Case-Control Study of Increased Mammographic Breast Density Response to Hormone Replacement Therapy

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Abstract

Previous studies have demonstrated an association between current hormone replacement therapy (HRT) use and increased mammographic breast density. Many of these studies have also shown that only 20–35% of women initiating HRT respond in this manner. This subgroup of HRT responders may be at an increased risk of breast cancer. We performed a case-control study to investigate how women who experience increased density in response to HRT (cases) differ from women who do not experience an increase in density with HRT use (controls) with regard to breast cancer risk factors, type of HRT, weight change, and baseline breast density. Participants were female residents of Olmsted County, Minnesota who received routine screening mammograms at the Mayo Clinic. Cases included 172 women identified between the years 1998 and 1999 by Mayo radiologists as having a HRT response. Controls were women who did not experience an increase in mammographic density with HRT use and were matched to cases on age (±3 years), menopausal status, duration of HRT, month of initiation of HRT, and months between baseline and follow-up mammograms. Mammograms were obtained from cases and controls before and during HRT therapy. Breast density was read as a four-category Bi-Rads density grade measure and as a quantitative percentage estimate, using a computer-assisted method. Risk factor information was obtained from both chart review and a mammography database of patient-provided information. There was no association between HRT response and first-degree family history of breast cancer [odds ratio (OR), 0.8; 95% confidence interval (CI), 0.4–1.5], parity (OR, 0.8; 95% CI, 0.4–1.7), later age at first birth (OR, 0.8 for age >25 years *versus* nulliparous women; 95% CI, 0.4–1.8), or history of biopsy (OR, 0.9; 95% CI, 0.6–1.5). There was also no association with baseline weight or change in weight between a woman's baseline and follow-up mammograms. However, there was evidence of an association between HRT response and type of HRT used; women who experienced a mammographic increase in density with HRT had 2.3 greater odds (95% CI, 1.4–3.7) of having taken estrogen-progestin combined therapy than estrogen alone, compared with controls. This association was stronger among women with a baseline weight below the median (OR, 5.2; 95% CI, 1.6–17.6). Also, there was an inverse association between HRT response and baseline density. Because all risk factors examined accounted for only 26% of the variation in the HRT response, genes or other unmeasured factors are thought to be involved.

Introduction

Given the hormonal etiology of breast cancer, there is considerable clinical interest in HRT[2] and its possible relationship to the risk of the disease. Numerous studies and several meta-analyses have been performed (1–4) suggesting that the relative risk (RR) associated with use of HRT is likely to be modest. However, because HRT is so widely prescribed, even small increases in risk could translate to a significant population attributable risk.

Differences in the relative amounts of fat, connective and epithelial tissues in the breast lead to variations in mammographic appearance. Fat, which is radiolucent, appears dark on mammograms, and connective and epithelial tissues, which are radiodense, appear light. Breasts with greater amounts of connective and epithelial tissue appear more dense than those with greater amounts of fat and thus have a greater proportion of mammographic density. Percentage of mammographic breast density, or the proportion of nonfatty tissue on the mammographic image, is associated with a 3–5-fold risk of breast cancer for categories of increased *versus* no density (5–7). This association holds true for both categorical and quantitative estimates of breast density (5, 7).

Mammographic breast density has been shown to increase with initiation of HRT (8–12). This association appears to be dynamic because levels of breast density decrease with discontinuation of HRT use (13). There is evidence that the increases are greater with combination therapy *versus* estrogen alone (8, 10, 11, 14). However, there is heterogeneity in a woman's breast tissue response to the use of these exogenous hormones. Studies suggest that only 20–35% of women experience increases in breast density upon initiation and continuation of HRT (8, 10, 11, 14).

Taken together with the evidence that breast density is a risk factor for breast cancer, these observations suggest the

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[2] The abbreviations used are: HRT, hormone replacement therapy; OR, odds ratio; CI, confidence interval; BMI, body mass index; RR, relative risk; GRHA, gonadotropin-releasing hormone agonist.

hypothesis that the subset of women who experience increases in mammographic breast density when exposed to HRT may be at increased risk of breast cancer, compared with those on HRT who do not experience an increase in density. No studies to date have examined whether a woman's mammographic response to HRT is associated with breast cancer risk. As a first test of this hypothesis, we performed a case-control study to characterize the differences between women who experience an increase in breast density in the presence of HRT and those who do not experience increases. Specifically, we explored whether differences in family history of breast cancer, parity, age at first birth, history of biopsy, baseline weight, weight change, baseline density, and type of prescribed HRT are associated with an increased breast density in response to HRT. Characterization of this mammographic response to HRT is important because a woman's response to HRT may provide a visual tool to identify women for whom use would be contraindicated.

Materials and Methods

Study Population. Participants were identified from female residents of Olmsted County, Minnesota who received routine screening mammograms at the Mayo Mammography Clinic. A database of all women who have received mammograms since 1986 at the Mayo Clinic is maintained for clinical and research purposes. This database includes the clinic number of the patient, the type of mammogram performed (screening/diagnostic), date, findings on the mammogram, and research authorization status. Also included for each mammogram visit is patient-reported information on first-and second-degree family history of breast cancer, menopausal status at time of mammogram, parity, age at first birth, total years on HRT, and history of breast biopsy. Only women who had previously signed an authorization to review their medical records were eligible (98% of all women). This study was approved by the Mayo Clinic Institutional Review Board.

From this database, we selected all residents of Olmsted County who received a screening mammogram at the Mayo Clinic between the years of 1998 and 1999 and reported 4 or fewer years on HRT at the time of their screening mammogram ($n = 17,285$). We subset this group of women to those who had screening mammograms available before initiation of HRT ($n = 3,010$). From this group, we identified all women who had been diagnosed by a radiologist as having a HRT response, or an increase in mammographic breast density corresponding to the initiation of HRT since the time of their last screening mammogram. In making this diagnosis, the radiologist compares the percentage of breast density in the mammogram under review (in our case, 1998–1999) with the previous screening mammogram. If there is an increase, the radiologist consults a card completed by the technician with the patient's self-reported duration of HRT. If the patient reports starting HRT sometime between the two mammograms, the radiologist classifies this increase in breast density as a HRT response. Type (patch, pill, cream) and formulation (estrogen alone, estrogen and progestins combined, and so forth) of HRT are not available to the radiologist at time of classification.

Two hundred and ten women of the 3010 eligible (7%) were defined as cases. Only eight of these cases (4%) were excluded due to missing information on menopausal status. The potential pool of controls, or women who were not diagnosed as experiencing an increase in mammographic density with HRT use, consisted of 2766 women (menopausal status was unavailable for 34 women). Of the total 3010 women eligible, the average duration of HRT use was 2.1 years (SD = 1.1 years), and the average interval between mammograms before and during HRT use was 2.9 years (SD = 1.6 years). For potential cases ($n = 210$), the average number of years on HRT was smaller (average = 1.5 years, SD = 0.81 year) than the average number of years for the pool of controls (average = 2.2 years, SD = 1.1 years); consequently, the average interval between mammograms before and during HRT use was also slightly smaller for cases (average = 2.1 years, SD = 1.1 years) than controls (average = 3.0 years, SD = 1.6 years).

One control was matched to each case on age at screening mammogram during the 1998–1999 period (±3 years), duration of HRT use (±0.5 year), date of screening exam in 1998–1999 (±90 days), menopausal status at the screening exam during the period 1998–1999 and the screening exam before HRT use, and the time interval between the two mammograms. Of the 202 potential case and control pairs with known menopausal status, mammograms were not available on 5 cases and 6 controls (5.9%); 3 of the selected cases and 2 selected controls had implants in both breasts (2.5%), and 1 case had a history of breast cancer (0.5%). Additionally, because of the strict matching criteria, a suitable control could not be identified for 13 cases (6.4%). Thus, the final sample set included 172 of the eligible 210 (82%) potential case-control sets.

Estimation of Breast Density. Mammograms were obtained on all cases and controls for periods before and during their use of HRT. The right cranio-caudal view was digitized on a Lumisus model 85 digitizer; the left craniocaudal view was digitized if the right was unavailable. Percentage of breast density was estimated from the mammographic image using two methods: (*a*) a four-category Bi-Rads density grade estimate (15) read by a trained radiologist (K. R. B.); and (*b*) a quantitative measure estimated with a computer-assisted thresholding algorithm [Cumulus software (16, 17)] by a trained programmer. The Bi-Rads density estimate is similar to the four-category classification originally proposed by Wolfe (18) and comprises levels of mostly fatty (level 1) to mostly dense tissue (level 4). The quantitative estimate of density is calculated by setting two thresholds that segment the breast area from the background and the dense from nondense regions within the breast; the total number of pixels representing the dense area is divided by the total number of pixels comprising the entire breast area to arrive at a percentage of breast density estimate. The Cumulus program used for the quantitative estimation has been described elsewhere in detail (16, 17). Both methods have been used reliably to estimate density, although the quantitative percentage of density estimate is more strongly associated with breast cancer risk (5, 7).

Intrareader reliability was high for both methods in the current study (κ for Bi-Rads density estimation on 150 repeat mammograms = 0.75; R^2 for quantitative estimation on 150 repeat mammograms = 0.95). For this study, the readers were blinded to case-control status and risk factor information.

Risk Factor Information. Breast cancer risk factor information was obtained from the mammography database as described previously and from data abstracted from medical records. Family history of breast cancer in a first- or second-degree relative, nulliparity, age at first birth, history of breast biopsy, and years on HRT were all self-reported by the women at time of their mammogram and entered into the mammography database. Confirmation of duration of HRT; the prescribed types of HRT (estrogen, estrogen and progestin, progestin alone); date of hysterectomy, bilateral oophorectomy or unilateral oophorectomy; and weight (kg) and height (m) measures corresponding to dates of mammograms before and during

HRT use were abstracted from the women's medical charts. The sample of women in the study received their usual care at the Mayo Clinic, thus the HRT information available in the medical records was expected to be complete.

Statistical Analyses. Descriptive analyses were performed to evaluate the success of the matching criteria for cases and controls. Univariate associations of case and control status with family history of breast cancer, parity, age at first birth, history of breast biopsy, baseline weight, weight change, history of hysterectomy or oophorectomy, type of prescribed HRT, and baseline density were performed using conditional logistic regression. A generalized R^2 statistic (19) was computed for each univariate analysis to determine how much variability in the HRT response (case/control status) could be attributed to that factor. We evaluated family history as first- and/or second-degree relatives with breast cancer. Parity and age at first birth were both categorized for analyses. History of biopsy was analyzed as an ever/never variable. History of hysterectomy and/or oophorectomy was classified into three categories: no surgery or hysterectomy only; hysterectomy with unilateral oophorectomy, or unilateral oophorectomy alone; and bilateral oophorectomy alone or a hysterectomy with bilateral oophorectomy. Only surgeries performed before the initiation of HRT were considered. Type of prescribed HRT included estrogen alone, estrogen and progestin combined, or progestin alone. No data were collected on hormone dosage. Breast density was analyzed using both the four-category Bi-Rads classification of breast density (1–4) and the quantitative thresholding estimate of breast density categorized into quartiles based on the distribution of breast density in controls. Change in breast density was calculated as an absolute change in the Bi-Rads estimate or percentage of breast density estimate between the mammograms before and during HRT use. Quartiles of baseline weight and weight change were also created from the control distribution, and an additional category was added for women with missing weight values.

The associations of breast cancer risk factors and case-control status were also examined with adjustment for baseline breast density because we hypothesized that baseline breast density may be a potential confounder. If a woman had a high level of breast density before using HRT, then the absolute amount of density that she could increase would likely be smaller than that for a woman at a low level of density. The adjustment, then, attempts to remove variability related to the initial baseline density of cases and controls. Consequently, the ORs adjusted for baseline density describe the association between breast cancer risk factors and change in breast density (with HRT use) accounting for the level of a woman's breast density before HRT use. We next examined the association of case and control status with all variables that were statistically significantly associated with HRT response. Additionally, we compared the estimation of breast density change with use of HRT, using the Bi-Rads classification *versus* the thresholding technique. For the analyses involving type of HRT, we included history of hysterectomy and/or oophorectomy in the models.

All statistical tests were two-sided. Analyses were carried out using the SAS (SAS Institute, Inc., Cary, NC) software system.

Results

Initial classification of women as cases and controls was based on a medical diagnosis indicating HRT response. We assessed the validity of this classification using both the qualitative and quantitative estimates of breast density change before and during HRT use. Fig. 1*A* depicts the Bi-Rads density category, and Fig. 1*B* shows the quantitative percentage of breast density for the cases and controls before initiation of HRT and during HRT use. Cases had a 0.6 (SD = 0.6) average increase in the Bi-Rads classification compared with a 0.1 (SD = 0.7) increase in controls and a 13.7% (SD = 11.9%) increase in the quantitative classification of breast density, compared with 2.8% (SD = 10.3%) in controls.

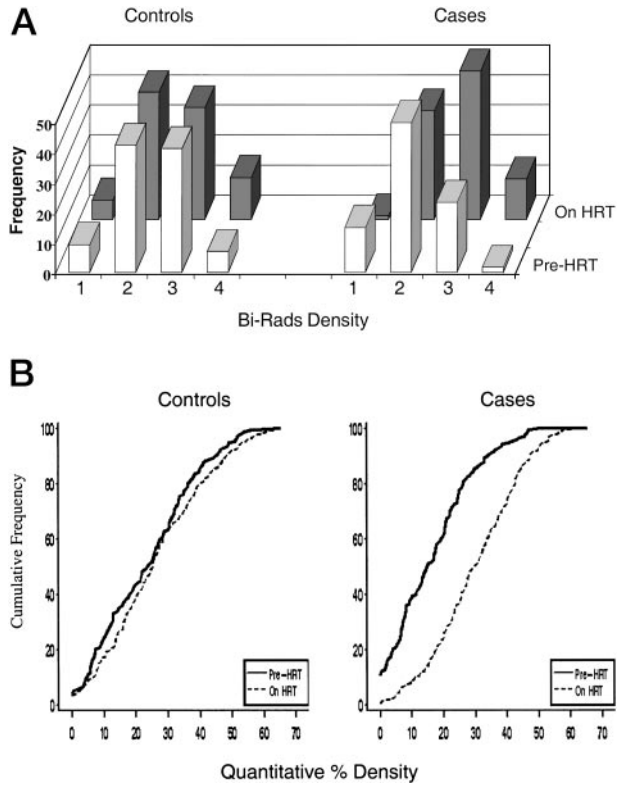

Fig. 1. *A*, change in breast density with HRT use: qualitative Bi-Rads breast density estimate. *B*, cumulative distribution function plots of change in breast density with HRT use: quantitative estimate using Cumulus software.

Cases and controls were tightly matched. Both cases and controls were, on average, 66.4 years of age. The time interval between mammograms at baseline and during HRT use was 2 years (SD = 1.0 year) for both groups [range for cases and controls respectively, 0.9–6.1 and 0.9–5.7 year(s)]. The average number of years on HRT was 1.5 years (SD = 0.8 year) for both cases and controls, and the average number of screening mammograms [7.9 (SD = 3.2) for cases and 7.3 (SD = 2.9) for controls] was also similar for cases and controls.

As shown in Table 1, there was no association between HRT response and first- and/or second-degree family history of breast cancer, parity, later age at first birth, or history of breast biopsy. All ORs were close to the null value of 1.0, and all CIs included the null. There was also no association with baseline weight or change in weight between a woman's baseline and follow-up mammograms. Adjusting for baseline level of breast density did not materially change these results.

We also examined whether or not a woman's breast density before initiation of HRT was associated with mammographic response As hypothesized, there was an association with baseline level of density and HRT response (Table 2). Women who had a lower baseline breast density, whether

Table 1 Association of breast cancer risk factors and weight change with HRT response

Variable name	Cases[a]	Controls[a]	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[b]
Family history of female breast cancer				
Mother or sister				
No	149	145	1.00[c]	1.00[c]
Yes	23	27	0.83 (0.45–1.52)	0.88 (0.46–1.67)
Mother, sister, daughter, aunt, grandmother				
No	122	122	1.00[c]	1.00[c]
Yes	50	50	1.00 (0.62–1.62)	0.87 (0.52–1.47)
Nulliparous				
Yes	17	14	1.00[c]	1.00[c]
No	155	158	0.80 (0.37–1.71)	0.71 (0.31–1.62)
Age at first birth (yrs)				
Nulliparous	17	14	1.00[c]	1.00[c]
≤20	29	25	1.02 (0.42–2.50)	0.69 (0.25–1.85)
21–25	80	83	0.85 (0.39–1.86)	0.69 (0.29–1.63)
>25	46	49	0.84 (0.38–1.84)	0.82 (0.35–1.94)
Breast biopsy				
Never	136	134	1.00[c]	1.00[c]
Ever	36	38	0.94 (0.56–1.55)	1.10 (0.65–1.93)
Baseline weight (kg)				
<62.0	38	36	1.00[c]	1.00[c]
62.0–69.8	31	38	0.77 (0.41–1.48)	0.70 (0.35–1.40)
69.9–80.3	33	36	0.92 (0.49–1.74)	0.77 (0.39–1.54)
>80.3	33	36	0.90 (0.47–1.73)	0.63 (0.31–1.28)
Missing	37	26	1.43 (0.71–2.88)	0.98 (0.46–2.11)
Weight change (kg)				
<−1.54	29	31	1.00[c]	1.00[c]
−1.54–0.60	38	30	1.28 (0.65–2.54)	1.44 (0.69–2.97)
0.61–2.72	29	34	0.84 (0.40–1.75)	1.06 (0.47–2.38)
≥2.73	19	28	0.74 (0.35–1.59)	0.81 (0.36–1.82)
Missing	57	49	1.18 (0.64–2.19)	1.32 (0.68–2.54)

[a] Data presented here as unmatched for descriptive purposes, but analyses were performed on matched pairs.
[b] Adjusted for baseline breast density.
[c] Reference group.

estimated qualitatively or quantitatively, had greater odds of experiencing increases in density with HRT use compared with women with a high baseline breast density.

The next set of analyses explored the association of type of HRT with HRT response. Of the 172 cases with an increased breast density response to HRT, 29.1% had been on estrogen alone, 64.5% had been on combined therapy, 2.9% had been on progestins alone, and type of therapy for 3.5% were missing. Unadjusted for other risk factors, women who experienced a mammographic increase in density with HRT had 2.3 greater odds of having taken estrogen-progestin combined therapy and 1.6 greater odds of having taken progestin than estrogen alone, compared with controls (Table 2). These results attenuated slightly with adjustment for baseline density. With further adjustment for hysterectomy/oophorectomy status, the association of HRT effect with combination therapy remained elevated (OR, 1.9), but the association with progestins was eliminated (OR, 1.0). Note that only 10 women (5 cases and 5 controls) received progestins alone.

The proportion of variance (R^2) in case and control status explained by the type of HRT was 7%; the proportion explained by baseline density (whether qualitative or quantitatively estimated) was 12%. The other variables accounted for <7% of the overall variance (data not shown).

Some studies suggest that HRT may be associated with a greater risk of breast cancer in lean women (1, 4). Therefore, a secondary analysis was performed to investigate whether the association between type of HRT and mammographic response differed according to weight at time of baseline mammogram. Stratifying by median weight among the controls (69.9 kg), we observed a stronger association between type of HRT and mammographic response among the leaner women (OR, 5.3; 95% CI, 1.6–17.6 for estrogen and progestins combined *versus* estrogen alone) compared with women in the upper median of weight (OR, 1.6; 95% CI, 0.5–5.0 for estrogen and progestins combined *versus* estrogen alone). Models were adjusted for quantitative baseline breast density and hysterectomy/oophorectomy status. Only 101 matched sets were used for these secondary analyses, due to missing data on weight.

Because of the qualitative nature of the Bi-Rads estimate and the wide range of densities included in each category, we were concerned about the sensitivity to capture small changes in density during HRT use. Therefore, analyses were performed to directly compare the two methods used to estimate change in mammographic breast density. The changes in breast density between mammograms before and during HRT measured by the qualitative Bi-Rads and quantitative thresholding techniques were highly correlated (Table 3), but the quantitative estimate offered greater precision.

Discussion

There is heterogeneity in a woman's breast tissue response to the presence of exogenous hormones (8, 10, 11, 14, 20). Our study explored factors that may be associated with this heterogeneity in mammographic response. None of the traditional breast cancer risk factors examined was significantly associated with whether a woman was identified as having experienced a

Table 2 Association of baseline density and type of HRT with HRT response

Variable name	Cases[a] (*n*)	Controls[a] (*n*)	Unadjusted OR (95% CI)	Adjusted OR (for baseline density)[b] (95% CI)	Adjusted OR[c] (95% CI)
HRT					
Estrogen alone	50	79	1.00[d]	1.00[d]	1.00[d]
Estrogen and progestin	111	79	2.27 (1.41–3.67)	2.15 (1.30–3.57)	1.89 (0.92–3.55)
Combined					
Progestin alone	5	5	1.64 (0.45–6.00)	1.26 (0.34–4.74)	1.01 (0.24–4.36)
Missing	6	9	1.02 (0.35–2.99)	0.77 (0.23–2.61)	0.67 (0.18–2.42)
Baseline density by qualitative Bi-Rads					
1	26	16	1.00[d]	N/A[e]	1.00[d]
2	103	73	0.94 (0.47–1.87)		1.03 (0.50–2.12)
3	40	71	0.38 (0.18–0.79)		0.41 (0.19–0.89)
4	3	12	0.19 (0.05–0.77)		0.17 (0.04–0.72)
Baseline density by quantitative % density					
<10.45%	68	43	1.00[d]	N/A[e]	1.00[d]
10.45–23.54%	57	43	0.73 (0.42–1.28)		0.77 (0.43–1.38)
23.55–33.44%	29	43	0.38 (0.19–0.74)		0.40 (0.19–0.82)
>33.44%	18	43	0.24 (0.11–0.50)		0.24 (0.11–0.53)

[a] Data presented here as unmatched for descriptive purposes, but analyses were performed on matched pairs.
[b] Models adjusted for baseline density as quartiles of quantitative estimation.
[c] HRT is adjusted for baseline density as a quantitative variable and hysterectomy and/or oophorectomy status. Hysterectomy and oophorectomy status corresponds to dates preceding the initiation of HRT. Baseline density models are adjusted for HRT.
[d] Reference group.
[e] N/A, Adjustment not applicable.

Table 3 Comparison of estimated breast density change for cases and controls combined by Bi-Rads and quantitative computer-assisted techniques

	Bi-Rads estimate of change in breast density with HRT use				
	−2	−1	0	1	2
Mean change in quantitative breast density (%)	−24.7	−3.7	4.1	14.6	25.2
SD (%)		9.1	10.1	10.0	11.6
n	1	19	193	110	21

response to HRT. The variable response in breast density to HRT could not be explained by the confounding effect of weight change over the same period or the baseline level of weight at initiation of HRT. In fact, only type of HRT and baseline breast density were significantly associated with whether or not a woman had a HRT response, and all of the variables examined accounted for <26% of the variability in the response to HRT.

In our study, the type of HRT explained 7% of the variability in change in percentage of breast density. This was largely due to combination therapy being positively associated with a HRT effect. This observation is consistent with most previous studies of HRT and breast density (8, 10, 11, 14, 20). This is important, given the emerging evidence that women taking estrogen and progestins combined have been reported to be at greater risk of breast cancer than women taking estrogens alone (1, 2, 21–23). Also, there is evidence from human and animal models that there is greater breast cell proliferation when breast tissue is exposed to progestins in combination with estrogen than when it is exposed to estrogen alone (24–27). These proliferative responses have been seen mainly in the breast epithelial cells (24, 25, 27).

The stronger association of combined therapy (*versus* estrogen alone) with breast density response among leaner women is consistent with a recent study of type of HRT and breast cancer (1). Schairer *et al.* (1) found an overall association of breast cancer with estrogen only therapy as well as combined therapy. However, the association between duration of HRT in recent users and breast cancer risk was only present among lean women, defined as BMI ≤ 24.4 kg/m^2. Of particular note, there was a 0.12 (0.02–0.25) increase in RR for breast cancer per year of estrogen-progestin use and a 0.03 (0.01–0.06) increase in RR per year of estrogen only use. The large reanalysis of 51 epidemiology studies also found a greater risk with long-term HRT use *versus* never use among lean women (defined by BMI and weight), but data were not presented on the type of therapy within strata of BMI or weight (4).

If use of exogenous estrogens (in combination with progestins or alone) results in an increased breast density response only in a subset of women, one might expect to see that administration of antiestrogens or estrogen agonists would result in decreases in breast density only in a subset of women also. There have been several studies reporting decreases in breast density in response to administration of tamoxifen and gonadotropin-releasing hormone agonists (GRHAs) (28–32). Interestingly, several of these studies suggest that there is heterogeneity in response to these treatments also; not all women on tamoxifen or GRHAs experience reductions in breast density, and the size of the reductions vary (29, 30, 32, 33). To date, however, these studies have been small and do not provide information as to whether those women on tamoxifen or GRHA who experience decreases in density are at a reduced risk of breast cancer.

Strengths of this study lie in the standardized mammograms from one institution, abstracted chart data, and closely matched cases and controls on initiation and duration of HRT use. One primary limitation to this study is the absence of data on dose of HRT and whether combined therapy was cyclical *versus* continuous. We also lacked information on alcohol intake, which has been shown to be associated with small increases in breast density (34). The design of the study precluded the ability to estimate the lag time between HRT initiation and

change in breast density. Potentially this could be important and should be considered in future investigations.

Also, our use of the diagnosis of estrogen effect by the radiologist to determine case and control status is not ideal. We would have liked to use a less subjective selection of HRT responders and nonresponders from the Mayo mammography screening population, potentially using the quantitative estimate. However, this process is not feasible at this time, due to the large numbers of screening mammograms that would have to be digitized before selection of cases and controls (or before a cohort study was undertaken). The selection of cases and controls based on the radiologists' classification may have introduced ascertainment bias to our study. Because the radiologist subjectively diagnoses HRT response, he/she may be more likely to identify women who have a lower breast density pre-HRT use, such that the visual change in breast density is more apparent compared with women with a higher pre-HRT breast density. Fig. 1 shows a greater percentage of cases with lower pre-HRT Bi-Rads than controls; 75% of cases compared with 52% controls have a pre-HRT Bi-Rads of 1 or 2. Among cases, the magnitude of the breast density increase with HRT use does decrease with increasing baseline density [average change ($\pm$SD) for increasing quartiles of baseline density = 17% (11%), 16% (11%), 6% (13%), and 5%(6%)], illustrating the presence of a thresholding effect. We attempted to control for this potential bias by adjusting all analyses for baseline density; this did not materially change the results.

The results from this case-control study are generalizable to Midwestern women in a mammography screening clinic who have been on HRT for 4 years or less and who had a mammogram before the initiation of HRT. Several exclusions were made in our selection of cases and controls that could potentially introduce selection bias and compromise external validity. As reviewed in the methods, these exclusions for cases and controls included missing menopausal information, availability of mammograms, breast implants, and a history of cancer. Additionally, for a few cases, there was no control available due to the strict matching criteria. Although we cannot rule out the possibility, we do not expect these exclusions to largely influence these results because only 18% of potential case-control sets were excluded. Also, for the largest proportion of the exclusions (unavailability of menopausal status or mammograms), the association between risk factors and HRT response should not differ from those included in the study. This may not be true for those excluded due to implants or a history of cancer, but these only comprise 6 of the total 38 excluded sets.

It is reassuring, though, to see that after digitizing and estimating breast density for the mammograms before and after HRT use, there is consistency between the calculated change in density using both the qualitative and quantitative estimates of breast density and the radiologist's classification of estrogen effect. As might be expected, estimating change in density using the qualitative Bi-Rads estimate was less precise than using the quantitative breast density estimate from Cumulus. Although the two estimates of change were highly correlated, a woman could have had a 5% increase in breast density with HRT use but could have no increase in the change of Bi-Rads density estimates. This suggests that using the Bi-Rads density estimates are more appropriate for identifying women with large changes in breast density, whereas the quantitative estimate can identify women with small or large changes in breast density.

The subset of women who experience increases in breast density with HRT use may be susceptible to harmful effects of HRT on the breast. Similarly, decreased breast density with the initiation of tamoxifen may reflect those in whom tamoxifen will be an effective preventive agent for breast cancer. If this is indeed true, the mammogram image would permit the identification of those women most likely to benefit from these therapies. Cohort studies that have detailed HRT (or tamoxifen) history, serial screening mammograms, and sufficient follow-up for breast cancer events could address the association between breast density response and breast cancer risk. The results of such studies may have significant implications for the reduction of breast cancer risk by modification of HRT dosage, prescription of alternative drugs for management of menopausal symptoms, or closer surveillance.

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