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Breast density, body mass index, and breast cancer risk: Implications for clinical and public health settings.

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Breast density, body mass index, and breast cancer risk: Implications for clinical and public health settings.

by

Natalie J. Engmann

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of the

Dedication

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Approved: _____ Karla Kerlikowske, MD, Dissertation Chair

[1] Engmann NJ, Golmakani MK, Miglioretti DL, Sprague BL, Kerlikowske K. Population attributable risk proportion of clinical risk factors for breast cancer. *JAMA Oncology.* 2017 Sep 1;3(9):1228-1236.

BREAST DENSITY, BODY MASS INDEX, AND BREAST CANCER RISK: IMPLICATIONS FOR CLINICAL AND PUBLIC HEALTH SETTINGS.

Natalie J. Engmann

Abstract

Breast density and obesity are two of the most common risk factors for breast cancer among women in the United States. However, the importance of these risk factors and their individual and joint effects on breast cancer risk in individuals and on a population level is relatively unknown.

The first chapter of my dissertation provides a population perspective on the impact of breast density and obesity on breast cancer incidence in U.S. women. This study used the population attributable risk proportion (PARP) to estimate of the proportion of premenopausal and postmenopausal breast cancer cases that can be attributed to breast density, obesity and their combined effects. Results from this study suggest that breast density alone accounts for 39% of premenopausal and 26% of postmenopausal cancers, and combined with obesity, accounts for 43% of postmenopausal cancers.

Volumetric breast density software measures the three-dimensional volume of breast tissue and is increasingly used in clinical and research settings. Dense tissue volume may mediate or moderate the effect of obesity on breast cancer risk; thus, identifying the joint effects of obesity and breast density on breast cancer can improve risk stratification and provide insights into pathways driving breast cancer incidence. The second chapter evaluates if the effects of volumetric breast density on breast cancer risk are greater in obese compared with non-obese women. This study finds that the effect of volumetric density on breast cancer risk is

dramatically higher in obese compared with non-obese women, with the most pronounced effects in postmenopausal women. The third chapter evaluates how obesity and other risk factors affect longitudinal change in dense breast volume over the menopausal transition in healthy women. This study found no effect of obesity, but a strong effect of baseline dense volume on greater decline in volumetric breast density across the menopause transition.

As a body of work, my dissertation provides insights into the public health impact and clinical relevance of two of the most common breast cancer risk factors in U.S. women. It provides new evidence to improve clinical risk stratification and offers novel insights into the complex causal relationship between obesity, volumetric breast density and breast cancer risk.

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Introduction

Breast density.

Breast density, a measure of the stromal and epithelial tissue in the breast, was first described by Wolfe in 1976[1] and has since emerged as one of the strongest and most common risk factors for premenopausal and postmenopausal breast cancer. Research has estimated that approximately 47% of women of screening age (ages 40-74 years) in the United States have dense breasts,[2] increasing their risk for breast cancer and reducing the sensitivity of mammography to detect cancers.[3] As of early 2018, legislation has been enacted or introduced in 31 states requiring clinicians to inform women if they have dense breasts.[4] Breast density has been incorporated into risk prediction models that estimate a woman's 5 or 10-year absolute risk of breast cancer,[5–7] and improves clinicians' ability to stratify women into low, average and high-risk categories for targeted prevention and screening efforts.[5,7]

Assessment of breast density is varied across clinical and research settings and includes both qualitative and quantitative measurement. The most commonly used measure in clinical practice is the qualitative measurement of the American College of Radiology's BI-RADS classification, which uses four categories (a, b, c, d) to measure the extent and pattern of dense tissue in the breast.[8] Quantitative assessment includes two-dimensional measurement of breast density that uses computer software to segment the breast area and dense tissue area, and subsequently calculates the percentage of the breast that is dense. The majority of these "area-based" two-dimensional measures require a trained user and impose a substantial time burden to implement, therefore their utility is typically limited to research settings. Recently, quantitative breast density measurement that measures the three-dimensional volume of dense tissue and breast tissue has emerged and uses automated software to capture volumetric breast density.

These measures have very high reproducibility and are easily integrated into full field digital mammography (FFDM) systems, thus enhancing their potential use in both clinical and research settings. Previous research has compared across qualitative and volumetric assessment and found that the different measures have robust and broadly similar associations with breast cancer risk.[9]

Another challenge in the field of breast density is the lack of clarity regarding which phenotype of breast density is most predictive of breast cancer risk. Both area-based and volumetric breast density software produces measures of the percentage of the breast that is dense ("percent density"), the absolute extent of dense tissue ("dense breast area" or "dense breast volume"), and the extent of non-dense or fatty tissue ("non-dense area" or "non-dense volume"). Percent and absolute density are both associated with breast cancer risk, and while area-based percent breast density has shown slightly stronger associations with breast cancer than absolute density,[10] it is hypothesized that absolute density is the more relevant indicator of breast cancer risk. This assertion is based on the fact that absolute dense tissue is likely to be strongly associated with the number of cells or tissue at risk of carcinogenesis, and should be independent of obesity or fatty tissue.[11] However, recent literature has found that the extent of non-dense or fatty tissue in the breast may be protective of breast cancer risk, though it is unclear if this is independent of absolute density.[10,12,13] This may explain the stronger association with percent density, as the percent density measure accounts for both the extent of dense tissue and non-dense tissue.[12] Much of the previous research examining phenotypes of breast density has used area-based density assessment; volumetric breast density has been hypothesized to capture a different underlying entity of breast density.[14] Therefore, volumetric assessment may be able to provide a greater understanding of which phenotype is most important for risk, and the biological pathways responsible for this difference.

Breast density and obesity.

In addition to breast density, obesity is another common risk factor for breast cancer.[15–17] Many of the risk factors for breast cancer, such as nulliparity or late age at first pregnancy, are also risk factors for high breast density; however this is not true of obesity, most commonly measured through body mass index (BMI). Prior research using area-based breast density assessment has consistently found that on average women with high BMI have lower dense breast area,[18,19] though BMI is positively associated with breast cancer.[15–17] Accordingly, epidemiologic studies have concluded that BMI is a negative confounder on an independent pathway to breast cancer,[20] but few have explored the joint effects of both risk factors. In contrast, new volumetric measures have found that dense breast volume is positively associated with BMI,[21,18,22] suggesting that dense breast volume may moderate or mediate part of the increased risk of breast cancer due to obesity through a shared pathway. This also suggests that a greater proportion of overweight and obese women will have high dense breast volume, two risk factors which have been well-established to independently affect risk, but for which the combination of effects is yet unknown.

Objectives and significance of the dissertation.

The overall goal of this dissertation is to describe the independent effects and joint effects of obesity and breast density, evaluating their relevance for prevention of breast cancer in the population and assessing their joint clinical relevance to inform individual breast cancer risk stratification.

Chapter 1 of this work evaluated the proportion of breast cancers attributable to breast density, obesity and commonly collected clinical risk factors in premenopausal and

postmenopausal women in the United States. I studied women from the Breast Cancer Surveillance Consortium (BCSC), a sample broadly representative of U.S. women, to demonstrate the population impact of these two risk factors on breast cancer in the U.S. As volumetric breast density assessment is increasingly used in clinical and research settings, Chapter 2 assessed the joint effects of volumetric breast density and BMI, hypothesizing that the effect of volumetric breast density on breast cancer risk is substantially higher in women who are overweight and obese. Chapter 3 measured the effect of BMI and other risk factors on changes in volumetric breast density across the menopausal transition, a time where many women, though not all, experience declines in breast density. As changes in breast density over time have been demonstrated to be predictive of breast cancer risk, Chapter 3 provides insights into potential interventions to modify changes over time as a way to reduce future breast cancer risk.

Taken together, this work provides new insights on the role of breast density as a contributor to breast cancer in the population, as well as informing clinical risk stratification and underlying biological pathways connecting obesity with dense tissue, non-dense tissue, and breast cancer risk. The use of novel measures of volumetric breast density ensures that our results have high relevance to future research and applications of breast density in the clinical setting.

Chapter 1: Population attributable risk proportion of clinical risk factors for breast cancer.

Natalie J. Engmann, Marzieh K. Golmakani, Brian L. Sprague, Diana L. Miglioretti, and Karla Kerlikowske

Abstract

Importance: Many established breast cancer risk factors are used in clinical risk prediction models, though the proportion of breast cancers explained by these factors is unknown.

Objective: To determine the population attributable risk proportion (PARP) for breast cancer associated with clinical breast cancer risk factors among premenopausal and postmenopausal women.

Design: Case-control with 1:10 matching on age, year of risk factor assessment, and Breast Cancer Surveillance Consortium (BCSC) registry.

Setting: Data collected prospectively from January 1996 through December 2015 from BCSC community-based breast imaging facilities.

Participants: 18,437 invasive breast cancer and ductal carcinoma in-situ cases and 184,309 matched controls among 58,146 premenopausal and 144,600 postmenopausal women aged 40-74 years undergoing mammography.

Exposures: Breast Imaging Reporting and Data System (BI-RADS) breast density (heterogeneously or extremely dense vs. scattered fibroglandular densities), first-degree family history of breast cancer, body mass index (>25 kg/m^2 vs. 18.5-25 kg/m^2), history of benign breast biopsy, and nulliparity or age at first birth (≥30 years vs. <30 years old).

Main measure: Population attributable risk proportion **(**PARP) of breast cancer.

Results: Overall, 89.8% of premenopausal and 95.1% of postmenopausal women with breast cancer had at least one breast cancer risk factor. The combined PARP of all risk factors was 52.7% among premenopausal women and 54.7% among postmenopausal women. Breast density was the most prevalent risk factor for both premenopausal and postmenopausal women and had the largest impact on the PARP; 39.3% of premenopausal and 26.2% of postmenopausal breast

cancers could potentially be averted if all women with heterogeneously or extremely dense breasts shifted to scattered fibroglandular breast density. Among postmenopausal women, 22.8% of breast cancers could potentially be averted if all overweight and obese women attained a body mass index of <25 kg/m^2.

Conclusions and Relevance: Most women with breast cancer have at least one breast cancer risk factor routinely collected at the time of mammography, and more than half of premenopausal and postmenopausal breast cancers are explained by these factors. These easily assessed risk factors should be incorporated into risk prediction models to stratify breast cancer risk and promote risk-based screening and targeted prevention efforts.

Introduction

One of the challenges in promoting the widespread utility of breast cancer risk prediction models has been the assertion most women who are diagnosed with breast cancer have no established clinical breast cancer risk factors or aren't considered high-risk.[23,24] While it is impossible to determine the cause of breast cancer in any individual case,[25] easily assessed risk factors that explain a substantial proportion of incident breast cancers can be used to stratify breast cancer risk for targeted screening[26] and primary prevention[27], and improve public health interventions to reduce breast cancer risk.

The population attributable risk proportion (PARP) represents the proportion of disease cases in a population that would not have occurred in the absence of a risk factor. The PARP can be calculated for a single risk factor or combinations of risk factors, and quantifies the proportion of cases averted if exposure to the risk factor was removed from the entire population, holding all other factors constant. The PARP incorporates both the prevalence of the risk factor and the magnitude of its association with disease, and therefore rare exposures with a high relative risk may explain a similar proportion of cases as common exposures with modest relative risks.

Previous studies of PARP have largely focused on quantifying the potential reductions in postmenopausal breast cancer incidence by intervening on modifiable factors.[28–35] Estimates of the proportion of postmenopausal breast cancers that could be averted through lifestyle interventions range from 26%[28,33] to 40.7%,[34] while estimates for combinations of non-modifiable factors range from 37.3% to 57.3%.[28,34,36] No studies have quantified the contributions of risk factors for premenopausal breast cancer, only one small study has included breast density as a risk factor,[37] and none have examined the PARP for breast density using the

Breast Imaging Reporting and Data System (BI-RADS) scale,[2] the standard for reporting breast density in clinical practice in the U.S.

We aimed to estimate the proportion of breast cancers attributable to breast cancer risk factors commonly collected in clinical practice and used in breast cancer risk prediction models, including BI-RADS breast density. We use data from a large cohort of women undergoing mammography at facilities participating in the Breast Cancer Surveillance Consortium (BCSC).

Methods

Study Population

Breast cancer cases and matched controls were selected from the BCSC, which is comprised of regional registries from across the U.S. that collect clinical characteristics and breast imaging data from community radiology facilities. Breast cancer diagnoses and tumor characteristics are obtained through linkage to pathology databases and regional Surveillance Epidemiology, and End Results (SEER) programs or state cancer registries. Each registry and the Statistical Coordinating Center (SCC) received institutional review board approval for either active or passive consenting processes or a waiver of consent to enroll participants, link data, and perform analytic studies. All procedures are Health Insurance Portability and Accountability Act (HIPAA) compliant and all registries and the SCC received a Federal Certificate of Confidentiality and other protection for the identities of women, physicians, and facilities. The BCSC cohort is described in further detail elsewhere.[38,39]

Five BCSC registries, New Hampshire, North Carolina, San Francisco, Vermont, and Group Health, contributed data for this analysis. Eligible cases were women ages 40-74 years diagnosed with invasive breast cancer or ductal carcinoma in situ (DCIS) between 1996 and 2015 and with a BI-RADS breast density measure and risk factor data available within 5 years

prior to their diagnosis. Risk factors and strength of associations with invasive cancer and DCIS are similar,[40,41] so both were included. Women with a prior history of breast cancer or missing menopausal status were excluded, as well as women with incomplete breast cancer risk factor data (Figure 1). We selected risk factor information associated with mammography examinations one year or more prior to diagnosis. For 4,499 of 18,437 women (24%), risk factor information was not available more than one year prior to diagnosis, and data from within a year of diagnosis was used. Risk factor information was on average collected 20.4 months (range: <1 to 60 months) prior to breast cancer diagnosis. As a sensitivity analysis, we excluded cases with risk factor information obtained within 1 year of diagnosis and our findings remained unchanged.

Ten controls were matched to each breast cancer case on menopausal status, age and year of risk factor assessment, and BCSC registry. Eligible controls had no breast cancer diagnosis between the year of risk factor assessment and the year of diagnosis of her matched case. For age and year of risk factor information, we matched to controls differing up to +/- 5 years, selecting controls with the closest match to the case. A total of 95.5% of cases matched to 10 controls on age and year exactly, and 16 cases matched to <10 controls. A total of 18,437 breast cancers and 184,309 matched controls were included.

Exposure Assessment

Demographics and breast cancer risk factors were obtained through questionnaires completed at each mammography visit. Questionnaires included birth date, race, ethnicity, height, weight, first-degree family history of breast cancer, menopause status, parity, and age at first birth. Body mass index was calculated as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), obese (30-34.4 kg/m^2), and obese II/III (≥ 35 kg/m^2).[42]

History of benign breast biopsy was obtained by self-report and through linkages with pathology databases. The American College of Radiology's BI-RADS system, assigned by clinical radiologists, was used to classify breast density as a=almost entirely fat, b=scattered fibroglandular densities, c=heterogeneously dense and d=extremely dense.[43]

Statistical Analysis

Analyses were stratified by menopausal status. We used descriptive statistics to assess differences in demographics and clinical characteristics for cases and controls. Risk factors selected *a priori* for analysis were dense breasts (heterogeneously dense or extremely dense), first-degree relative with breast cancer, history of benign breast biopsy, and nulliparity or age at first birth ≥30 years. We considered BMI ≥25 kg/m^2 to be a risk factor for postmenopausal breast cancer only. Multivariable conditional logistic regression, stratified by matched set, was used to estimate the odds ratios and 95% confidence intervals associated with each risk factor.

PARP was calculated using the generalized regression-based approach described by Bruzzi et al. (1985)[44] allowing for the calculation of joint PARP for combinations of risk factors. The multivariable combined PARP is measured by the equation, $1 - \sum_i \frac{pd_i}{RR_i}$; where pd_i is the proportion of cases in stratum *i* of the risk factor distribution, and RR_i is the multivariable adjusted relative risk associated with that stratum of the risk factor(s). Odds ratios from the multivariable conditional logistic regression models were used as relative risk estimates.[44] PARP was calculated for individual risk factors and combinations of risk factors. For each individual factor, the reference level shown in Table 1 is considered the low-risk category. For combinations of factors the PARP represents the proportion of cases eliminated in the population if everyone shifted to the referent category for all included variables. When the referent category

for PARP was not the lowest level of exposure, the lowest level of exposure was assumed to remain unchanged. Confidence intervals were calculated using bootstrapping.[45] All analyses were conducted in R Version 3.2.1.

Results

The mean age among premenopausal cases and matched controls was 46.3 years, and 61.7 years among postmenopausal women. The sample was predominantly non-Hispanic white (>75% of women) with smaller percentages of Asian, Hispanic and African American women. Breast cancer cases were more likely to have a first-degree family history of breast cancer, a history of benign breast biopsy, dense breasts, and an older age at first birth relative to controls (Table 1.1). Postmenopausal breast cancer cases were more likely to be overweight or obese.

Overall, 89.8% of premenopausal cases and 95.1% of postmenopausal cases had at least one risk factor, compared with 82% of premenopausal controls and 91% of postmenopausal controls. A majority of premenopausal cases (57%) had two or more risk factors compared with only 42% of premenopausal controls. Postmenopausal women on average had more risk factors, with 66% of cases having two or more risk factors compared with 55% of controls.

First-degree family history of breast cancer, history of benign breast biopsy, dense breasts, and nulliparity or age at first birth >30 years were associated with increased risk of breast cancer (Table 1.2). Obesity was not associated with breast cancer risk among premenopausal women, but overweight and obese postmenopausal women were at higher risk of breast cancer. This association showed a statistically significant positive trend, with overweight, obese I, and obese grade II/III women having 1.23, 1.39 and 1.54 times the odds of breast cancer relative to normal weight women.

Among premenopausal women, the largest individual PARP was for breast density, with 39.3% (95% CI: 36.6, 42.0) of breast cancers potentially removed by reducing breast density from BI-RADS heterogeneously or extremely dense breasts to scattered fibroglandular densities. The PARP for breast density increases to 65.5% (95% CI: 60.4, 70.6) if all premenopausal women reduced their breast density to the lowest category of fatty breasts. A more modest reduction of all women shifting to a single lower BI-RADS category would result in a PARP of 13.4% (95% CI: 11.0, 15.8). Among premenopausal women, the combination of first-degree family history, history of benign breast biopsy, age at first birth, and breast density had a PARP of 52.7% (95% CI: 49.1, 56.3) (Table 1.3).

Individual PARP's for first-degree family history, age at first birth and history of benign breast biopsy were similar for premenopausal and postmenopausal breast cancer. However, overweight and obesity accounted for a large proportion of postmenopausal breast cancers, with a PARP of 22.8% (95% CI: 18.3, 27.3) if all obese and overweight women achieved a normal BMI. The estimated PARP for shifting all postmenopausal women to the BI-RADS category fatty breasts was 43.9% (95% CI: 39.6, 48.2), whereas shifting only extremely or heterogeneously dense breasts to scattered fibroglandular densities was 26.2% (95% CI: 24.4, 28.0). The PARP was 12.7% (95% CI: 11.2, 14.3) for reductions of a single BI-RADS category. The combination of first-degree family history, history of benign breast biopsy, nulliparity or age at first birth >30 years, breast density (with scattered fibroglandular densities as reference) and BMI yielded a combined postmenopausal PARP of 54.7% (95% CI: 51.6, 57.8).

Discussion

We found that routinely collected clinical risk factors included in breast cancer risk models may explain 52.7% of premenopausal and 54.7% of postmenopausal breast cancers. A substantial proportion of breast cancers can be attributed to high breast density alone, suggesting behaviors or interventions that reduce breast density have the potential to eliminate a large proportion of breast cancers in both premenopausal and postmenopausal women. These easily assessed breast cancer risk factors are highly prevalent among premenopausal and postmenopausal breast cancer cases; over half of cases in the population are attributable to these factors and thus they offer promise for risk-based screening and prevention strategies.

Though breast density is a well-established, strong and prevalent breast cancer risk factor, few studies have quantified the PARP of breast density, and none have used the BI-RADS classification used in clinical practice. In a study of Canadian women, Boyd et al. (2007)[37] found a PARP of 16% if women with >50% breast density reduced their breast density to ≤50%,[37] and that this PARP was much greater, approximately 40%, for cancers detected within 12 months of a negative-screening exam, reflecting the increased probability of a masking effect in dense breasts.[46] We found a PARP roughly 2-fold higher than Boyd et al. if all women shifted to the BI-RADS scattered fibroglandular density category, and the PARP was unaltered in a sensitivity analysis excluding women with breast density measured within one year of diagnosis. Differences between our and Boyd's study may reflect distinctions between a classification of >50% density using quantitative measures that includes a smaller proportion of women with substantial amounts of density, whereas the qualitative BI-RADS classification of heterogeneously or extremely dense includes larger proportions of women.[47] Further, the use of ≤50% as a reference category is likely to attenuate the relative risk used in the PARP, as the

literature suggests women with 10%-50% breast density have an increased breast cancer risk relative to women with <10% density.[48]

We found reductions in breast density of a single BI-RADS category would avert roughly 13% of breast cancers among premenopausal and postmenopausal women, a reduction that would avert more cases than reducing any other risk factor in this study, with the exception of BMI in postmenopausal women. Studies of longitudinal changes in BI-RADS breast density suggest a reduction of a single BI-RADS category reduced breast cancer risk relative to density that remained stable or increases.[49,50] Our results suggest that shifting the distribution of breast density down a single category would still result in a substantial reduction in breast cancers in the population. Reductions of a single BI-RADS category could potentially be achieved through increased breastfeeding, as well as primary prevention with tamoxifen for those at highest risk.[51–54] These interventions effectively reduce breast density but must be carefully considered in the context of anticipated harms. Our results highlight the necessity for new approaches to reduce breast density that could be widely adopted without adverse consequences, as reductions in breast density have the potential to dramatically reduce the incidence of breast cancer.

No prior studies have evaluated the PARP of clinical breast cancer risk factors in combination with breast density. However, our results are broadly consistent with previous literature evaluating non-modifiable clinical risk factors, with PARP estimates from 37.2%[28] to 57.3%[34] combining risk factors age at menarche, menopause, and first full-term pregnancy, and parity, family history of breast cancer, and benign breast disease. Estimates of the PARP of BMI in postmenopausal women have been disparate across studies; Barnes et al.[28] estimated a PARP of 2% shifting all women to a BMI of <=22.4 kg/m^2, Mezzeti et al.[31] estimated a PARP of 10.2% shifting all women to a BMI of 23.3 kg/m^2, and three additional studies found PARP's of 8%,

9.5%, and 24.8% shifting women to <25 kg/m^2.[30,32,55] It is difficult to directly compare our results with previous findings because of different reference categories. Our finding of a PARP of 22.8% may reflect the high prevalence of overweight and obese postmenopausal women in the U.S. relative to studies in European populations. Our results suggest excess bodyweight plays an important role in postmenopausal breast cancer, further reinforcing the need for weight reduction and management to prevent a substantial proportion of breast cancers. In the absence of interventions, the PARP for obesity will increase with the prevalence of obesity in the U.S.[56]

Our study includes over 200,000 women from BCSC community breast imaging registries, broadly representative of the demographic composition of women in the U.S[39] and with clinical risk factor distributions nearly identical to the distributions estimated in the population-based National Health Interview Survey (Figures 1.1 and 1.2). The BCSC 5-and 10-year absolute risk calculator was developed within the same cohort of women,[5,57] though the use of breast cancer risk models to identify women for primary and secondary preventions has been controversial, with a commonly expressed concern most women with breast cancer have no known risk factors. We found only 10% of premenopausal and less than 5% of postmenopausal breast cancers in our study had no clinical risk factors. The impact of assessing clinical breast cancer risk factors in combination with breast density is considerable, explaining over half of premenopausal and postmenopausal breast cancers and identifying risk factors where targeted public health interventions would have the greatest impact. These factors represent clinically available information that can and should be used by clinicians to stratify breast cancer risk for improved risk-based screening and primary and secondary prevention efforts.[26]

Estimates of the PARP are sensitive to changes in category definitions that alter the prevalence and relative risk of the risk factor.[25,58] We chose categories based on clinical relevance but examined how robust our findings were to changes in the reference group corresponding to ideal compared with more realistic interventions to change risk factor distributions. Close attention to risk factor prevalence should be considered when applying our results to other populations. We were unable to measure other behavioral and genetic risk factors, thus our estimated PARP likely underestimates the joint PARP of all known risk factors. Our study uses risk factor and breast density information from 1996-2015, a time period when the BI-RADS density category definitions changed. Despite these changes, there is no evidence of a difference in the distribution of breast density over time in the BCSC.[43] Studies have found mixed results for inter-rater and intra-rater reliability of the BI-RADS categories;[59–62] however, relative risks for breast cancer are similar comparing BI-RADS to more objective quantitative density measurements.[9] Most importantly, BI-RADS is currently the only measure of breast density used routinely in clinical practice, thus using BI-RADS enhances the clinical utility of our estimates for risk stratification and screening and prevention efforts.

Our study has several strengths, including collection of clinically available breast cancer risk factors. We provide novel insights into the contributions of breast density on a population level, reinforcing existing interventions to reduce breast density among high-risk women, and the need for acceptable behaviors and novel interventions to reduce risk in high and average risk women. Finally, we provide the first estimate of PARP for clinical risk factors in premenopausal women, and our results suggest with the exception of BMI, the PARP of most risk factors is similar among premenopausal and postmenopausal women.[63]

In the largest study, to our knowledge, of PARP in U.S. women, we find a majority of premenopausal and postmenopausal breast cancer cases have at least one breast cancer risk factor, and that breast density and clinical risk factors may explain over half of breast cancer cases. These risk factors represent clinically available data that can and should be used to stratify risk using established risk models that include breast density to promote risk-based screening and targeted prevention efforts. Future research should assess if PARP estimates differ by molecular subtypes of breast cancer, where the magnitude and direction of risk factors may differ.[64–67]

Table 1.1. Characteristics of breast cancer case and non-cases included in the study population, Breast Cancer Surveillance Consortium (1996-2015).

	Premenopausal Women		**Postmenopausal Women**	
	Control (n=52,860)	*Invasive & In-Situ Cancer (n=5,286)*	*Control (n=131,449)*	*Invasive & In-Situ Cancer (n=13,151)*
	N (%)	*N (%)*	*N (%)*	*N (%)*
Age				
40-49 Years	41120 (77.8)	4114 (77.8)	4711 (3.6)	471 (3.6)
50-59 Years	11740 (22.3)	1172 (22.2)	48868 (37.2)	4882 (37.1)
60-69 Years	NA	NA	54153 (41.2)	5415 (41.2)
70-74 Years	NA	NA	23717 (18.0)	2383 (18.1)
Race/Ethnicity				
White	40054 (75.8)	4091 (77.4)	104157 (79.2)	10832 (82.4)
Black	1295 (2.5)	122 (2.3)	3323 (2.5)	279 (2.1)
Asian	5670 (10.7)	548 (10.4)	11177 (8.5)	894 (6.8)
Hispanic	2719 (5.1)	208 (3.9)	5105 (3.9)	395 (3.0)
Other/mixed	3122 (5.9)	317 (6.0)	7687 (5.9)	751 (5.7)
Family History of Breast Cancer				
No	46020 (87.1)	4181 (79.1)	109827 (83.6)	10035 (76.3)
Yes	6840 (12.9)	1105 (20.9)	21622 (16.5)	3116 (23.7)
History of Benign Breast Biopsy				
No	45658 (86.4)	4193 (79.3)	102741 (78.2)	9252 (70.4)
Yes	7202 (13.6)	1093 (20.7)	28708 (21.8)	3899 (29.7)
Age at First Live Birth				
Nulliparous	11729 (22.2)	1240 (23.5)	20236 (15.4)	2350 (17.9)
Age < 30 years	29060 (55.0)	2615 (49.5)	97101 (73.7)	9168 (69.7)
Age ≥ 30 years	12071 (22.8)	1431 (27.1)	14112 (10.7)	1633 (12.4)
Body Mass Index				
<18.5 kg/m^2	924 (1.8)	106 (2)	2223 (1.7)	173 (1.3)
18.5-24.9 kg/m^2	23739 (44.9)	2642 (50.0)	45341 (34.5)	4194 (31.9)
25-29.9 kg/m^2	15123 (28.6)	1456 (27.6)	43937 (33.4)	4476 (34.4)
30-34.9 kg/m^2	7192 (13.6)	616 (11.7)	23321 (17.7)	2493 (19.0)
≥35 kg/m^2	5882 (11.1)	466 (8.8)	16627 (12.7)	1815 (13.8)

Table 1.1 (continued). Characteristics of breast cancer case and non-cases included in the study population, Breast Cancer Surveillance Consortium (1996-2015).

	Premenopausal Women		**Postmenopausal Women**	
	Control (n=52,860)	*Invasive & In-Situ Cancer (n=5,286)*	*Control (n=131,449)*	*Invasive & In-Situ Cancer (n=13,151)*
	N (%)	*N (%)*	*N (%)*	*N (%)*
BI-RADS Breast Density				
Almost entirely fat (a)	2764 (5.2)	95 (1.8)	16852 (12.8)	1014 (7.7)
Scattered fibroglandular densities (b)	17256 (32.6)	1248 (23.6)	62743 (47.7)	5749 (43.7)
Heterogeneously dense (c)	24479 (46.3)	2803 (53.0)	44686 (34.0)	5448 (41.4)
Extremely dense (d)	8361 (15.8)	1140 (21.6)	7168 (5.5)	940 (7.2)
Type of Cancer				
Invasive Cancer	N/A	3890 (73.5)	N/A	10313 (78.4)
In-Situ Cancer		1396 (26.4)		2838 (21.6)
Number of Risk Factors				
None	9749 (18.5)	539 (10.2)	11222 (8.5)	649 (4.9)
One	20793 (39.3)	1759 (33.3)	49661 (37.8)	3803 (28.9)
Two	17509 (33.1)	2039 (38.6)	46076 (35.1)	4807 (36.6)
Three	4365 (8.3)	821 (15.5)	19744 (15.0)	2914 (22.2)
Four or more	444 (0.8)	128 (2.4)	4746 (3.6)	978 (7.4)

Table 1.2. Odds ratios and population attributable risk proportion (PARP) of breast cancer risk factors in women undergoing screening or diagnostic mammography estimated by multivariable conditional logistic regression.

	Premenopausal Breast Cancer (n=58146)		Postmenopausal Breast Cancer (n=144600)	
	*OR (95% CI)**	*Population Attributable Risk %[δ] (95% CI)*	*OR (95% CI)**	*Population Attributable Risk %[δ] (95% CI)*
Family History of Breast Cancer				
No	*Reference*	8.7 (7.3, 10.1)	*Reference*	8.2 (7.2, 9.1)
Yes	1.71 (1.59, 1.84)		1.53 (1.46, 1.60)	
Body Mass Index (kg/m2)				
Underweight (<18.5)	0.93 (0.76, 1.15)	N/A	0.79 (0.67, 0.93)	22.8 (18.3, 27.3)[€]
Normal (18.5-24.9)	*Reference*		*Reference*	
Overweight (25.0-29.9)	0.99 (0.93, 1.07)		1.23 (1.17, 1.28)	
Obese I (30.0-34.9)	1.00 (0.91, 1.10)		1.39 (1.31, 1.47)	
Obese II/III (≥35.0)	1.05 (0.94, 1.18)		1.54 (1.45, 1.64)	
History of Benign Breast Biopsy				
No	*Reference*	6.9 (5.5, 8.4)	*Reference*	8.6 (8.0, 9.2)
Yes	1.50 (1.40, 1.62)		1.41 (1.35, 1.47)	
Age at First Live Birth				
Nulliparous	1.14 (1.05, 1.22)	8.7 (4.8, 12.7)	1.20 (1.14, 1.26)	5.2 (4.2, 6.2)
<= 30 Years	*Reference*		*Reference*	
> 30 Years	1.28 (1.19, 1.37)		1.23 (1.16, 1.30)	
BI-RADS Breast Density				
Mostly Fat, a	0.47 (0.38, 0.58)	39.3 (36.6, 42.0)[¥]	0.62 (0.58, 0.67)	26.2 (24.4, 28.0)[¥]
Scattered Fibroglandular Densities, b	*Reference*		*Reference*	
Heterogeneously Dense, c	1.57 (1.46, 1.69)		1.40 (1.34, 1.45)	
Extremely Dense, d	1.81 (1.65, 1.99)		1.58 (1.46, 1.71)	

** Odds ratios presented are adjusted for family history of breast cancer, body mass index, history of benign breast biopsy, age at first live birth, and BI-RADS breast density.*

[δ]PARP calculated using multivariable adjusted odds ratios (OR's).
[€]PARP calculated for shifting everyone to normal weight and holding underweight constant.
[¥]PARP calculated for shifting BI-RADS categories 'heterogeneously dense' and 'extremely dense' to 'scattered fibroglandular densities', and holding 'mostly fat' constant.

Table 1.3. Population attributable risk proportion (PARP) for individual risk factors and combinations of factors.*

	Premenopausal Breast Cancer	Postmenopausal Breast Cancer
	PARP (95% CI)	*PARP (95% CI)*
Two risk factors		
Family history of breast cancer, breast density	44.6 (41.3, 47.9)	32.2 (30.0, 34.4)
History of benign breast biopsy, family history of breast cancer	14.8 (13.3, 16.3)	16.0 (14.8, 17.1)
History of benign breast biopsy, breast density	43.5 (40.3, 46.7)	32.4 (30.8, 34.0)
Breast density, BMI	N/A	43.3 (39.3, 47.3)
Family history of breast cancer, BMI	N/A	29.1 (26.1, 32.1)
History of benign breast biopsy, BMI	N/A	29.5 (24.4, 34.2)
Nulliparous or age at first birth ≥ 30 years, BMI	N/A	26.9 (23.9, 29.8)
Nulliparous or age at first birth ≥ 30 years, family history of breast cancer	16.6 (13.5, 19.8)	13.0 (11.4, 14.5)
Nulliparous or age at first birth ≥ 30 years, history of benign breast biopsy	15.0 (12.3, 17.8)	13.3 (11.8, 14.9)
Nulliparous or age at first birth ≥ 30 years, breast density	44.6 (41.1, 48.1)	30.0 (28.1, 32.0)
Three risk factors		
Family history of breast cancer, history of benign breast biopsy, breast density	48.3 (44.6, 51.9)	37.8 (35.9, 39.7)
Family history of breast cancer, history of benign breast biopsy, nulliparous or age at first birth ≥ 30 years	22.2 (19.8, 24.6)	20.3 (18.8, 21.8)
Family history of breast cancer, history of benign breast biopsy, BMI	N/A	35.1 (30.2, 40.0)
Family history of breast cancer, nulliparous or age at first birth ≥ 30 years, breast density	49.4 (46.2, 52.5)	35.8 (33.9, 37.6)
Family history of breast cancer, breast density, BMI	N/A	47.9 (45.1, 50.7)
History of benign breast biopsy, nulliparous or age at first birth ≥ 30 years, breast density	48.4 (45.1, 51.7)	35.9 (34.3, 37.5)
History of benign breast biopsy, breast density, BMI	N/A	48.0 (44.7, 51.4)
Family history of breast cancer, nulliparous o rage at first birth ≥ 30 years, BMI	N/A	32.8 (29.9, 35.8)
History of benign breast biopsy, nulliparous or age at first birth ≥ 30 years, BMI	N/A	33.2 (28.8, 37.5)
Breast density, BMI, nulliparous or age at first birth ≥ 30 years	N/A	46.2 (42.5, 49.9)
Four risk factors		
Family history of breast cancer, history of benign breast biopsy, breast density, nulliparous or age at first birth ≥ 30 years	52.7 (49.1, 56.3)	41.0 (39.7, 42.3)
Family history of breast cancer, history of benign breast biopsy, breast density, BMI	N/A	52.2 (49.0, 55.4)
History of benign breast biopsy, nulliparous or age at first birth ≥ 30 years, breast density, BMI	N/A	50.7 (47.9, 53.6)
Family history of breast cancer, nulliparous or age at first birth ≥ 30 years, breast density, BMI	N/A	50.6 (46.5, 54.7)
Family history of breast cancer, nulliparous or age at first birth ≥ 30 years, history of breast biopsy, BMI	N/A	38.5 (36.4, 40.7)
Five risk factors		
Family history of breast cancer, history of benign breast biopsy, nulliparous or age at first birth ≥ 30 years, breast density, BMI	N/A	54.7 (51.6, 57.8)

**BMI: Body Mass Index ≥25 kg/m²*

Family history of breast cancer: first-degree family history only.
Breast Density: Heterogeneously or extremely dense breasts with scattered fibroglandular densities as reference.

Figure 1.1. Prevalence of clinical risk factors among premenopausal women in the National Health Interview Survey 2010 compared with premenopausal women in the Breast Cancer Surveillance Consortium (BCSC). Data were obtained from the 2010 NHIS Cancer Control Supplement, and included 7,662 women aged 40-74 years without breast cancer weighted to represent the broader population of U.S. women. *Family history* refers to first-degree family history; *History of breast biopsy* includes benign breast biopsy only.

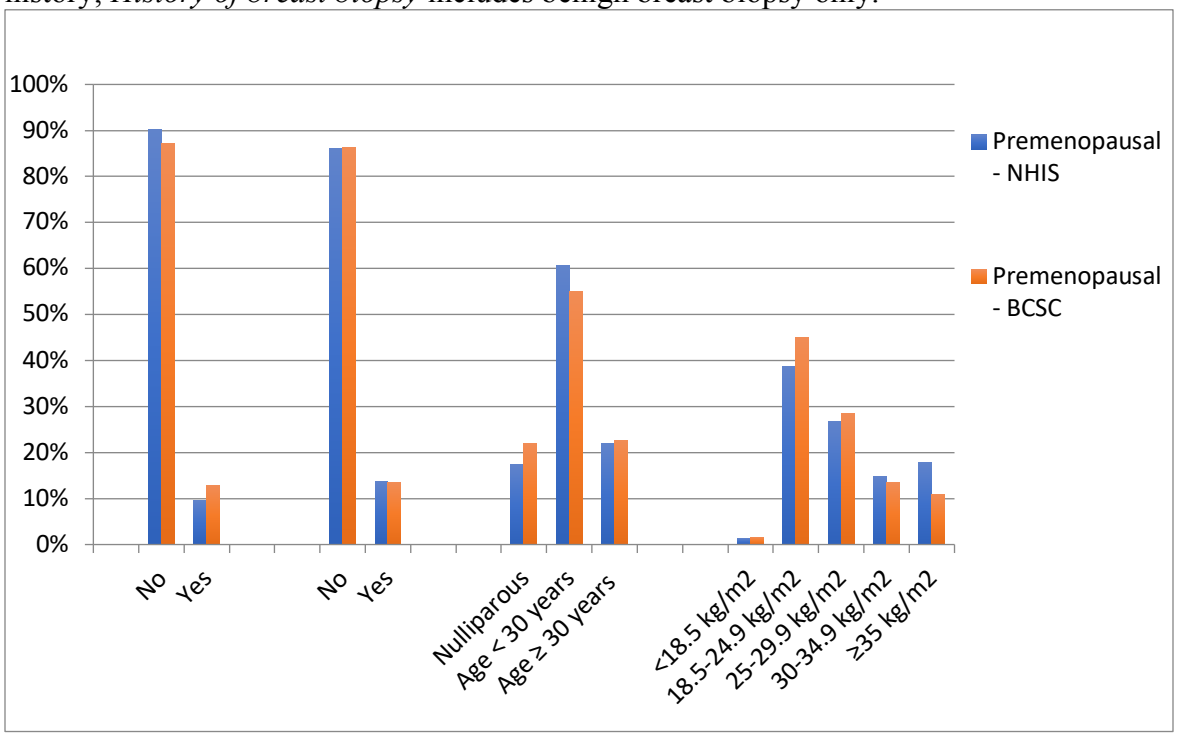
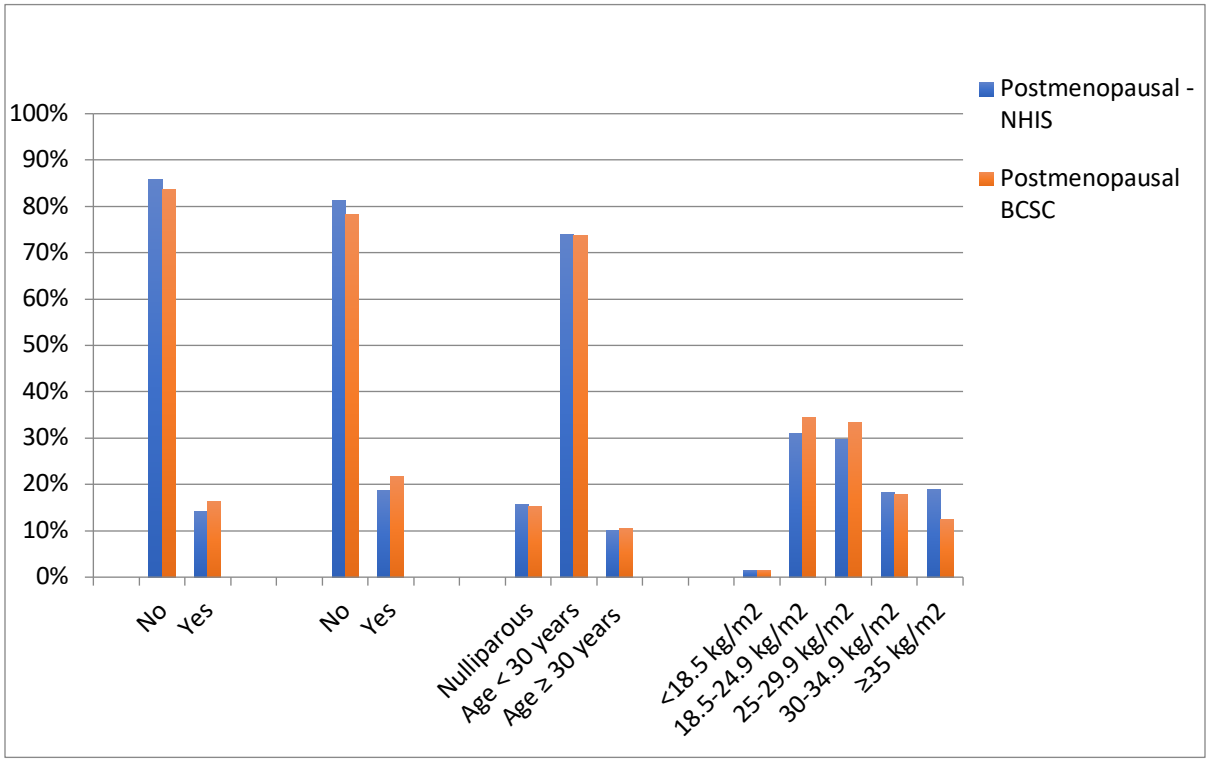


Figure 1.2. Prevalence of clinical risk factors among postmenopausal women in the National Health Interview Survey 2010 compared with postmenopausal women in the Breast Cancer Surveillance Consortium (BCSC). Data were obtained from the 2010 NHIS Cancer Control Supplement, and included 7,662 women aged 40-74 years without breast cancer weighted to represent the broader population of U.S. women. *Family history* refers to first-degree family history; *History of breast biopsy* includes benign breast biopsy only.



Chapter 2: Combined effect of volumetric breast density and body mass index on breast cancer risk.

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Abstract

Purpose: Breast density and body mass index (BMI) are used for breast cancer risk stratification. We evaluate whether the positive association between volumetric breast density and breast cancer risk is strengthened with increasing BMI.

Methods: The San Francisco Mammography Registry and Mayo Clinic Rochester identified 781 premenopausal and 1850 postmenopausal women with breast cancer diagnosed between January 2007 and 2015 that had a screening full-field digital mammogram at least 6 months and up to 5-years prior to diagnosis. Up to three controls (N=3535) were matched per case on age, race, date, mammography machine, and state of residence. Volumetric percent density (VPD) and dense volume (DV) were measured with Volpara^{TM} software. Breast cancer risk was assessed with logistic regression stratified by menopause status. Multiplicative interactions were fit between BMI categories [<25(normal), 25-29(overweight) and ≥30(obese) kg/m^2] and VPD and DV, and trend tests assessed for increasing odds ratios (OR) with increasing BMI.

Results: The increased risk of breast cancer associated with VPD got stronger with increasing BMI for both premenopausal ($p\text{-}_{trnd}$=0.0007) and postmenopausal (p_{trend} =0.0001) women. For a BMI of <25, 25-30, and ≥30 kg/m^2, ORs for cancer risk for a 10% increase in VPD are 1.39. 2.19, and 2.88 for premenopausal women and 1.35, 2.03, and 3.60 for postmenopausal women, respectively. In contrast, the increased risk of breast cancer associated with DV got stronger with increasing BMI for postmenopausal (p_{trend}=0.01) but not premenopausal (p_{trend} =0.68) women. For BMI <25, 25-30 and ≥30 kg/m^2, a 1 SD increase in DV was associated with ORs of 1.39, 1.33, and 1.51 in premenopausal women and 1.31, 1.34, and 1.65 in postmenopausal women, respectively.

Conclusion: The effect of volumetric percent density on breast cancer risk is strongest in overweight and obese women. These associations have clinical relevance for informing prevention strategies.

Introduction

Breast density is one of the strongest and most common risk factors for breast cancer with approximately 39% of premenopausal and 26% of postmenopausal breast cancer cases in the United States attributable to dense breasts.[68] Many techniques for measuring breast density are used in clinical and research settings, including two-dimensional ("area-based") and three-dimensional ("volumetric") assessment. Research suggests broadly similar associations with breast cancer risk across measurement techniques.[22,9,69]

Obesity is a well-established risk factor for postmenopausal breast cancer. Although obesity has been associated with no effect or reduced risk of breast cancer among premenopausal women,[70] some literature suggests that obesity is indeed a risk factor after adjusting for breast density.[20,67,71,72] Both Harris et al.[72] and Boyd et al.[20] found that adjustment for area-based percent density reversed the protective association between obesity and premenopausal breast cancer risk. Kerlikowske et al.[67] also found that body mass index (BMI) measured during the premenopausal period was a risk factor for ten-year breast cancer risk after adjusting for breast density as assessed by the American College of Radiology's BI-RADS categories.[8] These findings suggest that overweight and obese women with dense breasts, whether premenopausal or postmenopausal, may have a higher risk of breast cancer than currently appreciated. Three previous studies, all using area-based breast density, have evaluated if the effect of breast density is stronger among women with high BMI, with two studies finding no evidence of an interaction, and the third finding much stronger effects of percent density in overweight and obese postmenopausal women.[73]

Volumetric breast density software can be used in the clinical setting for breast cancer risk stratification. Therefore to optimize risk prediction, it is important to understand if obesity

modifies the effect of volumetric breast density on risk. We assessed if the effect of volumetric breast density on breast cancer risk is modified by obesity in a population of women from two large breast screening cohorts.

Methods

Study Population

Cases and controls were sampled from breast-screening practices, the San Francisco Mammography Registry (SFMR) and the Mayo Clinic Breast Screening practice. Each study has institutional ethics approvals and is described briefly below and in detail elsewhere.[9]

San Francisco Mammography Registry (SFMR)

The SFMR is a diverse, multi-facility mammography screening registry that collects demographic, risk factor, and mammography data from breast screening facilities in the San Francisco Bay Area. Cancer outcomes are obtained annually through linkage to the California Cancer Registry (CCR), which pulls from the Northern and Southern California Surveillance Epidemiology and End Results (SEER) programs. Passive permission to provide data for research is obtained at each mammography visit for all women. The SFMR has collected raw "for processing" digital mammograms from Hologic-Selenia since 2006 from four facilities, and only women from these facilities were eligible for the nested case-control study. Women with a breast cancer diagnosis (invasive cancer or ductal carcinoma in situ) between January 2007 and 2015, and a screening full-field digital mammogram (FFDM) at least 6 months prior to diagnosis were included as cases. Two controls without breast cancer were matched to each case on age, date of earliest mammogram, race/ethnicity, mammography facility, and mammography unit.

Mayo Clinic, Rochester, Breast Screening Practice

The Mayo Clinic, Rochester, has collected and stored raw FFDM images acquired with Hologic-Selenia FFDM units since April 2008. Women presenting for breast screening at the Mayo Clinic who reside in the tri-state area of MN, IA and WI, were eligible for inclusion in the Mayo case-control study. Previous studies have shown that women presenting for routine screening mammography who reside in the tri-state area are likely to return to Mayo to receive their diagnosis and primary treatment for breast cancer.[74] Thus, breast cancers were identified through linkage to the Mayo Clinic Tumor Registry. All women presenting for screening have the option of providing authorization to use their medical records, images and diagnostic information for research; 93% provided authorization. Women residing in the tri-state area who were diagnosed with breast cancer at Mayo with a FFDM screening exam at least 6 months prior to diagnosis were included as cases. Three controls from the screening practice without breast cancer were matched on age, race / ethnicity, date and mammography unit of earliest mammogram, state of residence and date of last mammogram.

Mammogram Selection

A total of 2912 cases and 6538 controls from the SFMR and Mayo Clinic were eligible for inclusion in the analysis. For cases, we selected the earliest available mammogram between 6 months and 5.5 years prior to the cancer diagnosis. Forty-five cases (1.5%) had no mammograms within 5.5 years of diagnosis; therefore, the most recent mammogram outside 5.5 years was used (mean years prior to diagnosis = 6.3). For matched controls, we selected the mammogram from the date closest to the case mammogram included; we excluded 29 controls (0.4%) that had no

images within 365 days of the case mammogram. We excluded 168 cases and 246 controls with missing covariate data and 8 cases and 16 controls with missing menopause status. The total sample included 781 premenopausal and 1868 postmenopausal cases, and 1730 premenopausal and 4298 postmenopausal controls. The mean time from mammogram to case diagnosis was 3.05 (SD: 1.4) years for cases and 3.1 (SD: 1.4) years for controls.

Covariate Data

Demographic and risk factor data were self-reported on a clinical questionnaire at each mammography visit. BMI was calculated from self-reported height and weight SFMR and was obtained from the medical record closest to the time of mammogram for Mayo [median(range) days between BMI and mammogram: 2 (0-364)]. Covariates used for analysis include age, BMI, race/ethnicity, menopause status, age at first birth, parity, first-degree family history of breast cancer and current use of postmenopausal hormone therapy (HT). Menopause status was classified according to the complex definition in Phipps et al. (2010),[75] whereby women who self-reported as premenopausal and not on HT were classified as premenopausal, and women who were ≥55 years, self-reported as postmenopausal, or self-reported as premenopausal but were on HT were classified as postmenopausal. BMI was calculated as normal weight (<25 kg/m^2), overweight (25-29 kg/m^2), and obese (≥30 kg/m^2). Given previous literature suggesting that very obese women (≥35 kg/m2) may represent a unique population of women at high risk,[17] we also included a four category classification of BMI including BMI ≥ 35 kg/m^2 (obese II/III) for postmenopausal women only; sample size did not allow for this classification in premenopausal women. Age at first birth and parity were combined, with levels for nulliparous (no births), age at first birth ≤30 years, and age at first birth >30 years.

Breast Density Measurement

Raw ("for processing") FFDM images were available at both sites and Volpara™ (Matakina Technology) software was run on all four views for cases and controls.

Volpara Software

Volpara™ (Version 1.5.3, Matakina Technology, New Zealand) is a fully-automated software that measures volumetric breast density on FFDM machines. The Volpara proprietary algorithm identifies an area of the breast that is entirely fatty tissue and uses this reference point to estimate the thickness of dense tissue at each pixel in the image, not including the skin. Estimates of dense breast volume (DV) are obtained by summing the estimated dense tissue across all pixels in the breast image and estimated total breast volume is determined by multiplying the estimated breast area by the breast thickness. Volumetric percent density (VPD) is obtained by dividing the estimated DV from the total breast volume, and nondense volume (NDV) is obtained by subtracting DV from total breast volume. We measured breast density on the cranio-caudal (CC) and medio-lateral oblique (MLO) views for both left and right breasts for each woman. The estimates from all 4 views were averaged to obtain the final density values.

Statistical Methods

Characteristics of the cases and controls by menopause status are summarized by frequency and percentage or median and quartiles. We used unconditional logistic regression models, stratified by menopause status, adjusted for matching factors and covariates family history of breast cancer, age at first birth / parity, and HT (postmenopausal models only).

Sensitivity analyses comparing conditional and unconditional models found similar results, therefore unconditional models adjusting for matching factors were used. Models for DV were additionally adjusted for NDV, and NDV models were adjusted for DV. We fit interaction terms between BMI categories [<25 (normal weight), 25-30 (overweight) and >30 (obese) kg/m2] with each density measure [VPD (10% increase), DV and NDV (per standard deviation)] and used interaction p-values to test whether association of density measures was differential by BMI categories. Ordinal trend tests, assuming a linear effect across BMI categories, were used to assess whether odds ratios for the effect of density measures showed evidence of monotonic trends across BMI categories. All analyses were conducted in SAS 9.3.

Results

Characteristics of cases and controls by menopause are reported in Table 2.1. Cases had greater VPD and DV than controls among both premenopausal (VPD: 14.9% vs. 12.0%, DV: 74.1 cm^3 vs. 64.4 cm^3) and postmenopausal women (VPD: 6.8% vs. 6.1%, DV: 53.4 cm^3 vs. 48.0 cm^3)(all p-values<0.001). There were no significant differences in NDV between cases or controls for premenopausal or postmenopausal women (Table 1).

Higher BMI was associated with decreased VPD and increased DV for both premenopausal and postmenopausal women (Table 2.2). The median VPD was 16.7%, 9.7% and 5.3% and DV was 63.8, 73.0, and 71.4 cm^3 for normal, overweight, and obese premenopausal women, respectively. Similar trends were seen for postmenopausal women, who had 9.1%, 5.5% and 4.4% VPD, and 43.7, 49.7 and 58.3 cm^3 DV, respectively. Non-dense volume increased substantially with BMI: the median NDV was 402.7, 808.7, and 1316.7 cm^3 for premenopausal,

and 507.9, 873.6, and 1307.2 cm^3 for postmenopausal normal, overweight, and obese women, respectively.

VPD and DV were positively associated with breast cancer risk in premenopausal women, and the association increased strongly with increasing BMI for VPD (p_{trend}=0.0007) but not DV (p_{trend}=0.68). Odds ratios (OR) for breast cancer risk for a 10% increase in VPD among normal weight, overweight, and obese women were 1.39 (95% CI: 1.2-1.6), 2.19 (95% CI: 1.5-3.1) and 2.88 (95% CI: 1.5-5.5) respectively. For DV, a 1 SD increase corresponded to ORs of 1.39 (95% CI: 1.2-1.5), 1.33 (95% CI: 1.2-1.5) and 1.51 (95% CI: 1.2-1.8) for normal weight, overweight and obese women, respectively, though the trend was not significant.

Similar effects were seen in postmenopausal women; however for these women the association between breast density and cancer risk increased significantly with increasing BMI for both VPD (p_{trend}=0.0001) and DV (p_{trend}=0.01). ORs for breast cancer risk for a 10% increase in VPD were 1.35 (95% CI: 1.2-1.6), 2.03 (95% CI: 1.5-2.7), 3.60 (95% CI: 2.1-6.2) and for a 1 SD increase in DV were 1.31 (95% CI: 1.2-1.4), 1.34 (95% CI: 1.2-1.5), 1.65 (95% CI: 1.4-1.9) for normal weight, overweight, and obese women, respectively. In sensitivity analyses including BMI≥35 kg/m2, the ORs for VPD were 1.35 (95% CI: 1.2-1.6), 2.03 (95% CI: 1.5-2.7), 3.47 (95% CI: 1.8-6.7), and 5.18 (95% CI: 1.8-15.0), for normal, overweight, obese and obese II/III, respectively (p_{trend}<0.0001).

There were no statistically significant differences in the effect of NDV on risk by BMI category for premenopausal or postmenopausal women. Among premenopausal women, a 1 SD

increase in NDV was associated with a decreased risk of breast cancer, with ORs of 0.74 (95% CI: 0.6-1.0), 0.72 (95% CI: 0.5-1.0) and 0.86 (95% CI: 0.7-1.1) for normal, overweight and obese women, though there was no evidence of a trend (p_{trend}=0.52)(Table 2.3). For postmenopausal women, higher NDV showed greater reductions in breast cancer risk in women in the lowest two BMI categories, with ORs of 0.77 (95% CI: 0.7-0.9), 0.80 (95% CI: 0.7-0.9), and 0.92 (95% CI: 0.8-1.0) for normal weight, overweight and obese women, respectively, though this observed trend did not reach statistical significance. (p_{trend}=0.07).

Discussion

Our findings suggest higher VPD was associated with increased breast cancer risk, and the magnitude of this effect was significantly greater in both premenopausal and postmenopausal overweight and obese women. The risk of breast cancer with high DV was also greater in overweight and obese women, though only significant for postmenopausal women. Higher NDV was associated with reduced risk of breast cancer, with greater apparent protection in postmenopausal women BMI<30 kg/m^2, though the trend was not significant.

Previous research evaluating differences in the effect of breast density on breast cancer risk by adiposity have used qualitative or area-based density assessment and have had mixed findings. Two of three studies found no effect modification by BMI in premenopausal and postmenopausal women.[73,76,77] One study of postmenopausal Chinese women found that breast cancer risk for women with >75% vs. <10% area-based density was 9.5-fold higher for women with BMI >=26.7 kg/m^2, compared with 3.5-fold higher in women with BMI<26.7 kg/m^2.[77] A subsequent paper hypothesized that these findings were due to decreased image contrast resulting

from the increased compressed breast thickness in overweight women, leading to an underestimate of the extent of breast density.[78] While it is difficult to directly compare our study given different classification of BMI and area-based vs. volumetric breast density measures, our study finds similarly high breast cancer risks among overweight and obese women with high breast density.

Differences between our study and previous research may reflect different scales of measurement between area-based and volumetric assessment, or the potential that the two measurement types capture different underlying entities of breast density.[14,79,80] Inverse associations between BMI and absolute dense area have been commonly reported,[18,72,81,82] whereas studies of dense volume, including ours, show positive associations with BMI.[83–85] The reason for these opposing associations on area measures is unclear but is thought to be due to distortions of dense and fat tissue from the projection of a three-dimensional to a two-dimensional image. Consequently, in our study women with both high BMI and high dense volume may represent a different population than identified in previous research using two-dimensional assessment. For example, where previously an obese woman may have been classified with low dense breast area, in our study she may be classified as having high dense volume. Alternatively, the volumetric measurement may truly capture a different underlying entity of breast density in the same women, [14,79,80] potentially explaining why we found strong effect modification by BMI where area-based density did not.

We found that interaction between BMI and VPD was stronger than for DV, suggesting that it is not only the amount of dense tissue that is relevant but the microenvironment of the

surrounding breast. Indeed, if both DV and NDV provide important information about breast cancer risk, it is not surprising that VPD, which incorporates both measures, is the most strongly associated with risk. In further support of this hypothesis, we found that the effect of DV on breast cancer risk was substantially strengthened when adjusted for NDV, and that NDV was protective for breast cancer risk even after adjustment for DV and BMI. This finding is consistent with previous research using area-based measures of breast density, [48,86–88] though we are the first study to our knowledge to report using volumetric assessment. Biologically, the protective effect of NDV may be explained by increased breast involution in fat tissue, vitamin-D3 induced growth regulation of the ductal epithelium, or decreased extracellular matrix stiffness that leads to reduced cancer risk.[12] However, the protective effect of NDV may also be dependent on obesity. A recent paper in *Cancer* found that mammary fat tissue in obese women had increased myofibroblasts compared to lean women, which contributes to extracellular matrix stiffness and can promote carcinogenesis.[89] Though we did not find significantly different associations of NDV by BMI on risk, the OR's trended towards greater protection of NDV in women with BMI<30 kg/m^2, lending some support to the hypothesis that NDV in overweight and obese women may have different cellular properties that promote carcinogenesis.

The biological mechanism supporting the stronger effect of DV in women with high BMI is unclear. One potential explanation is that the inflammatory effects of adiposity are mediated through increases in DV, which would explain the positive association between obesity and DV in our study. Obesity is associated with hyperinsulinemia, increased circulating adipokines and inflammatory markers that may upregulate cellular proliferation, and postmenopausal obesity is associated with increased circulating estrogens from adipose tissue.[90] These biological effects of

adiposity may contribute to increased DV on the causal pathway to breast cancer risk, explaining why obese women with higher DV had higher risk compared to those with lower DV. Studies looking at weight change and breast density have had mixed findings, with some suggesting that weight gain is associated with increased absolute dense tissue, [90,91] though the only study to use volumetric breast density found inverse associations with weight gain.[92] Further research is needed to evaluate the biological mechanisms by which NDV and DV are associated with breast cancer risk in both obese and non-obese women.

Our findings have strong clinical implications, as breast density is increasingly used in the clinical setting for risk prediction. Currently, breast cancer risk models including the Breast Cancer Surveillance Consortium (BCSC) model[5] incorporate BI-RADS breast density assessment into 5- and 10-year breast cancer risk prediction, and a recent study suggests that including volumetric measures in these models increases the ability to stratify risk.[93] Volumetric breast density will be progressively incorporated into risk prediction, and as such, our results are timely as they inform additional risk due to the interaction between BMI and volumetric density that may allow further risk stratification for primary and secondary prevention. In our data 18% of premenopausal and 24% of postmenopausal controls were overweight or obese and had a DV above the mean, representing a high clinical and public health relevance.

Our study is the first to report a greater effect of volumetric breast density in overweight and obese women, and benefits from a large sample of premenopausal and postmenopausal women with raw FFDM images. Our study limitations include the use of self-reported BMI for SFMR and menopausal status. Our use of broad categories of BMI should mitigate substantial

misclassification, though misclassification of menopause status is possible. However, misclassification of menopause would likely be non-differential, leading to an underestimate of the interaction effects in both pre- and postmenopausal women.

In summary, we found that the effect of percent volumetric density and dense volume on breast cancer risk was higher in overweight and obese women than women with normal weight, suggesting the potential to further stratify women for targeted primary and secondary prevention. Future research should confirm this finding and investigate the contribution of differences in measures of density and biological mechanisms by which obesity and volumetric density interact to increase breast cancer risk.

Table 2.1. Characteristics of the study sample by menopause and case status.

	Premenopausal		**Postmenopausal**	
	Control (n=1730)	*Case (n=781)*	*Control (n=4298)*	*Case (n=1868)*
	N (%)			
Age				
<45 Years	780 (45.1)	371 (47.5)	31 (0.7)	10 (0.5)
45 to 64 Years	950 (54.9)	410 (52.5)	2289 (53.3)	1005 (53.8)
≥65 Years	NA	NA	1978 (46.0)	853 (46.7)
Body Mass Index (BMI)				
Normal (<25 kg/m^2)	1062 (61.4)	518 (66.3)	2025 (47.1)	828 (44.3)
Overweight (25-29 kg/m^2)	382 (22.1)	172 (22.0)	1293 (30.1)	573 (30.7)
Obese I (≥30 kg/m^2)	286 (16.5)	91 (11.7)	980 (22.8)	467 (25.0)
Race				
Caucasian	1271 (73.5)	561 (71.8)	3582 (83.3)	1514 (81.1)
Asian	362 (20.9)	176 (22.5)	536 (12.5)	274 (14.7)
Black	28 (1.6)	18 (2.3)	77 (1.8)	42 (2.3)
Hispanic	69 (4.0)	26 (3.3)	103 (2.4)	38 (2.0)
Family History of Breast Cancer				
No	1455 (84.7)	571 (74.3)	3402 (79.2)	1313 (71.0)
Yes	263 (15.3)	198 (25.7)	892 (20.8)	537 (29.0)
Unknown	12	12	4	18
Age at First Birth / Parity				
Nulliparous	515 (29.8)	277 (35.5)	976 (22.7)	504 (27.0)
<30 Years	623 (36.0)	210 (26.9)	2703 (62.9)	1080 (57.8)
≥30 Years	592 (34.2)	294 (37.6)	619 (14.4)	284 (15.2)
Current Hormone Therapy				
No	1724 (100)	736 (100)	3536 (83.1)	1412 (80.0)
Yes	NA	NA	717 (16.9)	354 (20.0)
Unknown	6	45	45	102
	Median (IQR)			
Age, years	45.0 (6.3)	45.0 (6.0)	63.4 (14.0)	63.0 (13.4)
Volumetric Percent Density, *%*	12.0 (11.3)	14.9 (11.2)	6.1 (5.2)	6.8 (5.8)
Dense Volume, *cm^3*	64.4 (43.4)	74.1 (53.8)	48.0 (29.0)	53.4 (32.9)
Nondense Volume, *cm^3*	552.4 (547.8)	538.1 (505.6)	766.5 (610.6)	777.3 (655.8)

Table 2.2. Distribution of volumetric breast density by BMI category in premenopausal (n=1,730) and postmenopausal (n=4,298) controls.

	N	Volumetric Percent Density (VPD)	Dense Volume (DV) *Median (IQR)*	Nondense Volume (NDV)
Premenopausal Controls				
Body Mass Index (BMI)				
Normal (<25 kg/m2)	1062	16.6 (10.4)	62.4 (41.9)	397.9 (278.5)
Overweight (25-29 kg/m2)	382	8.9 (6.0)	68.5 (51.9)	811.2 (395.0)
Obese (≥30 kg/m2)	286	5.1 (3.0)	68.1 (37.3)	1297.8 (753.7)
Postmenopausal Controls				
Body Mass Index (BMI)				
Normal (<25 kg/m2)	2025	8.8 (7.1)	42.6 (28.8)	514.3 (366.7)
Overweight (25-29 kg/m2)	1293	5.3 (3.1)	48.4 (28.0)	883.4 (437.6)
Obese (≥30 kg/m2)	980	4.3 (1.8)	55.7 (26.1)	1310.9 (702.7)

Table 2.3. Breast cancer risk (odds ratios [OR], 95% confidence intervals [CI]) by volumetric breast density and body mass index.

		Volumetric Percent Density (VPD)	**Dense Volume (DV)**	**Non-Dense Volume (NDV)**
	Cases/ Controls	*OR (95% CI) for 10% Increase*	*OR (95% CI) for 1 SD Increase*	*OR (95% CI) for 1 SD Increase*
Premenopausal (n=2,511)				
<25 kg/m²	518/1062	1.39 (1.2, 1.6)	1.39 (1.2, 1.5)	0.74 (0.6, 1.0)
25-29 kg/m²	172/382	2.19 (1.5, 3.1)	1.33 (1.2, 1.5)	0.72 (0.5, 1.0)
≥30 kg/m²	91/286	2.88 (1.5, 5.5)	1.51 (1.2, 1.8)	0.86 (0.7, 1.1)
p, interaction*		0.01	0.58	0.56
p, ordinal**		0.0007	0.68	0.52
Postmenopausal (n=6,166)				
<25 kg/m²	828/2025	1.35 (1.2, 1.6)	1.31 (1.2, 1.4)	0.77 (0.7, 0.9)
25-29 kg/m²	573/1293	2.03 (1.5, 2.7)	1.34 (1.2, 1.5)	0.80 (0.7, 0.9)
≥30 kg/m²	467/980	3.60 (2.1, 6.2)	1.65 (1.4, 1.9)	0.92 (0.8, 1.0)
p, interaction*		0.0003	0.03	0.12
p, ordinal**		0.0001	0.01	0.07
Postmenopausal (n=6,166)				
<25 kg/m²	828/2025	1.35 (1.2, 1.6)	1.31 (1.2, 1.5)	0.77 (0.7, 0.9)
25-29 kg/m²	573/1293	2.03 (1.5, 2.7)	1.35 (1.2, 1.5)	0.80 (0.7, 0.9)
30-34 kg/m²	274/586	3.47 (1.8, 6.7)	1.53 (1.3, 1.9)	0.88 (0.7, 1.1)
≥35 kg/m²	193/394	5.18 (1.8, 15.0)	1.81 (1.4, 2.3)	0.93 (0.8, 1.1)
p, interaction*		0.0005	0.05	0.33
p, ordinal**		<0.0001	0.01	0.32

CI=confidence interval; SD=standard deviation; VPD= volumetric percent density; DV= dense volume; NDV=non-dense volume.
Logistic regression models were adjusted for age, study site, race, family history of breast cancer, parity / age at first birth, and hormone replacement therapy (postmenopausal only). DV models were additionally adjusted for NDV; NDV models were additionally adjusted for DV.

**p-value for interaction between BMI categories and continuous VPD (per 10% increase), DV or NDV, (per 1 SD).*
***p-ordinal tests for trend in the OR's by BMI category.*

Chapter 3: Longitudinal changes in volumetric breast density in healthy women across the menopausal transition.

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Abstract

Purpose: Many women experience declines in mammographic breast density during menopause. We assessed changes in volumetric breast density across the menopausal transition and factors that influence these changes.

Methods: Women without a history of breast cancer, mastectomy or implants who had full field digital mammograms during both pre- and postmenopausal periods, at least 2 years apart, were sampled from 4 facilities within the San Francisco Mammography Registry from 2007 to 2013. Dense breast volume (DV) was assessed using Volpara^{TM} on all available digital mammograms for each woman across the time period. Annualized change in dense volume from pre- to post-menopause was estimated using linear mixed models, adjusting for age, dense volume and body mass index (BMI) at baseline, and BMI change from baseline and including woman as a random effect. Multiplicative interactions were evaluated between baseline risk factors and time to determine if these covariates modified the annualized changes.

Results: Among the 2586 women included, 1766 had two mammograms, 655 had three, and 165 had four. Women experienced an annualized decrease in DV (-2.2 cm^3) over the menopausal transition. Annualized declines were greater among women with a baseline DV above the median DV of 54 cm^3 vs. below (DV: -3.4 cm^3 vs. -1.0 cm^3, $p<0.0001$). Other breast cancer risk factors had no effect on change in DV over the menopausal transition.

Conclusion: High baseline dense breast volume was a strong predictor of greater reductions in dense volume across the menopausal transition. Future research should examine if declines in dense volume across menopause are associated with decreased breast cancer risk.

Introduction

Breast density is a measure of the stromal and epithelial tissue in the breast and is a strong risk factor for breast cancer.[10,94,95] Breast density is strongly influenced by age, and many women experience a natural decline in dense breast tissue with aging. The most accelerated declines are often observed over the menopausal transition, corresponding with Pike's hypothesis that the rate of breast tissue aging decreases over the menopause, and that the magnitude of this decrease may be influenced by exposure to breast cancer risk factors.[96]

Previous studies of longitudinal changes in breast density have primarily used area-based breast density assessment to estimate the decline in percent density, and found an average annual decline of 0.5-2%, with the greatest reductions occurring over the menopausal transition.[97–101] Fewer studies have examined changes in area-based absolute density, which is believed to be the more etiologically relevant phenotype of breast density for breast cancer risk, as it reflects the amount of tissue at risk of carcinogenesis.[11] One study estimated that women undergoing menopause had a decline in dense area that was 3.39 cm^2 larger than age-matched women who remained premenopausal during the same time period.[99] Cross-sectional studies comparing breast density in premenopausal and postmenopausal women support this finding, with a recent study including women from 22 countries estimating that postmenopausal women had a mean dense area that was 3.5 cm^2 lower than premenopausal women.[102]

Not all women experience a decline in breast density with menopause, however, and previous research has identified few factors that modify longitudinal changes in breast density. Current research suggests that women with higher baseline breast density have accelerated declines,[97,98] and combined postmenopausal hormone therapy (HT) users have attenuated declines or increases over time and across the menopausal transition.[97,98,103] Findings on the

effects of reproductive-related factors and obesity on change in density over time however, have been mixed across studies.[97–101]

Longitudinal changes in breast density are associated with breast cancer risk, and women who experience the greatest declines over time have a reduced risk of breast cancer.[49,104] Therefore identifying factors influencing change across the menopausal transition may improve targeted prevention efforts. Automated, volumetric breast density measures are increasingly used in clinical settings and can monitor changes in breast density over time; however, literature quantifying longitudinal change in breast density using volumetric density assessment is sparse.

The objective of our study was to use volumetric breast density assessment to measure changes in breast density over the menopausal transition in healthy, cancer-free women, and identify risk factors that affect change during this time period.

Methods

Study Population

Participants were sampled from the San Francisco Mammography Registry (SFMR), a population-based mammography registry collecting demographic, risk factor, and mammographic information on women undergoing mammography at 22 facilities in the San Francisco Bay Area. We included four SFMR facilities that have obtained raw digital images from Hologic-Selenia mammography machines since 2006. Passive permission to participate in research is obtained at each mammography visit.

Participants

Eligible women had at least two mammograms between 2007 and 2013, with one mammogram prior to self-reported menopause ("baseline mammogram") and at least one subsequent postmenopausal mammogram. At least two years were required between the premenopausal and postmenopausal mammogram. The "menopausal transition" is defined as the time between the premenopausal and postmenopausal mammogram for each woman. Women with a personal history of breast cancer, breast implants, or mastectomy and women without cranio-caudal mammogram views were excluded. There were a total of 2,586 women and 6,157 mammograms (mean: 2.4 per woman) included in the analysis. All mammograms between the baseline and postmenopausal mammogram were collected for the current analysis.

Covariate Data

Demographics, risk factor data and menopause status were self-reported at each mammography visit. Menopause status was self-reported at each mammography visit and measured by asking women if their menstrual periods had stopped and if they were using oral contraceptives or postmenopausal hormone therapy. Women were classified as postmenopausal if they reported their periods had stopped, for any reason, or if they reported use of postmenopausal HT, regardless of their self-report of menstrual periods. All other women were considered premenopausal. Covariates collected at the time of the baseline mammogram included age, race/ethnicity (Caucasian, Asian, Other), body mass index (BMI; continuous [kg/m2] and categories [<25kg/m2, 25-29 kg/m2, ≥30 kg/m2]), first-degree family history of breast cancer (yes/no), parity (yes/no), age at first birth (nulliparous, <30 years, ≥30 years), and current alcohol use (none, ≤1 drink per day, ≥2 drinks per day). Use of postmenopausal HT was collected at all mammograms subsequent to the baseline mammogram and was classified as

unknown, no current use, and current use. Among current users, further classification by formulation (estrogen vs. estrogen & progesterone) was available. BMI was collected at each mammogram and change in BMI (kg/m^2) was calculated between baseline and each subsequent mammogram.

Breast Density Measurement

Raw ("for processing") mammogram image formats were collected and stored, and Volpara™ automated breast density software was run on all mammograms across the menopausal transition.

Volpara Software

Volpara™ (Version 1.5.3, Matakina Technology, New Zealand) is a fully-automated software that measures volumetric breast density on full field digital mammography (FFDM) machines. The Volpara proprietary algorithm identifies an area of the breast that is entirely fatty tissue and uses this reference point to estimate the thickness of dense tissue at each pixel in the image, not including the skin. Further detail on the Volpara algorithm is published elsewhere.[105] Estimates of dense breast volume (DV) are obtained by summing the estimated dense tissue across all pixels in the breast image and estimated total breast volume is determined by multiplying the estimated breast area by the breast thickness. Breast density was assessed on cranio-caudal (CC) mammography views. For women with both CC views available on all mammograms (n=2,551), we calculated breast density on the CC view of a randomly chosen side, using the same side for each subsequent mammogram. Among women who had CC views from only a single side, we used the available side (n=35) for all images.

Statistical Methods

Characteristics of the study sample at the baseline mammogram are summarized by frequency and percentage or median and quartiles. Linear regression, adjusted for age, was used to estimate the effects of baseline risk factors (race / ethnicity, BMI, family history of breast cancer, parity, age at first birth and alcohol use) on DV at the baseline mammogram. We fit linear mixed effects models including all available mammograms across the menopausal transition to estimate the annualized change in DV accounting for the correlation between women over time with woman-specific random effects. The association between baseline and time-updated (HT only) covariates on annualized change in DV were assessed by fitting an interaction between each risk factor and time (years) since baseline mammogram. Separate models were fit for each covariate interaction, and were adjusted for age, time (years), baseline BMI, change in BMI, and DV at the baseline mammogram. We found that baseline DV was strongly predictive of annualized changes in DV and baseline risk factors (e.g., BMI) were strongly associated with baseline DV; therefore, all longitudinal mixed models were additionally adjusted for the interaction between DV and time. All analyses were conducted in SAS 9.4.

Results

Characteristics of the study sample at the baseline mammogram are reported in Table 3.1. Of the 2,586 women included, 1,766 had two mammograms, 655 had three, and 165 had four. The median age of women at the baseline premenopausal mammogram was 51 (IQR: 49-52) years. The median BMI at the baseline mammogram was 23.3 (IQR: 21.2-26.5) kg/m^2 and the

median change in BMI from premenopausal to postmenopausal mammogram was 0 (IQR: -0.5, 0.9) kg/m^2.

Associations between demographics and baseline risk factors with DV at the baseline mammogram are shown in Table 3.2. Older age, parity, and younger age at first birth were associated with lower DV (all p's<0.01). Women with a first-degree family history of breast cancer had greater DV compared with women without a family history (p<0.001), and women who reported ≥2 drinks per day of alcohol consumption had greater DV compared to women consuming <2 drinks per day or women reporting no alcohol use (p<0.001). The greatest differences in DV were seen comparing women with a BMI <25 kg/m^2, who had a mean DV of 57.7 cm^3, to women with BMI's of 25-29 kg/m^2 or >30 kg/m^2, who had a mean DV of 70.5 cm^3 and 73.9 cm^3, respectively (p<0.001). DV also varied by race/ethnicity, with Caucasian women having the highest mean DV of 67.8 cm^3, compared to Asian women, who had the lowest DV of 52.0 cm^3, and women of other racial/ethnic groups who had a mean of 66.2 cm^3 (p<0.001).

The estimated decline in DV per year across the menopausal transition was 2.2 (95% CI: -2.7, -1.7) cm^3 with a median time of 3.1 (IQR: 2.6-3.5) years from premenopausal to postmenopausal mammogram (Figure 3.1). The median DV at the baseline mammogram was 54 (IQR: 37.7-77.8) cm^3, and women above the median DV at the premenopausal mammogram had greater declines across the menopausal transition, with average annualized declines of 3.41 (95% CI: -4.14, -2.68) cm^3 compared with 0.93 (95% CI: -1.67, -0.20) cm^3 in women above compared with below the median baseline DV (p-interaction<0.0001).

Asian women experienced attenuated change over time compared with Caucasian women (-1.68, 95% CI: -2.56, -0.81 cm^3 vs. -2.36, 95% CI: -3.05, -1.68 cm^3), though these differences did not reach statistical significance (p-interaction=0.36). The estimated decline in DV in

overweight and obese women was slightly higher (-2.02, 95% CI: -2.64, -1.39 cm^3 vs. -2.54, 95% CI: -3.64, -1.44 cm^3) compared with normal weight women (-2.33, 95% CI: -3.86, -0.80 cm^3), though these differences were not statistically significant (p-interaction=0.70). There were no differences in the rate of change in DV over time by parity, age at first birth, family history of breast cancer, or current alcohol use (Table 3.3).

Use of postmenopausal HT use during the menopausal transition trended towards larger declines in DV per year compared with non-users, with women on HT on average having decline of 3.29 cm^3 compared with 2.13 cm^3 in non-users, though this difference was not significant (p=0.24). Further breakdown of HT use by formulation showed no differences between non-users (-2.11 cm^3), estrogen-only users (-2.97 cm^3), and users of estrogen & progestin combination therapy (-2.58 cm^3)(p-interaction=0.76).

Discussion

This longitudinal analysis of dense breast volume across the menopausal transition found a decline in DV of 2.2 cm^3 per year across the menopausal transition. We found that baseline DV was a strong predictor of greater annualized declines in DV, and that other risk factors, including baseline BMI and postmenopausal HT using during the menopausal transition had no significant effect on annualized changes in DV.

Our finding of a decline of 2.2 cm^3 per year across the menopausal transition is broadly consistent with the annualized decline in dense breast area estimated by Boyd et al. of 6.8 cm^2 in healthy women across 5 years during the menopausal transition (approximately 1.36 cm^2 per year).[99] No studies, to our knowledge, have serial measures of volumetric breast density across the menopause, preventing direct comparisons of our findings. However, our own recent study

found annualized declines of 0.28 cm^3 in premenopausal women and 0.82 cm^3 in postmenopausal women who were not selected for proximity to the menopausal transition.[106] The mean decline in our study is significantly greater per year during the menopausal transition, which is consistent with longitudinal research using area-based measures finding that the greatest annualized reductions occur during the peri-menopausal years.[98]

Consistent with previous research using area-based density assessment, we found that higher baseline breast density is a strong predictor of greater annualized declines in breast density,[97–100] and that race/ethnicity, family history of breast cancer, parity, age at first birth and alcohol use at baseline did not significantly modify the effect on longitudinal change in breast density.[97,98,100] We adjusted for differences in baseline DV, which are strongly affected by these demographic characteristics and baseline risk factors, therefore it is possible that these factors have no effect on change in breast density aside from their effect on the baseline density.

We found no differences in reduction in DV over time among overweight and obese women compared with normal weight women. Some,[97,98] but not all,[100,101] longitudinal studies using area-based density assessment have found attenuated reductions over time in overweight and obese women, though all examined percent density, while we examine absolute dense volume. BMI is strongly inversely associated with area-based percent density, therefore it is possible that previous research found attenuated declines in overweight and obese women because the baseline breast density in these women was lower, thus allowing for relatively smaller changes over time.[97] In our study, overweight and obese women had the highest baseline dense volume, therefore we would expect these women to experience greater reductions in density over time. However, once adjusting for baseline dense volume, we found no additional effect of baseline BMI to slow declines in density. While the focus of our study was to identify

baseline predictors of decline in DV, it is probable that changes in BMI over time are more relevant for influencing change in DV. One prior study using volumetric measurement found that reductions in BMI were associated with subsequent decreases in DV[107]; this finding needs replication in future research.

Postmenopausal HT use is associated with increased breast density in cross-sectional studies, and has been associated with attenuated declines or increases over time and across the menopausal transition.[103,108] Our findings that postmenopausal HT had no significant effect on changes in DV were unexpected, particularly the finding of no distinction between formulations of HT, which have shown important differences with respect to breast density in other studies.[103,109,110] Maskarinec et al.[97] reported that combined HT users had attenuated declines in area-based percent density that were 3.3% less than declines in non-users, though declines in users of estrogen-only HT were only 1.6% less than declines in non-users, per decade of follow-up. Based on previous literature, we would expect that women on HT in perimenopause or menopause would increase, maintain, or at least experience attenuated reductions in breast density relative to non-users. However the effects on breast density are likely dependent, at least partially, on duration of use.[103,111] Though duration of HT use was not reported and thus was unavailable in our analysis, women were required to be non-users of HT at the baseline mammogram, therefore the maximum duration of use is limited. However, the median time of 3.1 years from premenopausal to postmenopausal mammogram in our study is substantially long that duration of use is unlikely to fully explain our results. It is possible that newer formulations of HT at lower doses have smaller effects of breast density, or that changes are less apparent when using volumetric assessment. Prior studies examining postmenopausal HT use and changes

in density using volumetric assessment are limited, therefore future research is needed to further examine this finding.

We restricted our analysis to assess changes in DV and did not assess changes in volumetric percent density or non-dense volume over time. The menopausal transition is typically characterized by decreases in dense tissue, but also weight gain, which can increase both non-dense tissue and total breast volume. We aimed to quantify the changes in absolute DV, with the hypothesis that the absolute dense tissue volume is reflective of the number of cells at risk of carcinogenesis, thus potentially serving as a better indicator of breast cancer risk compared to percent measures which are confounded by body size.[11] Furthermore, longitudinal assessment of changes in DV may be less influenced by mammography acquisition features, such as compressed breast thickness, which is known to be highly correlated with baseline factors such as BMI. As such, assessment of changes in measures of percent dense volume and total volume over time may potentially bias estimates of factors that influence changes in these measures over time. A fuller assessment of how acquisition parameters affects changes in different phenotypes of volumetric breast density over time is warranted to inform future longitudinal research and clinical applications.

Longitudinal changes in qualitative and area-based breast density have consistently been associated with breast cancer risk, with the greatest changes in breast density corresponding to the largest differences in risk.[49,100,101,111,112] Furthermore, Kerlikowske et al. demonstrated that the use of multiple longitudinal measures of breast density improved clinical risk stratification for breast cancer.[50] This suggests that longitudinal trajectories of breast density may be a more relevant indicator of changes in breast cancer risk than measurement at a single timepoint. As women tend to experience accelerated changes in breast density over the menopause, [97–101] these

changes may be relevant indicators of postmenopausal breast cancer risk, thus the ability to capture longitudinal trajectories across menopause may offer enhanced risk stratification in the clinical setting. However, to date, studies of change in breast density and associated changes in risk have focused exclusively on two-dimensional breast density. Given the potential for use in clinical decision-making, future research is required to identify what magnitude of change in volumetric breast density is meaningful to reduce breast cancer risk.

Our study has both strengths and limitations. A major strength of our study is the prospective collection of risk factor data and multiple mammograms in healthy women across the menopausal transition, and the use of automated volumetric breast density measurement. We are of the first few studies[106] to report on longitudinal changes in dense breast volume using automated, volumetric breast density assessment that have the potential for use in clinical settings. Our study as several important limitations, including the use of self-reported menopause status, which is subject to measurement error. Errors in self-report are unlikely to be dependent on baseline risk factors; however, these non-differential errors may have biased the effects of risk factors on changes in DV towards the null. Postmenopausal HT use and formulation were self-reported, and lack of duration information makes it difficult to determine if the lack of effect of DV over time is real, or if the short average duration of use, or newer lower dose formulations account for the lack of an effect of HT on changes over time.

In summary, we found that the mean change in DV over the menopause was 2.2 cm3, and that women with higher premenopausal DV experienced the greatest declines in DV over the menopausal transition. Future research is warranted to determine what magnitude of change and timing of these changes in volumetric breast density is relevant for breast cancer risk.

Table 3.1. Characteristics of 2,586 women in the study.

	N (%) or Median (IQR)
Age at premenopausal (baseline) mammogram	51 (49, 52)
Time between mammograms (years)	3.1 (2.6, 3.5)
Baseline BMI (kg/m2)	23.3 (21.2, 26.5)
Change in BMI* (kg/m2)	0 (-0.5, 0.9)
Baseline Dense Volume (cm3)	54.0 (37.7, 77.8)
Baseline BMI (kg/m2)	
Normal (<25 kg/m2)	1708 (66.4%)
Overweight (25-29 kg/m2)	562 (21.9%)
Obese (>=30kg/m2)	302 (11.7%)
Race (Excludes 3 unknown)	
Caucasian	1451 (56.2%)
Asian	861 (33.3%)
Other	271 (10.5%)
Family History Breast Cancer	
No	2093 (81.2%)
Yes	484 (18.8%)
Parous	
No	873 (33.8%)
Yes	1712 (66.2%)
Age at First Birth	
Nulliparous	873 (33.8%)
<30 Years	692 (26.8%)
30+ Years	1020 (39.5%)
Alcohol Use	
None	1278 (50.7%)
<= 1 drink/day	954 (37.9%)
>= 2 drinks/day	288 (11.4%)
Hormone Therapy at any mammogram	
No/Unknown	2207 (85.3%)
Yes	379 (14.7%)

Table 3.1 (continued). Characteristics of 2,586 women in the study.

	N (%) or Median (IQR)
Type of HRT (Known HRT-users only)	
Ever Estrogen Only	262 (69.1%)
Ever Estrogen + Progesterone	99 (26.1%)
Unknown	18 (4.8%)
Number of Mammograms	
2	1766 (68.3%)
3	655 (25.3%)
4	165 (6.4%)

**Change in BMI was calculated from baseline premenopausal to postmenopausal mammogram*

BMI: Body Mass Index; HRT: hormone replacement therapy.

Table 3.2. Associations between demographic and risk factors and baseline (pre-menopausal) dense volume (DV).

	N (%)	*Dense Volume Mean (95% CI)**
Age at premenopausal (baseline) mammogram		
<50 years	776 (30.0%)	65.6 (63.0, 68.3)
>=50 years	1810 (70.0%)	61.0 (59.3, 62.7)
p-value		0.004
Baseline BMI (kg/m^2)		
Normal (<25 kg/m^2)	1708 (66.4%)	57.7 (55.9, 59.5)
Overweight (25-29 kg/m^2)	562 (21.9%)	70.5 (67.4, 73.6)
Obese (>=30kg/m^2)	302 (11.7%)	73.9 (69.7, 78.1)
p-value		<.001
Race		
Caucasian	1451 (56.2%)	67.8 (65.9, 69.7)
Asian	861 (33.3%)	52.0 (49.5, 54.4)
Other	271 (10.5%)	66.2 (61.7, 70.6)
p-value		<.001
Family History Breast Cancer		
No	2093 (81.2%)	61.1 (59.5, 62.7)
Yes	484 (18.8%)	68.0 (64.6, 71.3)
p-value		<.001
Parous		
No	873 (33.8%)	68.3 (65.8, 70.8)
Yes	1712 (66.2%)	59.4 (57.6, 61.2)
p-value		<.001
Age at First Birth		
Nulliparous	873 (33.8%)	68.3 (65.8, 70.8)
<30 Years	692 (26.8%)	56.1 (53.3, 58.9)
30+ Years	1020 (39.5%)	61.6 (59.3, 63.9)
p-value		<.001
Alcohol Use		
No	1278 (50.7%)	59.6 (57.5, 61.6)

Table 3.2 (continued). Associations between demographic and risk factors and baseline (pre-menopausal) dense volume (DV).

	N (%)	*Dense Volume Mean (95% CI)**
<= 1 drink/day	954 (37.9%)	64.3 (61.9, 66.7)
>= 2 drinks/day	288 (11.4%)	68.4 (64.0, 72.7)
p-value		<.001

**Differences in mean dense volume by covariates estimated by linear regression adjusted for age.*

Table 3.3. Effect of covariates on change in dense breast volume across menopause. (N=2568 subjects with complete BMI data)

	Annualized Change in DV (95% CI) cm3*	*interaction p-value*
Overall Change	-2.19 (-2.70, -1.68)	NA
Baseline DV		
Below median (<=54 cm^3)	-0.93 (-1.67, -0.20)	<0.0001
Above median (>54 cm^3)	-3.41 (-4.14, -2.68)	
Baseline BMI		
Normal (<25 kg/m^2)	-2.33 (-3.86, -0.80)	
Overweight (25-29 kg/m^2)	-2.02 (-2.64, -1.39)	0.7
Obese (>=30kg/m^2)	-2.54 (-3.64, -1.44)	
Postmenopausal Hormone Therapy		
Not Current	-2.13 (-2.67, -1.59)	
Current	-3.29 (-5.22, -1.36)	0.24
Unknown	-4.24 (-7.37, -1.12)	
Postmenopausal Hormone Therapy		
Not Current	-2.11 (-2.65, -1.58)	
Current Estrogen	-2.97 (-5.32, -0.63)	0.76
Current Estrogen + Progesterone	-2.58 (-6.13, 0.98)	
Family History of Breast cancer		
No family history	-2.21 (-2.78, -1.65)	0.87
Family History	-2.11 (-3.28, -0.93)	
Parity		
Nulliparous	-2.21 (-3.10, -1.32)	0.92
Parous	-2.15 (-2.78, -1.53)	

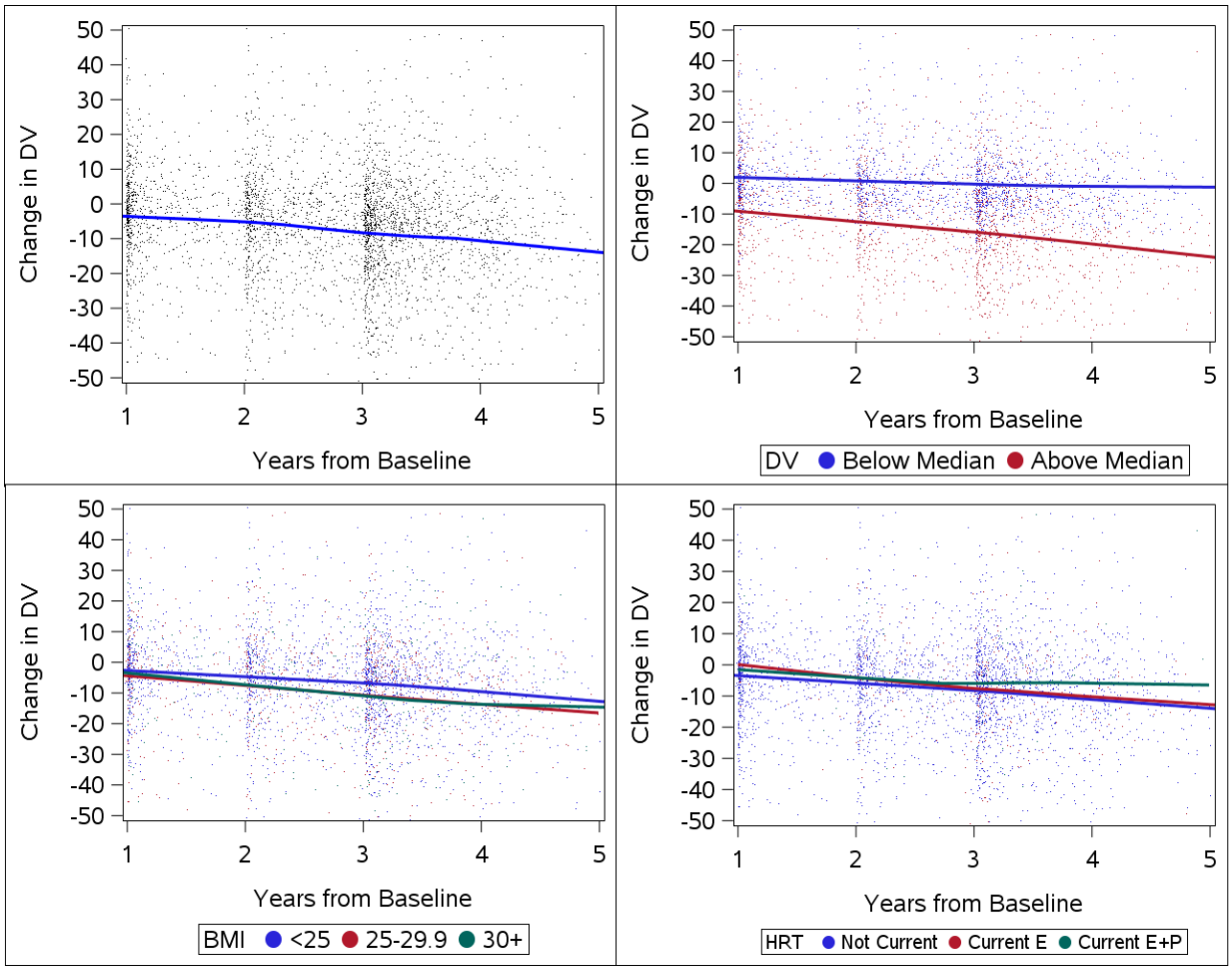
Table 3.3 (continued). Effect of covariates on change in dense breast volume across menopause. (N=2568 subjects with complete BMI data)

		Annualized Change in DV (95% CI) cm3*	*interaction p-value*
Age at First Birth			
	Nulliparous	-2.21 (-3.10, -1.32)	
	<30 Years	-2.45 (-3.46, -1.43)	0.77
	30+ Years	-1.98 (-2.76, -1.19)	
Race			
	Caucasian	-2.36 (-3.05, -1.68)	
	Asian	-1.68 (-2.56, -0.81)	0.36
	Other	-2.83 (-4.48, -1.17)	
Alcohol Use			
	None	-2.04 (-2.77, -1.32)	
	<=1/day	-2.76 (-3.59, -1.93)	0.10
	>=2 /day	-0.90 (-2.46, 0.67)	

*Coefficients estimated by linear mixed models adjusted for baseline age, BMI, log dense volume, BMI change across menopause, time from baseline mammogram and the interaction between dense volume and time from baseline and including a woman-specific random effect.

**The effect of each variable on change over time was estimated by fitting an interaction between time (years) and the baseline covariate of interest; p-values reflect overall interaction for each covariate.

Figure 3.1. Annualized changes in dense volume (DV) across the menopausal transition according to baseline characteristics.
Panel A: annualized changes in DV overall, *Panel B:* changes in DV according by baseline DV (above vs. below median DV), *Panel C:* changes in DV according to BMI; *Panel D:* changes by use of hormone replacement therapy. *BMI:* body mass index, *HRT:* hormone replacement therapy, *Current E:* current use of estrogen therapy, *Current E+P:* current use of combined estrogen & progesterone therapy.



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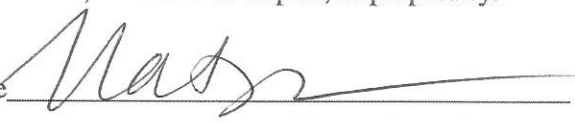
Author Signature  Date: March 14, 2018