to be a past or current smoker compared to women without depressive symptoms. Similarly, women using ADs at baseline were generally heavier and less physically active, more likely to be White, and more likely to be a past or current smoker as well as a past or current postmenopausal hormone (PMH) user compared to non-AD users.

During the study follow-up period, a total of 4,325 women were diagnosed with breast cancer. Results from the age-adjusted and multivariable-adjusted Cox proportional hazards regression models are presented in Table 2. After adjustment for confounders, but not AD use, women with depressive symptoms had a statistically significant 9% increased risk of total breast cancer (HR=1.09, 95% CI: 1.07-1.12), but we found no association between depressive symptoms and invasive breast cancer risk (HR=0.99, 95% CI: 0.74-1.31). Women with depressive symptoms at baseline had a non-significant 24% increase in risk of in situ breast cancer (HR=1.24, 95% CI: 0.53-2.90); however, this relationship became somewhat weaker after additional adjustment for mammogram screening during follow-up (HR=1.16, 95% CI: 0.49-2.71).

In multivariable models not adjusted for depression, current AD users had a significant 12% increased risk of total breast cancer (HR=1.12, 95% CI: 1.09-1.16), but we found no association between AD use and invasive breast cancer (HR=0.98, 95% CI: 0.69-1.38). Current AD users had a non-significant 80% increased risk of in situ breast cancer (HR=1.80, 95% CI: 0.85-3.83), which was also attenuated after adjustment for mammogram screening during follow-up (HR=1.48, 95% CI: 0.69-3.22).

No association was observed between depressive symptoms or current AD use at baseline and ER+ breast cancer risk; however, women with depressive symptoms (HR=1.39, 95% CI: 0.73-2.63) and current AD users (HR=1.61, 95% CI: 0.89-2.93) had a non-significant increase in ER- breast cancer risk.

We investigated the relationship between depressive symptoms and total breast cancer risk independent of AD use and observed no meaningful change in the estimate (HR=1.08, 95% CI: 1.05-1.10). In addition, we observed similar results when adjusting for depression in the multivariable model for current AD use at baseline and total breast cancer risk (HR=1.11, 95% CI: 1.07-1.14). While additional adjustment for AD use in the depression and breast cancer models and vice versa did not materially influence estimates for invasive breast cancer, ER+ breast cancer, or ER- breast cancer, the relationship between depressive symptoms and in situ breast cancer was attenuated after adjustment for AD use (HR=1.11, 95% CI: 0.47-2.64).

We observed significant variation in the risk of total breast cancer when the association was examined by the joint distribution of depressive symptoms and AD use at baseline (*P* for interaction=0.004, Table 3). Specifically, compared to non-AD users without depressive symptoms, a significant increase in total breast cancer risk was observed among women experiencing depressive symptoms without AD use (HR=1.09, 95% CI: 1.07-1.12), women using ADs without depressive symptoms (HR=1.13, 95% CI: 1.10-1.17), and women using ADs with depressive symptoms (HR=1.12, 95% CI: 1.07-1.19). The interaction between strata of current AD use and depressive symptoms at baseline was not significant for invasive breast cancer (*P* for interaction=0.11) or in situ breast cancer (*P* for interaction=0.78).

Next, we evaluated if the consistency of AD use varied by strata of depressive symptom status at baseline (Table 4). No significant relationships emerged related to consistency of AD use and total breast cancer at any time points other than baseline. Among current AD users at baseline, the majority of women reported using SSRIs (n=3,589, 68%) and/or TCAs (n=3,122, 59%). No significant associations emerged between consistency of SSRI use and total breast

cancer risk in women with depressive symptoms; however, women without depressive symptoms who were using TCAs or SSRIs at baseline had a 13-25% significant increase in breast cancer risk (Table 4).

Discussion

In this prospective cohort study of postmenopausal women, we observed a significant, though small, increase in total breast cancer risk among women experiencing depressive symptoms or using AD medication at baseline; however, no association was observed between AD use or depressive symptoms and invasive breast cancer. A non-significant positive relationship was observed with in situ breast cancer, which was attenuated after adjustment for mammogram screening during follow-up. When the exposures were evaluated together, women using ADs had a similarly elevated risk of total breast cancer whether or not they were concurrently experiencing depressive symptoms. This suggests that ADs confer a small but significant increase in risk while depressive symptoms appear to play less of a role in breast carcinogenesis. To our knowledge, this is the first study to examine the combined relationship between depressive symptoms and AD use and subsequent breast cancer risk, which is important given the overwhelming overlap in these exposures among US women.

Prior epidemiologic studies support a potential relationship between depression and breast cancer risk, but the data are inconsistent. The direct comparison of results from prior studies is complicated by the multitude of exposures ranging from depression, personality traits, psychosis, and dysthymia, as few studies have focused specifically on the association between depressive symptoms and breast cancer risk. In a meta-analysis of the available prospective data on depression and breast cancer risk, depressed women had a non-significant 59% increased risk of breast cancer (RR=1.59, 95% CI 0.74-3.44) compared to women without depression (42). However, results of the individual studies included in this meta-analysis varied widely, as few studies included information on important potential confounders, and follow-up time was limited

which is problematic as the impact of depression on breast cancer risk likely occurs over a period of several years. In the prospective studies with at least 10 years of follow-up, a 3-4 times increased breast cancer risk was observed among women with depression. Given that these studies did not account for AD use however, it is possible that the positive association observed between depression and breast cancer risk is due to AD use for treatment of depressive symptoms rather than depression itself.

The majority of the epidemiologic evidence on AD use and breast cancer risk is based on case-control studies, many of which have utilized pharmacy or healthcare databases (30-33, 43). These studies are limited as prescription databases contain sparse information on potential confounders, and participants may incorrectly recall prior AD use, which may contribute to the null findings observed. In the prospective New York University Women's Health Study, the use of any type of psychotropic medication at baseline was associated with a significant 39% increase in breast cancer risk (27), and over four years of SSRI use was associated with a significant 53% increase in breast cancer risk in another study using nationwide record linkage data from Finland (28). Taken together, this evidence from prospective studies further supports the potential role of ADs in breast carcinogenesis.

Despite the significant positive relationships observed between AD use and total breast cancer and depressive symptoms and total breast cancer, no associations emerged with regard to invasive breast cancer. The non-significant positive relationship between depressive symptoms and in situ breast cancer was attenuated after adjustment for AD use, which suggests that AD use contributed to the observed relationship between depression and in situ breast cancer. In contrast, while the association between AD use and in situ breast cancer was attenuated after adjustment for mammography screening, adjustment for depressive symptoms did not alter the effect estimate. Compared to healthy individuals, women with depressive symptoms and/or AD use maintain regular contact with their health care providers to fulfill treatment and prescription needs, and these women may be more likely to be referred for regular screening mammograms based on their

greater health care utilization (44). Further, women receiving more frequent screening may be more likely to receive an in situ breast cancer diagnosis compared to women with less overall contact with the health care system (45). Given the attenuation we observed after adjustment for mammography screening, the relationships observed with in situ breast cancer are likely attributable, at least in part, to screening artifacts rather than true etiologic associations. Future research is warranted to confirm these results as prior prospective studies have not examined the relationships between these exposures and breast cancer subtypes, nor have previous studies considered the potential impact of screening mammography specifically with regard to the relationships between depressive symptoms, AD use, and in situ breast cancer risk (23, 26-28).

The majority of AD users in this cohort reported SSRI and/or TCA use, and a significant increase in breast cancer risk was observed in women without depressive symptoms using SSRIs or TCAs at baseline. Prior studies support a significant increase in breast cancer risk associated with short-term AD use. In one meta-analysis, a significant increase in breast cancer risk was observed among women who used SSRIs for up to two years (OR=1.10, 95% CI: 1.02-1.19) that was attenuated with longer use (OR=1.04, 95% CI: 0.89-1.22) (46). In addition, *in vitro* evidence suggests that AD medications may promote biphasic cancer cell growth in rodents which has been correlated with early and late peaks in cancer incidence among AD users in epidemiologic studies (47).

The overall safety profile of SSRI and TCA medications in humans remains unclear. In an animal study, a statistically significant difference in breast tumor development was observed between rats exposed to clinically relevant doses of SSRI or TCA medication compared to rats receiving saline treatment, which supports the potential role of these medications in tumor development and growth (48). However, a later study conducted by the Food and Drug Administration found that SSRIs and TCAs did not significantly stimulate cellular proliferation, DNA synthesis, or colony formation in human breast adenocarcinoma cells (49). The epidemiologic evidence regarding the safety of SSRIs and TCAs is also inconsistent: one large

prospective study observed a 53% increased risk of breast cancer in SSRI users after four or more years of follow-up (28), while a meta-analysis including mainly retrospective studies found that neither TCA nor SSRI use were associated with an increased breast cancer risk (pooled OR=1.02, 95% CI: 0.96-1.08 (46). Given the conflicting evidence, additional research is needed to more fully understand the impact of SSRI and TCA medications in humans and how these drug classes may impact breast cancer risk.

Strengths of our study include the prospective design, large sample size, comprehensive data on potential confounders, and up to 17 years of follow-up. In addition, data for this study were collected when the prevalence of both depression and AD use were rising, and the large number of exposed women in this study is a significant strength over prior studies. We were limited by the small number of in situ breast cancer cases in this study and thus were constrained in our ability to investigate relationships between depressive symptoms, AD use, and in situ breast cancer risk. Further limitations of this study primarily relate to the measurement of depressive symptoms and AD use. Data on depressive symptoms were gathered via self-report instead of the gold standard structured psychiatric interview or clinical diagnosis of depression, and information on the duration of depressive symptoms and prior history of depression was not available. In addition, given that we only had data on depressive symptoms, not on clinical diagnoses of depression, we may have captured a less severe spectrum of depression in this study, which may have contributed to attenuation of our estimates. With regard to AD use, actual use may be underestimated if women failed to bring their pill bottles to visits, and we additionally lacked information on AD indication and compliance. If nondifferential misclassification of AD use and/or depressive symptoms occurred, it would attenuate our estimates. While depression is a heterogeneous condition, the Burnam scale captures data on a variety of short- and long-term symptoms; therefore, it is unlikely that participants would be incorrectly classified. In addition, the Burnam scale has been shown to have good to excellent sensitivity and specificity with a threshold score that is well correlated with clinical depression (Burnam 1988). Further, data on

AD use were collected in an objective manner, and while the validity of data collected using this method has not been assessed, we believe this is a reasonable approach to accurately capture this information.

In summary, AD use confers a significant, though small, effect on breast cancer risk regardless of depressive symptom status. This association appeared due largely to a positive association with in situ breast cancer that was based, at least in part, on increased mammographic screening among exposed women. No associations were observed between depressive symptoms or AD use and invasive breast cancer in this large prospective cohort of postmenopausal women. The findings from this study contribute considerably to a better understanding of the safety profile of ADs in women. Given the high prevalence of these exposures, the results of this study may provide some reassurance to the millions of women who are depressed and/or use ADs each year.

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Table 3.1. Distribution of baseline characteristics by depressive symptoms and AD use in the Women's Health Initiative Observational Study

Characteristic	Depressive symptoms	No depressive symptoms	Current AD user	Non-AD user
Number of participants	**8,021 (11.1)**	**64,100 (88.9)**	**5,282 (7.3)**	**66,839 (92.7)**
Mean age at enrollment (years, SD)	61.8 (7.4)	63.5 (7.3)	62.1 (7.3)	63.4 (7.3)
Mean age at menopause (years, SD)	47.2 (6.8)	48.4 (6.2)	46.9 (6.7)	48.4 (6.3)
Mean body mass index (kg/m^2, SD)	28.4 (6.4)	27.0 (5.5)	28.5 (6.2)	27.1 (5.6)
Race, White (%)	6,325 (78.9)	54,393 (84.9)	4,719 (89.3)	55,999 (83.8)
Mean parity (among parous women only, SD)	2.5 (0.7)	2.5 (0.7)	2.5 (0.7)	2.5 (0.7)
Age at first birth (%)				
<20 years	1,388 (17.3)	6,837 (10.7)	823 (15.6)	7,402 (11.1)
20-29 years	4,312 (53.8)	9,038 (60.9)	3,307 (57.5)	40,313 (60.3)
≥30 years	543 (6.8)	5,001 (7.8)	341 (6.5)	5,203 (7.8)
History of breastfeeding, ever (%)	3,516 (43.8)	29,627 (46.2)	2,418 (45.8)	30,725 (46.0)
Mean total energy expenditure (MET h/week, SD)	10.8 (13.0)	14.2 (14.5)	11.2 (13.2)	14.0 (14.4)
Smoking status, past or current (%)	4,232 (52.8)	31,122 (48.6)	2,891 (54.7)	32,463 (48.6)
Alcohol user, past or current (%)	7,103 (88.6)	56,921 (88.8)	4,749 (89.9)	59,275 (88.7)
PMH use, past or current (%)	6,007 (74.9)	46,230 (72.1)	4,506 (85.3)	47,731 (71.4)

Table 3.2. Hazard ratios (HR) and 95% CIs for depressive symptoms and AD use at baseline and the risk of breast cancer in the WHI-OS

			Age-adjusted	Multivariable model 1[a]	Multivariable model 2[b]
				HR (95% CI)	
	Depressive symptoms	**No depressive symptoms**			
Person years	82,012	693,251			
	No. of breast cancer cases				
Total breast cancer	428	3,897	1.14 (1.12-1.17)	1.09 (1.07-1.12)	1.08 (1.05-1.10)
Invasive breast cancer	357	3,162	1.17 (0.88-1.54)	0.99 (0.74-1.31)	0.99 (0.74-1.32)
In situ breast cancer	70	729	1.31 (0.59-2.88)	1.24 (0.53-2.90)	1.11 (0.47-2.64)
ER+ breast cancer	293	2,712	1.18 (0.84-1.66)	1.06 (0.75-1.49)	1.05 (0.73-1.50)
ER- breast cancer	68	518	1.17 (0.64-2.14)	1.39 (0.73-2.63)	1.28 (0.66-2.47)
	Current AD user	**Non-AD user**			
Person years	54,899	720,363			
	No. of breast cancer cases				
Total breast cancer	324	4,001	1.14 (1.11-1.17)	1.12 (1.09-1.16)	1.11 (1.07-1.14)
Invasive breast cancer	253	3,266	1.12 (0.80-1.57)	0.98 (0.69-1.38)	0.98 (0.69-1.39)
In situ breast cancer	71	728	1.81 (0.89-3.70)	1.80 (0.85-3.83)	1.78 (0.83-3.82)
ER+ breast cancer	216	2,789	1.09 (0.72-1.65)	0.95 (0.62-1.44)	0.93 (0.61-1.44)
ER- breast cancer	58	528	1.51 (0.86-2.65)	1.61 (0.89-2.93)	1.52 (0.83-2.81)

[a]Multivariable models for depression and antidepressant use adjusted for: age (years, continuous); smoking status (never, past, current); alcohol use (0, 0.1-<2, 2-<4, 4-<7, ≥7 servings/week), parity (nulliparous, 1 child, 2 children, ≥3 children); age at first birth (never had a term pregnancy, <20 years, 20-29 years, ≥30 years); breastfeeding (ever, never); oophorectomy (ever, never); PMH use (never, past, current); age at menopause (years, continuous); race (American Indian or Alaskan Native, Asian or Pacific Islander, Black or African American, Hispanic/Latino, White not of Hispanic origin, Other); physical activity (MET-hours/week); BMI (kg/m^2, continuous).
[b]Multivariable model for depression adjusted for covariates in model 1 and AD use; model for AD use adjusted for covariates in model 1 and depression.

Table 3.3. Hazard ratios (HR) and 95% CIs for the joint distribution of depressive symptoms and AD use at baseline and the risk of total breast cancer in the WHI-OS (1993-2010)

Group	No. of breast cancer cases	Multivariable model[a,b] HR (95% CI)
Non-AD user, no depressive symptoms	3,629	1.00 (ref)
Non-AD user, depressive symptoms	356	1.09 (1.07-1.12)
AD user, no depressive symptoms	256	1.13 (1.10-1.17)
AD user, depressive symptoms	84	1.12 (1.07-1.19)

[a]Multivariable model adjusted for: age; smoking status; alcohol use; parity; age at first birth; breastfeeding; oophorectomy; PMH use; age at menopause; race; physical activity; and BMI.
[b]*P* for interaction=0.004.

Table 3.4. Hazard ratios (HR) and 95% CIs for consistency of AD use overall and by drug class by strata of depressive symptoms and the risk of total breast cancer in the WHI-OS (1993-2010)

Group	Multivariable model[a] HR (95% CI)	
	Depressive symptoms	**No depressive symptoms**
Overall AD use		
Never use	1.00 (ref)	1.00 (ref)
Baseline only	1.12 (1.02-1.24)	1.15 (1.04-1.26)
Year 3 only	0.97 (0.89-1.04)	1.04 (0.93-1.15)
Consistent use	0.95 (0.83-1.07)	0.96 (0.82-1.09)
SSRIs		
Never use	1.00 (ref)	1.00 (ref)
Baseline only	1.02 (0.93-1.13)	1.25 (1.17-1.36)
Year 3 only	0.94 (0.86-1.04)	1.04 (0.98-1.10)
Consistent use	1.01 (0.85-1.20)	0.98 (0.83-1.11)
TCAs		
Never use	1.00 (ref)	1.00 (ref)
Baseline only	1.12 (0.97-1.27)	1.13 (1.06-1.22)
Year 3 only	0.98 (0.83-1.16)	1.04 (0.96-1.12)
Consistent use	0.89 (0.71-1.16)	0.87 (0.77-1.01)

[a]Multivariable model adjusted for: age; smoking status; alcohol use; parity; age at first birth; breastfeeding; oophorectomy; PMH use; age at menopause; race; physical activity; and BMI.

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