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The effects of menopausal vasomotor symptoms and changes in anthropometry on breast cancer etiology

Victoria Hart
University of Massachusetts Amherst

THE EFFECTS OF MENOPAUSAL VASOMOTOR SYMPTOMS AND CHANGES IN ANTHROPOMETRY ON BREAST CANCER ETIOLOGY

A Dissertation Presented

by

VICTORIA HART

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Approved as to style and content by:

_____
Katherine W. Reeves, Chair

_____
Susan R. Sturgeon, Member

_____
Nicholas G. Reich, Member

_____
Lynnette Leidy Sievert, Member

_____
Paula Stamps, Graduate Program Director
Public Health

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ABSTRACT

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VICTORIA HART

B.S., CORNELL UNIVERSITY

M.S., UNIVERSITY OF VERMONT

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Katherine W. Reeves

Much about the etiology of breast cancer remains unknown. One of the strongest predictors of breast cancer risk is mammographic density; however, the mechanisms relating density to breast cancer risk are not fully understood and this has limited the use of mammographic density as a marker for breast cancer susceptibility. Hormone fluctuations during the menopausal transition may influence declines in mammographic density and may also trigger the onset of menopausal vasomotor symptoms (VMS), which have been associated with lower breast cancer risk. The effects of hormone changes on density, VMS, and ultimately breast cancer risk are complicated by external factors such as changing body mass and exogenous hormone therapy use during the menopausal transition.

We evaluated the longitudinal association between change in BMI and change in breast density among 24,556 women in the Breast Cancer Surveillance Consortium using a novel volumetric measurement method. We found that an annual increase in BMI was associated with a decrease in both absolute dense volume (β=-1.01 cm^3, 95% CI -1.59, -0.42) and in percent dense volume (β=-1.17%, 95% CI -1.31, -1.04). Longitudinal studies of density and breast cancer, or those using density to reflect breast cancer risk, should thus consider controlling for BMI gain/loss to understand the independent relationship between density and risk. We further

investigated the association of VMS and percent mammographic density (PMD) among 833 women enrolled Study of Women's Health Across the Nation (SWAN) Mammographic Density Sub-study. We observed no overall association between VMS and PMD (β = -0.47%, 95% CI -1.39, 0.45), although some evidence of an inverse association was found among perimenopausal women (β = -1.29%, 95% CI -2.58, -0.001) and those using hormone therapy (β = -3.62%, 95% CI -7.17, -0.07). These results suggest that an association between VMS and breast cancer risk is via a pathway that is not strongly mediated by changes in breast density. Finally, we evaluated VMS and incident breast cancer risk within the full SWAN cohort of 3,098 women. VMS were associated with a 38% reduction in breast cancer risk (OR 0.62, 95% CI 0.39, 0.99). Adjustment for endogenous hormone levels in this analysis did not alter our results, suggesting that endogenous hormones may play a lesser role in the observed association between VMS and breast cancer risk than has been previously hypothesized.

The results of these studies further our understanding of breast cancer etiology. If confirmed, the association between VMS and breast cancer risk could propose VMS as an easily measured factor that may be used to enhance risk prediction in clinical practice. Our findings that this association is not strongly mediated through breast density nor endogenous hormone levels raise provocative questions regarding the biological mechanisms that may link VMS to breast cancer risk. Extending our knowledge of breast cancer etiology using new measurement methods and novel risk factors may lead to improved risk prediction and provide opportunities for disease prevention.

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CHAPTER 1

THE EFFECT OF WEIGHT CHANGE ON VOLUMETRIC MEASURES OF MAMMOGRAPHIC DENSITY: A LONGITUDINAL STUDY IN THE BREAST CANCER SURVEILLANCE CONSORTIUM

1.1 Abstract

The impact of changing body mass index (BMI) on breast composition is unclear. The association between BMI and breast density is important in evaluating adjustment for BMI gain/loss in longitudinal studies of density and breast cancer risk. In cross-sectional analysis, BMI has been associated with lower dense breast area but higher dense breast volume. No studies to date have explored a longitudinal association using volumetric methods to assess density.

We examined the association between change in BMI and change in volumetric density in a population of 24,556 women who received breast imaging at the San Francisco site of the Breast Cancer Surveillance Consortium from 2007-2013. Height and weight were self-reported at the time of the mammogram. Breast density was assessed using single x-ray absorptiometry (SXA) volumetric measurement. The cross-sectional and longitudinal associations between BMI and absolute dense volume (DV), absolute non-dense volume (NDV) and percent dense volume (PDV) were assessed using multivariable adjusted regression.

The study population was primarily Caucasian (66%) or Asian (25%). Most women were postmenopausal (64%) and normal weight (63%) at first mammogram. In cross-sectional analysis, BMI was positively associated with DV (β=2.95 cm^3, 95% CI 2.69, 3.21) and inversely associated with PDV (β=-2.03%, 95% CI -2.09, -1.98). By contrast, in longitudinal analysis an annual increase in BMI was associated with an annual decrease in both DV (β=-1.01 cm^3, 95% CI -1.59, -0.42) and PDV (β=-1.17%, 95% CI -1.31, -1.04). These findings were consistent between pre- and postmenopausal women. Among premenopausal women, the annual decrease in DV

was only observed among women who were initially overweight or obese ($p<0.01$ for interaction by initial BMI).

Our findings support an inverse association between change in BMI and change in percent density. Longitudinal studies of BMI or PDV and breast cancer risk should consider adjusting for change in the other factor. The association between increasing BMI and decreasing absolute DV is unexpected and will require confirmation using SXA and other volumetric methods.

1.2 Introduction

Percent dense area (PDA), the ratio of dense breast tissue compared to total breast tissue on a mammographic image, is a known risk factor for primary breast cancer (1-4). However, the relative contributions of dense and non-dense tissue to breast cancer risk are not fully understood. Dense tissue reflects epithelial and stromal tissue where malignant growth may initiate, and larger amounts may provide more opportunity for abnormal cell development (5). Androstenedione is converted to estrogen in non-dense adipose tissue, and greater estrogen exposure also has been associated with increased risk (6). In cross-sectional analysis, higher body mass index (BMI) has been strongly linked to higher non-dense area (NDA) and weakly linked to lower dense area (DA) on a mammographic image (2, 7, 8). However, cross-sectional studies assessing dense breast volume have found positive associations between BMI and both non-dense volume (NDV) and dense volume (DV) (9-12). Several methods of assessing breast volume have been proposed as enhancements over area assessment (11, 13-15). Single X-ray absorptiometry (SXA), which calculates breast volume using a calibrated phantom included in the mammographic image, may more accurately predict breast cancer risk than area methods (15). BMI and breast density may negatively confound each other in their associations with breast cancer risk (8, 16, 17). Understanding the impact of BMI on breast composition is necessary to determine how these factors should be included and controlled for in studies of risk.

Neither breast density nor BMI are static values. It is understood that PDA decreases during aging, and that the rate of decline is greatest during the menopausal transition (18-20). A recent study showed that PDA declined less over time for women who went on to be diagnosed with breast cancer than for age-matched controls (21). It is unclear how changing BMI influences the decline in density. A limited number of longitudinal studies have shown an inverse relationship between change in BMI and change in PDA (22, 23), and have reported either a null (22) or positive (23) association between change in BMI and change in absolute DA. To date, no studies have evaluated the longitudinal effect of change in BMI on change in density using volumetric methods. This relationship must be established to understand the impact of adjusting for unstable BMI in longitudinal studies that use change in breast density to reflect breast cancer risk.

We examined the cross-sectional and longitudinal associations between BMI and breast density using the SXA volumetric density measurement method. Our analysis was conducted in the Breast Cancer Surveillance Consortium, a large cohort of women undergoing breast imaging. Based on prior cross-sectional analyses using volumetric methods (9-12), we hypothesized that an increase in BMI over the study period would be associated with an increase in both dense volume (DV) and non-dense volume (NDV), and a decrease in percent dense volume (PDV).

1.3 Methods

1.3.1 Study population

The Breast Cancer Surveillance Consortium (BCSC) was established by the National Cancer Institute in 1994. At each of seven locations, screening mammography data are linked to cancer registry information at each site then pooled across sites to provide a large sample of women. The BCSC sites were selected to represent urban, suburban, and rural locations and a

diverse racial/ethnic pool. As of February 2013, the full BCSC database included approximately 9.5 million mammograms and over 113,000 breast cancer cases (24).

The current study used data from the San Francisco Mammography Registry (SFMR), one of two BCSC sites at which volumetric mammographic density has been measured since 2007. At the time of their screening mammogram, women completed a self-administered, one-page questionnaire providing basic demographic, medical, and reproductive history information. The questionnaire allowed women to opt out of having their data included in BCSC research. The average opt out rate for the facilities contributing to the SFMR is 1.8% (range: 0.8-3.3%). The eligible study sample included women age 18 and older with two or more mammograms in 2007-2013 spaced at least nine months apart. Women with history of breast cancer, mastectomy, breast implants, or breast surgery were excluded from the study sample. Mammograms that were performed within 6 months of a breast cancer diagnosis were also excluded. The eligible sample consisted of 30,000 women who contributed 75,489 mammograms. A total of 1,149 mammograms were excluded due to poor placement of the SXA phantom in the mammographic image resulting in volumetric density measurement error. After this exclusion, 368 women had only one mammogram to contribute to the analysis and were therefore also excluded. These exclusions resulted in an eligible study population of 29,632 women and 73,972 mammograms (average 2.54 mammograms per woman). The average time between first and last contributed mammogram was 2.4 years (range 0.8-5.9 years).

1.3.2 BMI assessment

Women self-reported their current height in inches and weight in pounds at the time of their screening mammogram, and these data were used to calculate BMI. Women were excluded from the study sample if their reported height between mammograms varied by more than 3 inches (470 women, 1,197 mammograms). BMI was analyzed both in its original continuous

form and categorized according to WHO guidelines: <18.5 kg/m^2 for underweight, 18.5-<25 kg/m^2 for normal weight, 25-<30 kg/m^2 for overweight, and >=30 kg/m^2 for obese.

1.3.3 Volumetric breast density assessment

Dense breast volume and percent dense breast volume were measured using the single x-ray absorptiometry (SXA) technique. A complete description of the specific SXA imaging methods, development, and calibration processes can be found elsewhere (25, 26). Briefly, a specialized SXA phantom was included in the x-ray field during the mammography examination and was used to convert pixel grayscale values into unique volumes of adipose (non-dense) and fibroglandular (dense) breast tissue for each given pixel value (26). The SXA phantom was specifically designed to not interfere with standard screening procedures and to account for tilt of the compression surfaces during the examination (15, 26). The mean difference in PDV measurements between repeat readings using this technique has been demonstrated to be less than 2.5% (15).

1.3.4 Covariate assessment

Data on demographics, reproductive and menstrual history, family history, and brief medical history were obtained via the one-page questionnaire administered at the time of the screening mammogram. The covariates selected as potential confounders or effect modifiers for this analysis were based on known predictors of mammographic density and availability within the BCSC dataset. Covariates assessed at the time of the first screening mammogram and considered unchanging included age at first mammogram, race, ever given birth, age at first birth, education, first degree family history of breast cancer, and prior history of breast biopsy. Time-varying covariates included menopausal status, hormone therapy use, and hormonal birth control use. Consistent with previous analyses using BCSC data, women were considered postmenopausal if they reported that menstrual periods had stopped for more than 12 months, if

they reported a bilateral oophorectomy, or if they reported a hysterectomy and were 55 years of age or older. Women were otherwise considered premenopausal. Current use of hormone therapy and hormonal birth control were assessed at the time of the screening mammogram. The questionnaire did not include specific formulations of hormone therapy or history of prior use.

1.3.5 Statistical analysis

We additionally excluded 4,606 women (11,122 mammograms) who were missing data on one or more covariates included in the multivariable model. There were no statistically significant differences in the remaining covariates between women who were excluded from the analysis and those who were retained (data not shown). The final analytic sample included 24,556 women who contributed 61,653 mammograms.

We calculated descriptive statistics for available demographic and reproductive characteristics. We assessed the cross-sectional association between BMI at first mammogram and volumetric density measures at first mammogram using a generalized linear regression model adjusted for all covariates. Log transformations of density variables were used to satisfy normality assumptions. In longitudinal analyses we calculated annual change in BMI and volumetric density measures as (Δ value / Δ time, days) $\times$ 365.25 days/year to account for varying time lapse between mammograms. We categorized BMI change over the study period based on change from initial BMI (at first mammogram): $\geq$10% loss, 5-10% loss, stable within $\pm$5%, 5-10% gain, and $\geq$10% gain. We used ANOVA to compare the percent gain/loss based on initial BMI, using the underweight or normal range (<25 kg/m^2) as the reference. We summarized the annual change in each volumetric density measure over the study period and calculated adjusted means and confidence intervals using generalized linear regression.

We assessed the association between annual change in BMI and annual change in DV, NDV, and PDV using a random intercept mixed effects model. The mixed effects model is appropriate for data in which each subject may contribute a varying number of observations, and

the random intercept allows for individual subject variation in baseline density measures (27). The model was adjusted for all covariates listed above and time-varying factors were updated at each successive mammogram. We stratified all analyses by menopausal status (premenopausal, postmenopausal, or transitioned from pre- to postmenopausal) because the association between BMI and breast cancer risk has been shown to vary between pre- and postmenopausal women (28, 29). Because analyses have shown that declines in PDA over the menopausal transition may be modified by initial BMI and hormone therapy use (18, 19), we tested for effect modification by BMI at the first mammogram and by hormone therapy (never user, consistent user, initiated use during the study period, discontinued use during the study period).

All analyses were performed using SAS Version 9.2 (SAS Institute, Cary, North Carolina).

1.4 Results

A majority of the study population was Caucasian (66.3%) with 25.1% Asian or Pacific Islander (Table 1). The average age at the first mammogram was 56.4 years, and 64.1% of women were postmenopausal. Over half of the study population was classified as normal weight at the first mammogram (62.6%), while 24.2% were classified as overweight and 10.9% were classified as obese. About 12% of the study population was using hormone therapy at the time of the first mammogram.

In cross-sectional analysis, BMI at first mammogram was positively associated with both DV (β=2.95 cm^3, 95% CI 2.69, 3.21) and NDV (β=51.03 cm^3, 95% CI 49.93, 52.13) at first mammogram, and was inversely related to PDV at first mammogram (β=-2.03%, 95% CI -2.09, -1.98) (Table 2). The associations with NDV and with PDV were stronger among women who were initially premenopausal compared to those who were initially postmenopausal (p value for interaction by menopausal status: p=0.79 for DV, p<0.01 for NDV, p<0.01 for PDV). No

significant interaction by hormone therapy (HT) use was observed (p value for interaction by HT use: p=0.60 for DV, p=0.15 for NDV, p=0.81 for PDV).

A majority of women maintained stable weight within ± 5% of their initial BMI during the study period (73.6%) (Table 3). Regardless of menopausal status, a higher proportion of women who were initally overweight or obese lost over 5% of their initial BMI compared to women who were initially lean (premenopausal: 6.1%, 16.4%, and 21.2% of normal, overweight, and obese women, respectively, p<0.01; postmenopausal: 9.3%, 18.3%, and 23.9% of normal, overweight, and obese women, respectively, p<0.01). A higher proportion of initially overweight or obese premenopausal women gained over 5% of their initial BMI compared to initially lean premenopausal women (13.9%, 17.6%, and 17.0% of normal, overweight, and obese women, respectively, p = 0.01). No difference in weight gain by initial BMI was observed among postmenopausal women (13.3%, 13.6%, 13.0% of normal, overweight, and obese women, respectively, p=0.86).

The mean annual change in DV, NDV, and PDV over the study period was -0.56 cm^3/year, 6.09 cm^3/year, and -0.81 %/year, respectively (data not shown). A 1 kg/m^2 annual increase in BMI was associated with a statistically significant decrease in DV (β=-1.01 cm^3, 95% CI -1.59, -0.42), increase in NDV (β=26.2 cm^3, 95% CI 23.5, 28.9), and decrease in PDV (β=-1.17%, 95% CI -1.31, -1.04) in the full study population (Table 4). Results were strongest among premenopausal women, although significant interaction by menopausal status was only observed for NDV and PDV (p value for interaction by menopausal status: p=0.43 for DV, p=0.01 for NDV, p<0.01 for PDV). When further stratified by initial BMI, the significant annual decrease in DV was observed among premenopausal women who were intitally overweight or obese, but not among premenopausal women who were initially underweight or normal BMI (p value for interaction by initial BMI: p<0.01). Among postmenopausal women, annual decrease in DV was not statistically significant after stratification by initial BMI and no interaction was observed (p value for interaction by initial BMI: p=0.67). Likewise, no interaction between DV and initial

BMI was oberved among the women who transitioned from pre- to postmenopausal (p value for interaction by initial BMI: p=0.52). Regardless of menopausal status, the significant annual decrease in PDV associated with an increase in BMI was strongest among initially lean women and progressively weaker among initially overweight and obese women (p value for interaction by intial BMI: p<0.01 for all menopausal status groups). We observed no significant overall interaction by HT use (p value for interaction by HT use: p=0.44 for DV, p=0.15 for NDV, p=0.40 for PDV).

1.5 Discussion

Consistent with our expectations, we observed positive cross-sectional relationships between BMI and both DV and NDV, and an inverse cross-sectional relationship between BMI and PDV. We also observed that DV and PDV declined on average over the study period. However, in contrast to our expectations, we found that an annual increase in BMI was associated with an annual decrease in DV in longitudinal analysis. This finding was consistent among pre- and postmenopausal women, and varied by initial BMI among premenopausal women. An annual increase in BMI was further associated with an annual decrease in PDV among all menopausal status groups, as a result of increases in NDV associated with increasing BMI.

Our finding of a positive cross-sectional relationship between BMI and DV is consistent with studies using the SXA method (12) and other volumetric techniques (9, 10, 30), but in contrast with studies using area assessment (8, 31). Unlike area methods, which rely on a dichotomous separation of dense and non-dense area, the SXA method calculates a continuous value for DV based on the comparison of each pixel on the mammographic image to a known phantom (26). Continuous assessment of DV may provide a more accurate assessment of dense tissue than dichotomous area methods. Further, the SXA method includes water in adipose tissue in its calculation of DV (15). A recent study comparing area and SXA volume measurements reported that correlations between DA and DV were stronger among lean women than among

obese women as a result of this inclusion (12). The contribution of water from adipose tissue may partially account for the positive cross-sectional association between BMI and DV.

Our observed 1.17% annual decline in PDV associated with a unit annual increase in BMI is similar in magnitude to the two previous studies of change in BMI and PDA over time, which reported annual declines of 0.36% (22) and 1.44% (23). However, our observation of a decrease in DV with increasing BMI does not support their findings of no association (22) or positive association (23) between change in BMI and DA. The association between change in BMI and change in DA observed by Reeves *et al* (22) was in the same direction as our finding, but their results among 833 women were not statistically significant. Although we observed a significant annual decrease in DV, it should be noted that the magnitude, approximately 1 cm^3/year, is small compared to the initial average DV in our study population (142.3 cm^3) and compared to the difference in DV that was associated with increased breast cancer risk in a case-control analysis of 864 women using SXA (women in the highest quintiles of DV (192+ cm^3) had significantly higher risk compared to those in the lowest quintile (<122.3 cm^3)) (15).

We observed that the longitudinal association between BMI and DV was strongest among premenopausal women who were initially overweight or obese. These women were more likely to gain more than 5% of their initial weight during the study period than their lean counterparts. It is possible that the association between change in BMI and DV was easiest to observe among women who gained more weight, since a larger change in BMI may allow us to see the associated small change in DV. The inverse association between BMI and DV also was observed among postmenopausal women, but did not vary by initial BMI. Likewise, no difference in weight gain by initial BMI was observed among postmenopausal women who were postmenopausal at baseline. The biological mechanism linking an annual increase in BMI to an annual decrease in absolute DV is unclear. Like other volumetric methods, the SXA technique calculates breast volume based on the two-dimensional mammographic image. It is possible that our finding may reflect differences in capturing dense breast tissue on a mammographic image for large- versus

small-breasted women as opposed to a true reduction in DV with increasing BMI. Future studies using SXA and other volumetric measurement techniques are necessary to confirm our results.

Joint cross-sectional analyses of BMI and PDA and breast cancer risk suggest that these are independent breast cancer risk factors in both pre- and postmenopausal women (8, 17, 32), and that the association of either factor with breast cancer risk is strengthened after adjustment for the other factor (8, 32). Therefore studies of either factor should control for the other to avoid negative confounding. The significant associations that we observed between change in BMI and change in density measures suggest that longitudinal studies of volumetric density and breast cancer risk should additionally consider adjusting for change in BMI (i.e. weight gain or loss) to fully understand the independent effect of change in volumetric density on breast cancer risk. Further, longitudinal studies using change in density outcomes as an indicator of changing breast cancer risk should carefully evaluate the potential for confouding by gain or loss in BMI and also consider adjustment as necessary.

Our study is strengthened by the large number of participants and by the fact that height and weight were reported at the time of the screening mammogram. In addition, volumetric density was assessed using a validated method that has been found to more accurately predict breast cancer risk than area assessment (15). Our results must be interpreted in context of the study limitations, however. First, height and weight were self-reported. Validity of self-reported height and weight measures has been assessed within a subset of the BCSC cohort (12) and the Spearman correlation coefficient between BMI from self-reported and measured values was 0.949 (95% CI 0.938, 0.957). Second, our ability to adjust for confounding variables is limited by the information collected on the BCSC questionnaire. The questionnaire was designed to minimize burden on women at their routine screening mammogram and therefore includes fewer questions than might be asked during a targeted research study. We have adjusted for confounding to the extent possible, but residual confounding by other factors related to BMI and breast density may exist. Lastly, our study sample was primarily Caucasian and Asian women. Both

mammographic density (33, 34) and body composition (28) have been shown to vary by racial/ethnic group, meaning that our findings may not be generalizable to a more diverse population.

The independent and collective roles of BMI and breast density in breast carcinogenesis are unclear and are complicated by the dynamic nature of both body and breast composition over time. This analysis supports the current understanding that an increase in BMI is associated with a decrease in percent density and suggests that change in PDV over time may differ based on starting BMI. Our unexpected finding of a decrease in DV with increasing BMI is contrary to our current understanding of the mechanisms relating dense breast tissue, BMI, and breast cancer risk. Confirmation using other volumetric techniques is required to ensure that our finding is not the result of chance or an artifact of our measurement method.

Table 1: Selected patient characteristics measured at first mammogram (N=24,556); BCSC SFMR 2007-2013

	Study population N (%)
General characteristics	
Age at mammogram (years); Mean (SD)	56.4 (10.9)
BMI (kg/m^2); Mean (SD)	24.5 (4.6)
Underweight: <18.5	583 (2.4)
Normal weight: 18.5 - <25	15,363 (62.6)
Overweight: 25 - <30	5,938 (24.2)
Obese: 30+	2,672 (10.9)
Race	
Caucasian	16,268 (66.3)
African-American	519 (2.1)
Asian / Pacific Islander	6,184 (25.2)
Other	1,585 (6.4)
Education level	
< High school	747 (3.0)
High school diploma	1,820 (7.4)
Some college	4,970 (20.2)
College degree	17,019 (69.3)
First degree family history of breast cancer	4,787 (19.5)
Previous breast biopsy	5,785 (23.6)
Reproductive history	
Menopausal status	
Premenopausal	8,355 (34.0)
Postmenopausal	15,734 (64.1)
Ever given birth	16,315 (66.4)
Age at first birth	
Nulliparous	8,183 (33.3)
< 20 years	835 (3.4)
20 - 29 years	7,681 (31.3)
30 - 39 years	7,031 (28.6)
40+ years	826 (3.4)
Hormone therapy use (at first mammogram)	3,001 (12.2)
Birth control hormone use (at first mammogram)	1,493 (6.1)
Tamoxifen or raloxifene use (at first mammogram)	204 (< 1.0)

Percentages may not add to 100% due to unknown values (<10% for any characteristic)

Table 2: Cross-sectional association between BMI and volumetric density measures at first mammogram; BCSC SFMR 2007-2013

	N (women)	Mean (SD) at first mammogram	β (SE) [a]	95% CI	P value
All women	**24,556**				
Dense breast volume (cm^3)		142.3 (76.7)	2.95 (0.13)	2.69, 3.21	<0.001
Non-dense breast volume (cm^3)		416.8 (331.0)	51.03 (0.56)	49.93, 52.13	<0.001
Percent dense breast volume (%)		32.5 (19.5)	-2.03 (0.03)	-2.09, -1.98	<0.001
Premenopausal at first mammogram	**8,355**				
Dense breast volume (cm^3)		166.7 (83.7)	3.05 (0.27)	2.51, 3.58	< 0.001
Non-dense breast volume (cm^3)		327.1 (303.2)	53.64 (0.98)	51.72, 55.55	< 0.001
Percent dense breast volume (%)		42.3 (21.3)	-2.88 (0.06)	-3.00, -2.77	< 0.001
Postmenopausal at first mammogram	**15,734**				
Dense breast volume (cm^3)		129.1 (69.1)	2.97 (0.16)	2.67, 3.28	< 0.001
Non-dense breast volume (cm^3)		465.6 (336.2)	49.96 (0.69)	48.61, 51.31	< 0.001
Percent dense breast volume (%)		27.3 (16.2)	-1.67 (0.03)	-1.73, -1.61	< 0.001

[a] Adjusted for age at first mammogram, race, education, first degree family history of breast cancer, history of breast biopsy, ever given birth (yes/no), menopausal status at first mammogram (all women), and hormone use at first mammogram

Stratified results exclude 467 women with unknown menopausal status at first mammogram

Table 3: Change in BMI from first mammogram to last mammogram, stratified by menopausal status and by BMI at first mammogram (kg/m^2); BCSC SFMR 2007-2013

	N (women)	> 10% loss N (%)	5-10% loss N (%)	Stable ±5% N (%)	5-10% gain N (%)	> 10% gain N (%)
All women	**24,556**	**839 (3.4)**	**2,177 (8.9)**	**18,059 (73.6)**	**2,584 (10.5)**	**897 (3.6)**
Premenopausal (all exams)	**7,266**					
Underweight or normal: <25	5,203	45 (0.9)	271 (5.2)	4,163 (80.0)	558 (10.7)	166 (3.2)
Overweight: 25 - <30	1,487	71 (4.8)	172 (11.6)	983 (66.0)	185 (12.4)	78 (5.2)
Obese: 30+	576	57 (9.9)	65 (11.3)	356 (61.8)	78 (13.5)	20 (3.5)
Postmenopausal (all exams)	**15,715**					
Underweight or normal: <25	9,682	168 (1.7)	735 (7.6)	7,489 (77.4)	956 (9.9)	331 (3.4)
Overweight: 25 - <30	4,093	239 (5.8)	513 (12.5)	2,787 (68.1)	417 (10.2)	137 (3.4)
Obese: 30+	1,940	191 (9.9)	272 (14.0)	1,224 (63.1)	181 (9.3)	72 (3.7)
Transition from pre- to postmenopausal	**1,089**					
Underweight or normal: <25	728	14 (1.9)	57 (7.8)	519 (71.3)	100 (13.7)	38 (5.2)
Overweight: 25 - <30	260	17 (6.5)	27 (10.4)	166 (63.9)	33 (12.7)	17 (6.5)
Obese: 30+	101	12 (11.9)	14 (13.9)	59 (58.4)	9 (8.9)	7 (6.9)

Stratified results exclude observations for 486 women with unknown menopausal status at one or more mammogram(s)

Table 4: Association between annual change in BMI and annual change in volumetric density measures, stratified by menopausal status and by BMI at first mammogram (kg/m^2); BCSC SFMR 2007-2013

	N	Annual change in dense breast volume (cm^3 per year)			Annual change in non-dense breast volume (cm^3 per year)			Annual change in percent dense breast volume (% per year)		
	(women)	β (SE) [a]	95% CI	p value	β (SE) [a]	95% CI	p value	β (SE) [a]	95% CI	p value
All women	**24,556**	**-1.01 (0.30)**	**-1.59, -0.42**	**0.001**	**26.2 (1.38)**	**23.45, 28.87**	**< 0.001**	**-1.17 (0.07)**	**-1.31, -1.04**	**< 0.001**
Premenopausal (all exams)	**7,266**	**-1.73 (0.73)**	**-3.17, -0.30**	**0.02**	**32.64 (2.63)**	**27.47, 37.80**	**< 0.001**	**-1.83 (0.14)**	**-2.10, -1.56**	**< 0.001**
Underweight or normal: <25	5,203	0.92 (0.74)	-0.52, 2.37	0.21	31.17 (2.65)	25.97, 36.37	< 0.001	-2.84 (0.23)	-3.30, -2.38	< 0.001
Overweight: 25 - <30	1,487	-2.71 (0.98)	-4.63, -0.80	0.01	33.56 (4.50)	24.74, 42.37	< 0.001	-1.47 (0.21)	-1.87, -1.07	< 0.001
Obese: 30+	576	-3.29 (1.90)	-7.01, 0.44	0.08	32.63 (6.48)	19.93, 45.32	< 0.001	-0.86 (0.17)	-1.18, -0.53	< 0.001
Postmenopausal (all exams)	**15,715**	**-0.74 (0.32)**	**-1.36, -0.12**	**0.02**	**24.08 (1.63)**	**20.89, 27.27**	**< 0.001**	**-0.95 (0.08)**	**-1.10, -0.80**	**< 0.001**
Underweight or normal: <25	9,682	-0.32 (0.32)	-0.95, 0.31	0.32	22.93 (2.95)	17.15, 28.70	< 0.001	-1.51 (0.20)	-1.91, -1.11	< 0.001
Overweight: 25 - <30	4,093	-0.94 (0.66)	-2.23, 0.36	0.16	31.09 (1.73)	27.70, 34.47	< 0.001	-1.08 (0.11)	-1.29, -0.87	< 0.001
Obese: 30+	1,940	-0.53 (0.61)	-1.73, 0.67	0.39	20.57 (2.56)	15.55, 25.58	< 0.001	-0.35 (0.09)	-0.52, -0.17	< 0.001
Transition from pre- to postmenopausal	**1,089**	**-1.40 (1.85)**	**-5.02, 2.23**	**0.45**	**24.20 (5.15)**	**14.11, 34.29**	**< 0.001**	**-1.48 (0.27)**	**-2.02, -0.94**	**< 0.001**
Underweight or normal: <25	728	-0.62 (1.22)	-3.02, 1.78	0.61	33.24 (4.76)	23.91, 42.58	< 0.001	-2.89 (0.43)	-3.73, -2.05	< 0.001
Overweight: 25 - <30	260	-2.81 (1.30)	-5.36, -0.27	0.03	22.80 (9.92)	3.35, 42.25	0.02	-1.25 (0.49)	-2.21, -0.29	0.01
Obese: 30+	101	0.21 (4.70)	-9.00, 9.43	0.96	17.94 (8.42)	1.44, 34.44	0.03	-0.35 (0.33)	-0.99, 0.29	0.28

[a] Adjusted for age at first mammogram, age at first birth, history of breast biopsy, education, ever given birth (yes/no), first degree family history of breast cancer, hormone use during study period, menopausal status (all women), and race

Stratified results exclude observations for 486 women with unknown menopausal status at one or more mammogram(s)

P value for interaction by menopausal status: DV p=0.43, NDV p=0.01, PDV p<0.01

P value for interaction by BMI at first mammogram: premenopausal women: DV p<0.01, NDV p=0.87, PDV p<0.01
postmenopausal women: DV p=0.67, NDV p<0.01, PDV p<0.01
transitioning women: DV p=0.52, NDV p=0.24, PDV p<0.01

CHAPTER 2

MENOPAUSAL VASOMOTOR SYMPTOMS AND MAMMOGRAPHIC DENSITY IN THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION

2.1 Abstract

Declines in endogenous estrogen during menopause have been independently linked to the onset of menopausal vasomotor symptoms (VMS) and to reduced breast cancer risk. Percent mammographic density (PMD) is viewed as a marker for breast cancer susceptibility. A relationship between VMS and PMD may improve understanding of breast cancer etiology and justify future investigations of VMS and breast cancer risk.

We investigated this association among 833 women enrolled in the Study of Women's Health Across the Nation (SWAN) Mammgraphic Density Substudy. Women were pre- or perimenopausal at enrollment and followed for six annual visits. VMS were self-reported at annual SWAN visits. PMD was ascertained from routine screening mammograms. A linear mixed effects model was used to evaluate the longitudinal association between VMS and PMD.

Women contributed a total of 4,748 mammograms (2-10 per woman) over a median 5.4 years of follow-up. We observed no overall association between VMS and PMD. Among perimenopausal women, VMS was associated with significantly lower PMD (β = -1.29%, 95% CI -2.58, -0.001). Similar results were observed among those with unknown menopausal status due to hormone use during follow-up (β = -3.62%, 95% CI -7.17, -0.07). Among women who transitioned to postmenopause without surgery, VMS was not associated with change in PMD across the menopausal transition.

Although our findings do not demonstrate a consistent relationship between VMS and PMD, we did observe an association among perimenopausal women and those using hormone therapy during the menopausal transition. Further prospective studies are needed to determine the

extent to which an observed decrease in breast cancer risk among women with VMS may be mediated by PMD.

2.2 Introduction

Menopausal vasomotor symptoms (VMS), which include hot flashes and night sweats, are frequently reported by women during menopause, occurring in up to 75% of women during and after the menopausal transition (35-38). Two recent case-control studies (39, 40) and one prospective study (Hart, in preparation) observed a 40-50% reduction in breast cancer risk among women who experienced VMS at any point during the menopausal transition compared to those who did not.

The mechanism that triggers the onset of VMS in symptomatic women may be related to a mechanism responsible for lower breast cancer susceptibility. Higher levels of endogenous estrogens have been positively associated with postmenopausal breast cancer risk (41); whereas the fluctuation and eventual decline in estrogen levels prior to menopause appears to be related to VMS onset. Previous work demonstrates that estrogen fluctuations may be responsible for a narrowing of the thermoneutral range (42), for a lack of responsiveness to thermal changes at the skin vasculature (43), and for changes in the regulation of central nervous system chemicals that trigger thermoregulatory response (44, 45). Hormone therapy (HT) has been shown to be a consistently effective treatment for VMS (46), indicating that the regulation of estrogen levels is important to the management of symptoms. However, non-hormonal treatments also have been shown to relieve VMS (47, 48), suggesting that VMS may be triggered by factors other than fluctuating hormone levels.

Percent mammographic density (PMD), the proportion of dense epithelial and connective breast tissue compared to total breast tissue on a mammographic image (2), has been consistently demonstrated as a strong risk factor for breast cancer (49), and high PMD is viewed as a marker

of breast cancer susceptibility (50). The role of menopausal hormone fluctuations on PMD is unclear, although studies have shown consistent declines in PMD and dense breast area with age and across the menopausal transition (18, 19, 51). Investigations also have found positive associations between PMD and circulating estradiol (52, 53). Declines in PMD during menopause appear to be modified by HT use, further suggesting hormonal influences on changes in breast tissue (18, 19). Common hormonal mechanisms affecting VMS and PMD provide justification for examining the relationship between these factors, and have the potential to provide prospective information regarding VMS and future breast cancer risk among healthy women.

We evaluated the association of VMS with PMD in the Study of Women's Health Across the Nation (SWAN), a large prospective cohort of women transitioning through menopause. We anticipated that VMS would be associated with lower PMD and indicative of a lower susceptibility to breast cancer. Specifically, we hypothesized that VMS would be inversely associated with PMD, overall and within each menopausal stage. We additionally hypothesized that women who experienced VMS while pre- or perimenopausal would have a greater decline in PMD across the menopausal transition compared to women who did not experience VMS while pre- or perimenopausal.

2.3 Methods

2.3.1 Study population

The Study of Women's Health Across the Nation (SWAN) was designed to characterize biological and psychosocial changes over the menopausal transition in a multiracial/ethnic cohort. A detailed description of the SWAN design and recruitment procedures is provided elsewhere (54). Briefly, each of seven SWAN sites recruited women starting in 1996, with certain locations oversampling from specific racial/ethnic groups to create a diverse cohort. Baseline eligibility

criteria for SWAN enrollment included being aged 42-52 years, having an intact uterus and at least one ovary, not being pregnant or lactating, not using oral contraceptives or hormone therapy, and having a menstrual cycle in the three months before enrollment. A total of 3,302 women were enrolled, and each participant provided written informed consent at the location of enrollment. Baseline clinical assessments were performed in 1996-1997 and annual follow-up assessments are on-going.

The current study involved SWAN participants enrolled in the ancillary Mammographic Density Sub-study. This sub-study was designed to examine factors related to mammographic density and changes in mammographic density over the course of the menopausal transition. Women at three SWAN sites were enrolled in the sub-study during follow-up visit 05 or 06 (N=1,055), representing four racial/ethnic groups. African-American women were enrolled from the Pittsburgh, PA site, Chinese women from the Oakland, CA site, and Japanese women from the Los Angeles, CA site. Caucasian women were enrolled from all three locations. Separate written informed consent was obtained from these women to obtain prior mammograms taken at routine screenings up to two years prior to the baseline SWAN visit through two years after follow-up visit 06. Women with a previous breast surgery in both breasts (i.e., breast augmentation, reduction or reconstruction) were not eligible for the Mammographic Density Sub-study. At least one mammogram was obtained from 95.5% of the eligible sub-study sample (N=1,007).

The current study excluded six women with a history of breast cancer at SWAN enrollment. A further 21 women were diagnosed with breast cancer during the follow-up period and were censored at the time of their diagnosis; however, ten of these women had no mammograms available prior to diagnosis and were therefore excluded completely. The outcome of the current study was change in PMD across the menopausal transition; therefore, the 139 women with only one eligible mammogram were excluded, leaving 852 participants in the study

population. Three women reported being pregnant or breastfeeding during the follow-up period, and information from these specific visits also was excluded.

2.3.2 Vasomotor symptom assessment

At the baseline and each follow-up visit, SWAN participants completed a self-administered questionnaire that included questions related to hot flashes and night sweats. The questions were worded as follows: "Thinking back over the last two weeks, how often have you had hot flashes or flushes / night sweats?" Response categories were: not at all, 1-5 days, 6-8 days, 9-13 days, every day. Consistent with previous analyses in the SWAN cohort (55-57), women who reported any hot flashes or night sweats (versus not at all) were classified as having VMS at that visit. Women who reported having hot flashes or night sweats on 6 or more days in the last two weeks were classified as having frequent VMS at that visit (38), while women who reported having hot flashes or night sweats 1-5 days in the last two weeks were classified as having infrequent VMS at that visit.

2.3.3 Mammographic density assessment

Mammographic density assessments for the obtained mammograms were performed by a single expert reader using a compensating polar planimeter (LASICO, Los Angeles, CA) to measure total breast area and dense breast area in cm^2 on the craniocaudal view of the right breast. Mammograms from the left breast were used for density assessment when a woman reported biopsy or other surgery in the right breast or when films from the right breast were unavailable. Percent density was calculated by dividing the area of dense breast tissue by the total area of the breast. A blinded random sample of mammogram films was sent to the reader for re-review to assess the reproducibility of the density assessments. The initial and repeat readings resulted in a within-person Spearman correlation coefficient of 0.96 and a mean difference in percent density assessment of 2.2% (22, 58).

Because mammograms were retrospectively collected from routine screening visits, mammogram dates did not typically coincide with SWAN visit dates. As a result, a difference of several months may have existed between the collection of VMS and covariate information and mammographic density assessment. We addressed this issue by matching each mammogram date to the closest SWAN visit date (before or after the mammogram) for mammograms that occurred within 90 days of a SWAN visit date (48.6% of eligible mammograms). For the remaining mammograms, we used a novel interpolation method to estimate mammographic density at the time of the SWAN visit dates using linear interpolation with multiple imputation to account for error in the estimation. This method was developed by Reeves *et al* for the study of changes in anthropometry with respect to mammographic density in SWAN and may provide more accurate estimations of mammographic density at the time of the SWAN visit by accounting for the lack of concordance between the timing of the mammogram and the timing of the SWAN visit. Details and validation of this method are provided elsewhere (22, 59).

2.3.4 Covariate assessment

Information on covariates was collected during annual SWAN follow-up visits as part of the clinical assessment or by interviewer- or self-administered questionnaires. The covariates selected as potential confounders or effect modifiers for this analysis were consistent with previous investigations of mammographic density in the SWAN cohort (22, 58, 60, 61). The following covariates were measured at baseline and considered unchanging: race/ethnicity, age at first birth, age at menarche, education, alcohol intake, smoking, and SWAN site. The following additional covariates were considered time-varying and updated at each follow-up visit: menopausal status, body mass index (BMI), parity, family history of breast cancer, hormone therapy (HT) use, and oral contraceptive (OC) use. We considered both active and passive exposure to smoking in our analysis, because previous investigations in the SWAN cohort have

observed differences in these exposures with respect to both VMS and PMD (38, 62). Consistent with the analysis of smoking and PMD by Butler *et al* (62), we categorized our smoking variable as: never smoked/no passive exposure, never smoked/with passive exposure, former smoker, current smoker.

Prior HT use was assessed during the baseline interview. By study design, women were not currently using HT at study enrollment but could initiate HT use during follow-up. Past year HT use was assessed at each annual follow-up interview. Women were asked to separately report the use of estrogen, progestin, and estrogen/progestin combination therapies and formulations were confirmed when possible using container labels. Menopausal status was classified in accordance with SWAN protocol (54): women with no change in menstrual regularity over the past year were considered premenopausal; women with decreased menstrual regularity in the past three months were considered early perimenopausal; women with no menstrual bleeding in the 3-11 months before the interview date were considered late perimenopausal; and those with no bleeding in the last 12 months were considered postmenopausal. Early and late perimenopause were collapsed into a single perimenopause category for this analysis. Women who reported bleeding in the previous 12 months and reported HT use in the previous year were reclassified as unknown menopausal status due to HT use for those visits. Postmenopausal women remained classified as such, regardless of HT use initiated after the final menstrual period. Women reporting a hysterectomy or bilateral oophorectomy were classified as surgically postmenopausal starting at the visit at which the surgery was reported.

2.3.5 Statistical methods

After applying the matching and interpolation methods described above, 19 women no longer had two eligible mammograms matched to SWAN visits and were therefore excluded from the study, leaving a total of 833 women. We calculated descriptive statistics for demographic and

reproductive characteristics of the study sample and summarized VMS experience in the population during the study period overall and by menopausal status.

Association of VMS with PMD: We used a linear mixed effects model to assess the longitudinal association between VMS and PMD across the study period while accommodating varying numbers of observations per woman and within-woman correlation. We included a random intercept term to account for differing baseline PMD values. We evaluated a random slope term to account additionally for differing changes in PMD over the study period; however, this term did not significantly enhance the model fit, so we performed the analysis using the simpler model. The multivariable model was developed using methods of best selection. We assessed the univariable associations between each covariate and the exposure and outcome to identify potential confounders. Confounders were retained in the multivariable model if they were statistically significant ($p<0.05$) in the model or if their removal resulted in a change in the regression coefficient for the VMS exposure of 10% or more.

To differentiate between symptomatic and non-symptomatic women, we modeled VMS at each study visit as VMS reported at that visit or any prior visit. Therefore, a woman reporting VMS at a study visit was considered symptomatic from that visit forward. This strategy was replicated for frequent VMS (i.e., a woman reporting frequent VMS at a study visit was considered symptomatic of frequent VMS from that visit forward). HT use was modeled similarly to comprehensively capture use of exogenous hormones during the study period. Because BMI is strongly associated with both VMS (38) and PMD (33), we created separate models with and without adjustment for BMI. We assessed interactions with VMS by race/ethnicity, HT use, and menopausal status using cross-product terms and where statistically significant interactions were observed, we stratified the results by levels of the interaction variable. Because hysterectomy and/or bilateral oophorectomy may be related to VMS (37), we repeated our analyses in the subset of women who had not undergone either of these procedures.

VMS and change in PMD: To assess the change in PMD over study period, we created an outcome variable to represent the difference between the earliest and latest PMD observation for each woman and an additional variable to quantify the time difference between these observations. We used linear regression to determine adjusted mean change in PMD over the study period and to test for differences by VMS experience, adjusting for the time difference in our analysis. This analysis was restricted to women who non-surgically transitioned from pre- or early perimenopausal to postmenopausal during the study period (N=426).

All analyses were performed using SAS Version 9.2 (SAS Institute, Cary, North Carolina).

2.4 Results

The 833 women included in the study population contributed a total of 4,748 mammograms (median 4, range 2-10 mammograms per woman). The average time between mammograms was a median of 469 days (interquartile range 385-728 days). On average, women were 47 years old at SWAN enrollment and were premenopausal (58%) (Table 5). A majority of the study population was Caucasian (49%) or Asian (44%), and most reported having a college education (54%). Overall, 51% of women reported any VMS during at least one SWAN study visit. This varied by menopausal stage, with 28%, 58% and 46% of women reporting having ever experienced symptoms while pre-, peri-, or postmenopausal, respectively.

Association of VMS with PMD: In the full study sample, no significant difference in PMD was observed between symptomatic and non-symptomatic women after adjustment for covariates including BMI (β = -0.47%, 95% CI -1.39, 0.45) (Table 6). Among women who reported HT use during the study period, results suggested an inverse relationship between VMS and PMD (β = -3.02%, 95% CI -5.59, -0.52), although this association was attenuated and not statistically significant after adjustment for BMI (β = -2.31%, 95% CI -4.83, 0.21) (Table 6).

No interaction was observed between VMS and race/ethnicity (p=0.19); however, a significant interaction was observed by menopausal status (p<0.01). During perimenopausal visits, symptomatic women had significantly lower PMD than non-symptomatic perimenopausal women, even following adjustment for BMI (β = -1.29%, 95% CI -2.58, -0.001) (Figure 1). This finding was stronger among women who experienced frequent VMS, although the confidence interval was wider and non-significant (β = -2.13%, 95% CI -4.39, 0.24). A similar, significant relationship was observed among women with unknown menopausal status due to HT use (β = -3.62%, 95% CI -7.17, -0.07) and was again strongest in women who were symptomatic of frequent VMS (β = -6.07%, 95% CI -11.4, -0.77). When grouped by type of HT use, the relationship was evident among women of unknown menopausal status using progestin or estrogen/progestin combination HT (β = -4.61%, 95% CI -8.38, -0.84), but not among similar women using estrogen only HT (β = 3.09%, 95% CI -9.23, -15.4) (Figure 1). Results were similar when the 54 women who reported a hysterectomy and/or bilateral oophorectomy during the study period were excluded from the analyses (data not shown).

VMS and change in PMD: A total of 426 women fully transitioned from pre- or perimenopause to postmenopause during the study period without a surgically induced menopause. Compared to women who did not transition to postmenopause during the study period, these women were slightly older (average age at enrollment 47.7 years compared to 45.2 years), reported fewer perimenopausal visits (average 3.4 visits compared to 4.9 visits), were more likely to report VMS during perimenopausal visits (50.4% of visits compared to 45.8% of visits), and were more likely to use HT during the study period (48% compared to 39%). Average age at menopause for these women was 52.7 years.

Although not statistically significant, symptomatic women had slightly higher starting and ending PMD than non-symptomatic women (starting PMD: average 43.5% compared to 42.0%; ending PMD: average 37.3% compared to 35.7%) (Table 7). No significant difference

was observed between symptomatic and non-symptomatic women in the change in PMD across the menopausal transition. The adjusted mean decrease in PMD was 5.3% for symptomatic women compared to an adjusted mean decrease of 5.4% for non-symptomatic women (p=0.97) (Table 7).

2.5 Discussion

In this racially/ethnically diverse prospective cohort, we observed no association between VMS and PMD in the overall study population or among premenopausal or postmenopausal women. Lower PMD was observed among women who were symptomatic for VMS in both the perimenopausal group and the group with unknown menopausal status due to HT use, which likely includes primarily women who are truly perimenopausal. Among women who transitioned to postmenopause without surgery during the study period, we observed no significant difference in the decline in PMD over the menopausal transition for symptomatic compared to non-symptomatic women.

Two case-control studies reported significantly lower breast cancer risk among women who experienced VMS during the menopausal transition compared to those who did not (39, 40). However, results from prospective studies have been mixed. No association was observed in a large prospective cohort with 13.7 years of follow-up and VMS assessment at three year intervals (63), but a significant 38% reduction in breast cancer risk was observed in a prospective investigation within the full SWAN cohort with 11.4 years of follow-up and annual VMS assessment (Hart, in preparation). The mechanisms linking VMS to breast cancer risk are not well understood, and PMD may offer one pathway through which this association may be observed. However, the biological connection between VMS and PMD, and ultimately breast cancer risk, is complex and may be influenced by numerous factors. Fluctuating estrogen levels during menopause may disrupt the thermoregulatory system that triggers VMS (42). However,

all women experience estrogen decline during menopause but not all women experience VMS, indicating that estrogen withdrawal alone cannot account for VMS onset. Significant associations have been observed between PMD and circulating estradiol (52, 53), but these findings are inconsistent (64-67); and two investigations of the combined effects of circulating estradiol and PMD concluded that hormone levels and PMD are independent risk factors for breast cancer and only weakly related to each other (68, 69). These associations are further complicated by HT use and BMI, which are each independently associated with VMS (38, 46), PMD (33, 70-72), and breast cancer risk (6, 41, 73). We have carefullly adjusted for these factors in our analyses, and our overall null results suggest that the previously reported breast cancer risk reduction among women symptomatic for VMS is not likely mediated through observable effects of these factors on PMD.

Although we did not observe an overall relationship between VMS and PMD, an association was found among perimenopausal women, when VMS are typically the most prevalent (35, 74), and among women with unknown menopausal status due to HT use, which is often prescribed for the management of menopausal symptoms (46). Thus our findings suggest that VMS may be associated with PMD while women are experiencing the most frequent or intense VMS. Use of progestin or estrogen/progestin combination HT has been shown to be associated with higher PMD (70-72). The significant association between VMS and lower PMD among women using HT, and particularly progestin or combination HT (Figure 1), may be observable because higher initial PMD makes it easier to witness a reduction in PMD; however, future investigation would be required to substantiate this hypothesis. The magnitude of the change in PMD associated with VMS in our study (4.6%) is similar to the decrease in PMD for parous versus nonparous women (approximately 2% per pregnancy (75)), but smaller than the typical decline in PMD for all women over the menopausal transition (mean 7.7%, 95% CI 1.9-14.4% (18)). Significant associations were not observed among postmenopausal women in our

study, regardless of HT use (Figure 1). These results may be partially explained by a lack of statistical power in these subgroups or by our definition of VMS, which classified a woman as symptomatic from the point at which VMS were first reported. If the effect of VMS on PMD was only evident during the time at which VMS were experienced, this definition of VMS could have contributed to the observed attenuation of results among postmenopausal women.

To our knowledge, no previous study has investigated the change in PMD in women undergoing the menopausal transition in relation to their VMS experience. Consistent with previous investigations (18, 19, 76), PMD declined on average over follow-up for our study sample; however, we observed no difference in the change in PMD for symptomatic versus non-symptomatic women. Our findings of significantly lower PMD among symptomatic women during perimenopause, when VMS may be most frequent or intense, suggest that differences in the rate of change in PMD may be restricted to the perimenopause phase of the menopausal transition. However, secondary analysis of the change in PMD across only perimenopausal visits did not show evidence of a difference by VMS experience (data not shown). These results support our assertion that the observed associations between VMS and breast cancer risk may act through a mechanism that is not strongly related to PMD.

Strengths of this study include the large, population-based cohort. In addition, menopausal status and hormone therapy use were carefully defined and monitored in SWAN, allowing us to stratify and examine associations within specific subgroups. Further, mammographic density was assessed by a single expert reader with high reliability. Our study also includes some limitations. First, the mammograms used for PMD assessment were not taken at the time of the annual SWAN visits at which VMS were assessed. Although considerable effort was made to minimize the effect of this time difference via matching and interpolation, some inaccuracy may have been introduced into our analysis. This inaccuracy was unlikely to be differential by VMS experience, meaning that our results would be attenuated and less significant

than we would have otherwise observed. Second, VMS experience was assessed at each annual SWAN visit, at which participants were asked to report their VMS experience over the past two weeks. Because the menopausal transition is a period of dynamic changes and symptoms may vary over the course of a year, this assessment may have failed to completely capture VMS. In addition, our description of VMS frequency based on number of symptomatic days in the past two weeks does not capture VMS severity, which may vary based on intensity and number of episodes per day. However, our VMS assessment was more comprehensive than that performed in the previous longitudinal evaluation of VMS and incident breast cancer risk. Previous analysis has shown that self-report of VMS differs significantly by racial/ethnic group (77, 78), and Asian women in particular are less likely to report VMS than Caucasian or African American women (79). Our study sample was approximately 44% Asian. Although we observed no evidence of an interaction by race/ethnicity, our findings may not be generalizable to populations of women with a considerably different racial/ethnic mix. Finally, because of multiple stratified analyses, we cannot rule out the possibility that our findings may be due to chance.

In summary, this is the first study of which we are aware to examine the relation of menopausal VMS to PMD. Findings from case-control and prospective studies of a 40-50% reduction in breast cancer risk associated with VMS have substantial public health implications, including the potential use of VMS as an easily measurable addition to current breast cancer risk prediction models. Our findings of no overall association between VMS and PMD indicate that PMD is unlikely to mediate the observed associations between VMS and breast cancer risk. However, understanding the mechanism that does link VMS to breast carcinogenesis is critical to realizing the impact of VMS as a possible marker of risk. This study provides evidence that severe VMS may affect PMD while symptoms are present. Further research is necessary to confirm these findings and evaluate the extent to which they are informative in explaining the observed association between VMS and breast cancer risk.

Table 5: Selected patient characteristics measured at baseline (N=833); SWAN Mammographic Density Study

	Study population N (%)
General characteristics	
Age, years: mean (SD)	46.5 (2.68)
BMI, kg/m^2: mean (SD)	25.4 (5.87)
Underweight/normal: < 25	491 (58.9)
Overweight: 25 - <30	203 (24.4)
Obese: 30+	131 (15.7)
Race	
Caucasian	406 (48.7)
African-American	62 (7.4)
Asian	365 (43.9)
Education level	
< High school	136 (16.3)
High school or some college	250 (30.0)
College graduate	222 (26.7)
Post-college	225 (27.0)
Family history of breast cancer (mother or sister)	74 (8.9)
Previous breast biopsy	104 (12.5)
Reproductive history	
Menopausal status at baseline	
Premenopausal	482 (57.9)
Early perimenopausal	346 (41.5)
Age at menarche (years)	
< 12	171 (20.5)
12	234 (28.1)
13	251 (30.1)
14+	173 (20.8)
Age at first birth (years)	
No children	148 (17.8)
< 20	55 (6.6)
20 - 29	395 (47.4)
30+	234 (28.1)
Number of live births	
0	147 (17.6)
1	139 (16.7)
2	354 (42.5)
3+	192 (23.1)
Hormone therapy use before baseline (ever)	110 (13.2)
Oral contraceptive use before baseline (ever)	604 (72.5)

Percentages may not add to 100% due to unknown values (< 3% for any characteristic)

Table 6: Regression coefficients and 95% confidence intervals (CI) for percent mammographic density in relation to the presence and frequency of self-reported VMS; SWAN Mammography Density Study

	N (mammograms)	Model 1 [a] β	Model 1 [a] 95% CI	Model 2 [b] β	Model 2 [b] 95% CI
All women (N=833)	4,746				
Non-symptomatic (no VMS)	1,632	Ref	Ref	Ref	Ref
Symptomatic (any VMS)	3,114	-0.69	-1.61, 0.23	-0.47	-1.39, 0.45
Infrequent (1-5 times in past two weeks)	2,410	-0.60	-1.61, 0.41	-0.47	-1.47, 0.53
Frequent (6+ times in past two weeks)	704	-0.97	-2.56, 0.63	-0.47	-2.05, 1.12
HT use during study period (N=250)	1,203				
Non-symptomatic (no VMS)	222	Ref	Ref	Ref	Ref
Symptomatic (any VMS)	981	-3.02	-5.59, -0.52	-2.31	-4.83, 0.21
Infrequent (1-5 times in past two weeks)	692	-2.71	-5.47, 0.04	-2.21	-4.90, 0.47
Frequent (6+ times in past two weeks)	289	-3.88	-7.53, -0.23	-2.59	-6.22, 1.04

[a] Model 1: Age at SWAN visit, education, dietary fat intake, first degree family history of breast cancer, hormone therapy (HT) or oral contraceptive (OC) use during study, OC use before baseline, race, age at menarche, and menopausal status

[b] Model 2: Adjusts for variables included in Model 1, plus BMI

Table 7: Mean percent mammographic density (PMD) at earliest and latest observation and change in PMD by VMS experience among women who naturally transitioned from pre/perimenopausal to postmenopausal (N=426); SWAN Mammographic Density Study

	N (women)	Starting PMD [a] Mean (SD) %	Ending PMD [a] Mean (SD) %	Change in PMD Adj. mean % [b]	P value
Non-symptomatic (no VMS)	95	43.5 (22.7)	37.3 (20.2)	-5.3	Ref
Symptomatic (any VMS)	331	42.0 (20.1)	35.7 (17.9)	-5.4	0.97
Ever, infrequent (1-5 times in past two weeks)	153	41.8 (19.4)	36.5 (18.4)	-4.8	0.69
Ever, frequent (6+ times in past two weeks)	178	42.2 (20.8)	35.1 (17.5)	-6.1	0.65

[a] Starting PMD is PMD at earliest observation; ending PMD is PMD at latest observation.

[b] Least squares means adjusted for: age at SWAN enrollment, age at first birth, use of HT or OC before baseline, use of HT or OC during the study period, BMI at baseline, alcohol use at baseline, menopausal status at baseline, education, dietary fat intake, first degree family history of breast cancer, age at menarche, starting PMD, and time difference between starting and ending PMD.

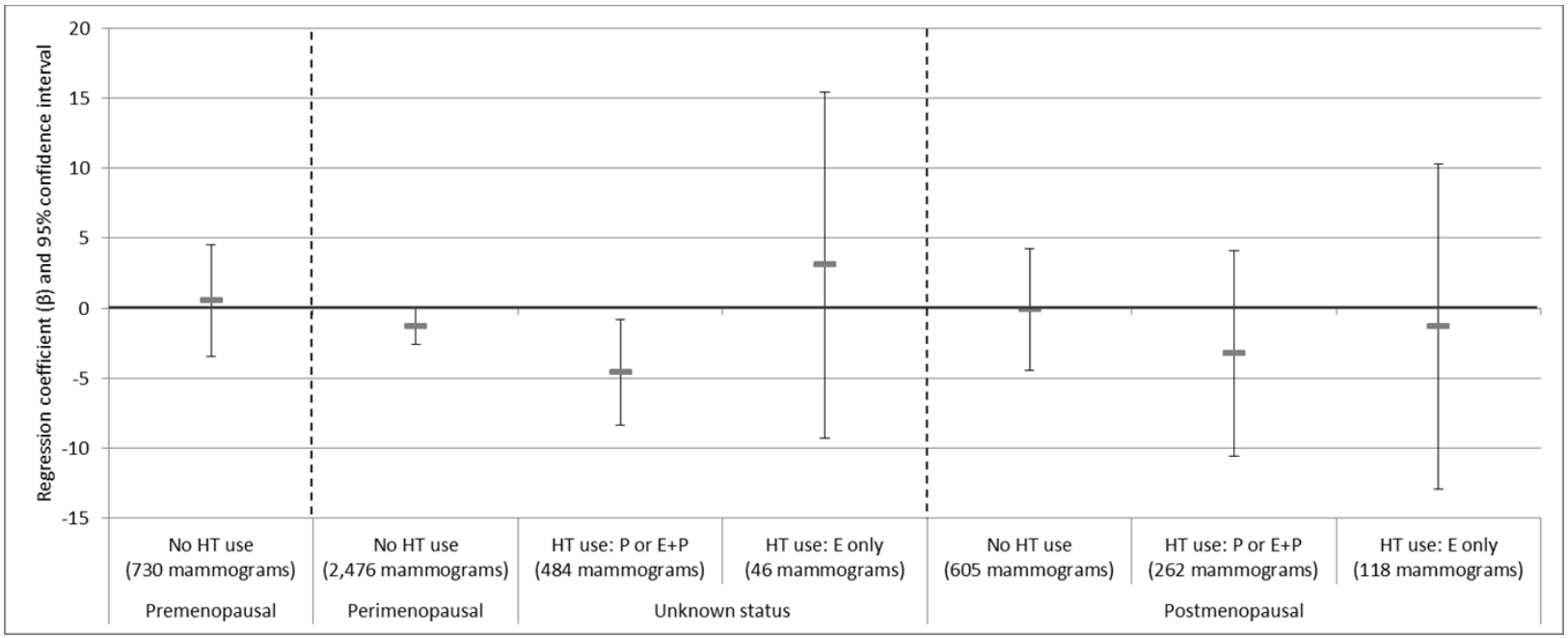

Figure 1: Risk estimates and 95% confidence intervals for the association between VMS and percent mammographic density by menopausal status and hormone therapy use (N=833)
HT, hormone therapy; P, progestin; E/P, estrogen/progestin

CHAPTER 3

MENOPAUSAL VASOMOTOR SYMPTOMS AND INCIDENT BREAST CANCER RISK IN THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION

3.1 Abstract

Two case-control studies have reported a 50% decreased breast cancer risk among women who experienced menopausal vasomotor symptoms (VMS), but one cohort study found no association. VMS may be triggered by declining estrogen levels during menopause, whereas elevated estrogen levels have been associated with increased breast cancer risk. VMS may thus be indicative of lower susceptibility to breast cancer.

We evaluated this relationship in the longitudinal Study of Women's Health Across the Nation (SWAN), which collected annual data on VMS, endogenous hormone levels, and breast cancer occurrences in 3,098 women who were pre- or early perimenopausal at enrollment. We evaluated the relation of VMS to breast cancer risk using discrete survival analysis.

Over an average 11.4 years of follow-up, 129 incident breast cancer cases were self-reported, and approximately 50% of participants experienced VMS. Symptomatic women had a reduced risk of breast cancer compared to non-symptomatic women (OR 0.62, 95% CI 0.39, 0.99). The association was stronger in the subgroup of women who fully transitioned to postmenopause during follow-up (N=80 cases, OR 0.45, 95% CI 0.26, 0.77). Associations were unchanged with adjustment for endogenous hormone levels.

VMS appear to be a marker of reduced breast cancer risk. Our findings may suggest a more limited role of endogenous hormones in this association than previously hypothesized. Future research is needed to understand the biology underlying this relationship.

3.2 Introduction

Two recent case-control studies found an approximate 50% reduction in breast cancer risk for women who reported any menopausal vasomotor symptoms (VMS), defined as hot flashes and/or night sweats, prior to their diagnosis compared to those who did not (39, 40). However, this provocative result was not supported by a recent, large, prospective cohort study (63). A clear inverse relationship between VMS and breast cancer risk could identify an easily measurable factor that may be used to enhance current risk prediction methods. The inconsistent findings from previous studies warrant further prospective investigation of this relationship.

An inverse relationship between VMS and breast cancer risk is plausible, given the common hormonal mechanisms underlying both VMS and breast carcinogenesis. Many identified breast cancer risk factors are associated with an increased lifetime exposure to sex hormones, particularly estrogen, such as early menarche, lower parity, and late age at menopause (80, 81). Further, use of certain exogenous hormone therapies has been shown to increase risk (82). While the exact etiology remains unclear, dramatic fluctuations in endogenous sex hormones during the menopausal transition may trigger the onset of VMS (45, 83, 84). Exogenous hormone therapy is effective in relieving VMS in many women (46), implying that hormone levels may be important to the management and etiology of VMS.

Prior studies have not adjusted for endogenous sex hormone levels to determine the extent to which an association between VMS and breast cancer risk is mediated by hormone levels (39, 40, 63). We prospectively investigated this association in the longitudinal Study of Women's Health Across the Nation (SWAN), a large, racially/ethnically diverse cohort of women who have been followed across the menopausal transition, and on whom annual demographic, reproductive, and endogenous sex hormone data are available. We hypothesized that menopausal VMS would be associated with reduced breast cancer risk. We further hypothesized that this association would be attenuated after adjustment for endogenous sex hormone levels.

3.3 Methods

3.3.1 Study population

The design and recruitment procedure of the SWAN cohort has been described in detail elsewhere (54). Briefly, the study enrolled women from seven locations during 1996-1997, with some locations oversampling from different racial groups to create a diverse cohort. Women were aged 42-52 years at enrollment and were pre- or early perimenopausal (i.e., bleeding within the last three months). Women using hormone therapy or oral contraceptives in the prior three months were excluded from enrollment, as were women who were pregnant or breastfeeding or who did not have an intact uterus and at least one ovary. In total, 3,302 women were enrolled and each provided written consent at the location of recruitment. All sites obtained institutional review board approval for the study protocol. Annual follow-up visits are on-going and include clinical assessments and questionnaires. Data through follow-up visit 13 are currently available.

The current study uses data from the full SWAN cohort. We excluded 21 women with a history of breast cancer at enrollment and a further 183 women who did not participate in any follow-up visits beyond the baseline enrollment visit and therefore had no incident breast cancer information. Women who reported that they were pregnant or breastfeeding during the study period were excluded for those specific visits (four women, five study visits). After these exclusions, the study population included 3,098 women who contributed data from 34,905 SWAN visits.

3.3.2 Vasomotor symptom assessment

At the baseline and each annual follow-up visit, SWAN participants reported the frequency of VMS in the prior two weeks via a self-administered questionnaire in the language appropriate to the participant (English, Spanish, Cantonese, or Japanese) using a standard questionnaire. Questions related to hot flashes and night sweats were worded as follows:

"Thinking back over the last two weeks, how often have you had hot flashes or flushes / night sweats?" Response categories were: not at all, 1-5 days, 6-8 days, 9-13 days, every day. Consistent with previous analyses in the SWAN cohort (55-57), women who reported any hot flashes or night sweats (versus not at all) were classified as being symptomatic of VMS at that visit. Women who reported having hot flashes or night sweats on 6 or more days in the last two weeks were classified as being symptomatic of frequent VMS at that visit (38), while women who reported having hot flashes or night sweats 1-5 days in the last two weeks were classified as being symptomatic of infrequent VMS at that visit.

3.3.3 Breast cancer assessment

SWAN participants self-reported cancer history during the baseline enrollment interview. At each subsequent follow-up, women were interviewed to assess their medication and health status since the previous assessment. Interviews were administered by trained personnel in the language appropriate to the participant (English, Spanish, Cantonese, or Japanese) using standard interview questions. During the interview, women were asked if they had been told that they had breast cancer by a health care provider since their last assessment. Responses were recorded as "Yes," "No," or "Don't know."

3.3.4 Endogenous sex hormone assessment

Endogenous sex hormone and gonadotropin levels (estradiol, E2; sex hormone binding globulin, SHBG; follicle stimulating hormone, FSH) were assessed using blood samples collected from SWAN participants at each of their annual clinical assessments. Samples were collected in the morning following a 12-hour overnight fast, and were scheduled within 2-5 days of the onset of the menstrual cycle (the early follicular phase (EFP)) for pre- and perimenopausal women. Two attempts were made to obtain an EFP sample. If a timed sample could not be obtained, usually due to menstrual unpredictability during perimenopause, a random fasting sample was

taken within 90 days of the annual SWAN visit date. Blood samples were refrigerated prior to centrifugation, which was performed 1-2 hours after the blood draw (85). Serum was shipped to a central laboratory (SWAN Central Ligand Assay Satellite Services Laboratory, University of Michigan, Ann Arbor, MI) for processing. Assays were performed on the ACS-180 automated analyzer (Bayer Diagnostics Corporation); the assay protocol has been described in detail elsewhere (86). All assay processing was blinded with respect to VMS and breast cancer status. Inter-assay and intra-assay coefficients of variation (CV) were 6.4% and 10.6% respectively for E2, 12.0% and 6.0% respectively for FSH, and 9.7% and 11.3% respectively for SHBG (87).

3.3.5 Covariate assessment

Information on covariates was collected during the baseline and annual SWAN follow-up visits as part of the clinical assessment or by interviewer- or self-administered questionnaires. Demographic and reproductive variables that were considered unchanging in the current analysis included: race/ethnicity, education, age at first birth, age at menarche, age at menopause (if observed), alcohol consumption, physical activity, active and passive smoking exposure, dietary fat and fiber intake, and SWAN site. Covariates that were considered time-varying and updated at each follow-up visit included: menopausal status, body mass index (BMI), parity, family history of breast cancer, hormone therapy use, bilateral oophorectomy or hysterectomy, sex hormone levels, and cycle day of blood draw for hormone level assessment (day 2-5 versus other).

Menopausal status was classified in accordance with standard SWAN protocol (54): women with no change in menstrual regularity over the past year were considered premenopausal; women with decreased menstrual regularity who had had a menstrual period in the past three months were considered early perimenopausal; women with no menstrual bleeding in the 3-11 months before the interview date were considered late perimenopausal; and those with

no bleeding in the last 12 months were considered postmenopausal. Early and late perimenopause were collapsed into a single perimenopausal category for this analysis. Women who reported bleeding in the previous 12 months and reported hormone therapy (HT) use in the previous year were classified as unknown menopausal status due to HT use for those visits. Postmenopausal women remained classified as such if HT use was initiated after the final menstrual period. Women reporting a hysterectomy or bilateral oophorectomy were classified as postmenopausal starting at the visit at which the surgery was reported.

By study design, women were not taking HT at baseline enrollment. At each follow-up interview, women were asked to report the use of estrogen, progestin, or combination estrogen/progestin hormone therapy since their last study visit. When possible, interviewers verified medication from the container label. Lifestyle variables, including alcohol consumption, physical activity, smoking, and dietary fat and fiber intake were assessed in accordance with previous SWAN publications (38, 57, 62). Total alcohol consumption was categorized as: <1 drink/month, 1 drink/month - <2 drinks/week, 2+ drinks/week. Total physical activity was assessed using a continuous variable that incorporated the proportion of the year in which activity was performed, a standardized intensity score, and the duration of each bout of activity. Development of the physical activity score has been described in detail in previous SWAN publications (61). Smoking was categorized as: never smoked/no passive exposure, never smoked/with passive exposure, former smoker, current smoker. Baseline dietary fat and fiber intake were assessed using composite variables that reflected total average consumption based on a validated food frequency questionnaire (88).

3.3.6 Statistical methods

After omitting study visits that occurred after a breast cancer diagnosis, a total of 31,712 observations from 3,098 participants contributed to the current analyses. Descriptive statistics

included means and standard deviations for continuous variables and percentages for categorical variables. We calculated relative risks of breast cancer diagnosis for baseline covariates using logistic regression.

We assessed the longitudinal association between VMS and incident breast cancer risk using logistic regression for discrete survival analyses. This statistical approach is appropriate for data in which the outcome is recorded at discrete time intervals (i.e., SWAN visits) and the actual date of the outcome is unknown (89). Women could contribute varying numbers of observations, and observations were omitted after the visit at which the event occurred (i.e., the visit at which a breast cancer diagnosis was reported) or continued to the end of the study period if no event occurred. As in other survival analysis approaches, the inclusion of each interval in the dataset was conditional on the participant having "survived" past the previous intervals. To avoid temporal concerns with breast cancer treatment affecting VMS, we reverse-lagged the breast cancer diagnosis to ensure that only VMS occurring before the diagnosis were considered (e.g., if breast cancer was reported at visit 08, VMS and covariate information up to visit 07 were included in the analysis).

To differentiate between symptomatic and non-symptomatic women, we modeled VMS at each study visit as VMS reported at that visit or any prior visit. Therefore, a woman was considered symptomatic from the first visit at which she reported VMS. Frequent VMS was modeled similarly, meaning that a woman was considered symptomatic of frequent VMS from the first visit at which frequent VMS was reported. HT use during the study period was modeled in the same fashion. We developed the multivariable logistic regression models using the method of best selection. Due to the limited number of breast cancer outcomes, we developed a parsimonious model, retaining covariates only if they were significant in the model, or if their exclusion resulted in a change in the odds ratio for the VMS exposure of 10% or more. Because HT use may act as a confounder or intermediary in the relationship between VMS and breast

cancer, we repeated our analysis among women who did not use HT during the study period. We assessed interactions by race/ethnicity, HT use, BMI, and menopausal status using cross-product terms.

To assess the effect of VMS through the complete menopausal transition on breast cancer risk, we performed a secondary analysis of VMS during pre- or perimenopause and postmenopausal breast cancer outcomes. This analysis was restricted to women who fully transitioned to postmenopause without surgery during the study period and had a discernable final menstrual period because they were not using HT (N=2,468). Only VMS reported during visits at which the woman was pre- or perimenopausal and only breast cancer diagnoses reported during visits at which the woman was postmenopausal were included in the analysis. Women reporting pre- or perimenopausal breast cancer were censored at the visit before their breast cancer was reported. A parsimonious multivariable model was built for this analysis using the same methodology described above.

We performed a preliminary analysis to assess the association between sex hormone levels and breast cancer risk in our study population. To evaluate the effect of sex hormone levels on the association between VMS and breast cancer, we adjusted for each hormone (E2, FSH, SHBG) individually in the multivariable model, as well as for all hormones together. Because of the uncertain association between VMS and endogenous hormones and because hormone levels fluctuated during perimenopause, we adjusted using several methods: hormone level at the current visit, hormone level at the first perimenopausal visit, average hormone level and change in hormone level over perimenopausal visits. Log transformations of hormone values were used to satisfy normality assumptions. All but two women had endogenous hormone data for at least one study visit. However, many women (N=2,579, 83%) had missing hormone data during at least one follow-up. Overall, hormone data were captured for 25,806 of 31,712 study visits (81%). Among visits in which women were pre- or perimenopausal, 63% of hormone data

were captured during the first 2-5 days of menses (EFP) and we controlled for cycle day (day 2-5 or other) in all analyses. Hormone data were not available at the visit preceding diagnosis for 41 breast cancer cases, and these cases were excluded from the analysis that adjusted for hormone level at the current visit.

Due to incomplete data collection, information from the New Jersey location was missing for SWAN visits 07, 08, 10, and 11. This affected 345 study participants, including all Hispanic women and one breast cancer case. We thus performed a sensitivity analysis by excluding all women from the New Jersey location and comparing our results to those including these participants.

All analyses were performed using SAS Version 9.2 (SAS Institute, Cary, North Carolina).

3.4 Results

On average, women were approximately 46 years old at enrollment. The majority was Caucasian (47%), with 29% African American, 17% Asian, and 7% Hispanic (Table 8). Most women had completed some college education (76%). Over an average of 11.7 years of follow-up, 129 breast cancer cases were self-reported. Women who reported a breast cancer diagnosis during follow-up were more likely to be Caucasian and college-educated. Overall, VMS were reported at 51% of SWAN visits, and the frequency of ever having experienced VMS increased from pre- to peri- and postmenopausal (32%, 56%, 52% of visits, respectively). More African American women reported VMS than Caucasian and Hispanic women (63.2% versus 48.8% of visits, $p<0.01$), while fewer Asian women reported VMS than Caucasian and Hispanic women (40.6% versus 48.8% of visits, $p<0.01$).

We observed that symptomatic women had a 38% reduced risk of breast cancer compared to non-symptomatic women (OR = 0.62, 95% CI 0.39-0.99) (Table 9). This

association was modified by race/ethnicity ($p_{interaction}$=0.07). After stratification, we found that the relationship between VMS and breast cancer risk was strongest and statistically significant among Caucasian women (OR = 0.45, 95% CI 0.22-0.79) (Table 9) and attenuated and non-significant among African American and Asian women. We observed no significant interaction of the protective relation of VMS with breast cancer by menopausal status (p=0.22), HT use (p=0.74), or BMI (p=0.43). We found similar results when looking at infrequent versus frequent VMS, although confidence intervals were wider due to smaller numbers of breast cancer cases in each group.

In the secondary analysis of pre- and perimenopausal VMS and postmenopausal breast cancer (N=2,468 women, 80 cases), we observed that symptomatic women had a significant 55% reduction in risk of postmenopausal breast cancer compared to non-symptomatic women (OR = 0.45, 95% CI 0.26-0.77) (Table 10). We observed no significant interaction of this protective relation by race/ethnicity (p=0.30), HT use (p=0.73), or BMI (p=0.42). Among women who transitioned to postmenopause with no use of HT (N=1,348, 40 cases), results were similar although non-significant (OR = 0.50, 95% CI 0.23-1.10) (Table 10).

Sex hormone levels were not statistically significantly associated with breast cancer risk when adjusted for age, menopausal status, HT use, cycle day (day 2-5 or other), and SWAN visit (OR = 1.17, 95% CI 0.91-1.49 for E2; OR = 0.83, 95% CI 0.64-1.09 for FSH; OR = 0.87, 95% CI 0.60-1.25 for SHBG). In the longitudinal analysis of VMS and breast cancer risk, adjustment for hormone levels did not appreciably change the observed associations for symptomatic women compared to non-symptomatic women (Table 11). These findings were consistent and non-significant among the 2,049 women with no HT use during the study period (Table 11). Results were less precise but did not otherwise change when the observations of pre- and perimenopausal hormone levels that were collected outside the EFP window were excluded from the analysis (data not shown).

Results of our analysis did not change after exclusion of women from the New Jersey location. We have therefore presented results from the full study population.

3.5 Discussion

Three prior investigations have examined the relationship between menopausal VMS and breast cancer risk (39, 40, 63). Our findings are consistent with two case-control studies that both concluded an approximate 50% reduction in risk for women who experienced VMS at any point during the menopausal transition (39, 40). However, a recent prospective investigation observed no association between VMS and breast cancer risk (63). This study followed 11,297 women with a mean age of 47.6 years at enrollment for an average of 13.7 years. During follow-up, 34% reported hot flashes, 26% reported night sweats, and 348 incident breast cancer cases were recorded. Participants were surveyed at three-year intervals and asked to report VMS in the past 12 months; thus VMS reporting was not captured for two out of every three years, and women could contribute a maximum of six observations. It is likely that some short-term VMS were not captured due to the infrequency of the data collection in this study. This may explain why the percentage of women reporting VMS was considerably lower than general estimates of 50-80% (35, 36, 38), and may have contributed to the observed null findings, particularly if VMS were not recorded during perimenopause when symptoms were most frequent and severe (38). In contrast, our study participants provided annual assessments of VMS over the past two weeks. Although some VMS may still have been missed, this protocol was more likely to capture VMS at least once during each menopausal stage, and allowed for up to 13 observations per woman.

Another unique feature of the present study was the inclusion of a racially/ethnically diverse study sample. We found that the inverse relationship between VMS and breast cancer risk was strongest among Caucasian women and weaker among African American and Asian women. After stratification, the number of breast cancer cases in each racial/ethnic subgroup was

limited (70, 32, and 26 cases for Caucasian, African American, and Asian, respectively). Low statistical power may thus have contributed to the attenuated results among African American and Asian women. It is interesting, however, that the association between VMS and breast cancer risk was strongest among Caucasian women, despite the limited number of cases. Race/ethnicity may influence self-reporting of VMS. African American women tend to self-report more frequent (38) and severe symptoms than Caucasian women (77, 78), although these results have not been consistent (90, 91). In contrast, Asian women have demonstrated less self-reporting of VMS than Caucasian women in some studies (79). Consistent with these findings and previous analysis of SWAN VMS data (38), African American women in our study reported more VMS and Asian women reported fewer VMS than Caucasian women. Our findings suggest a difference in the effect of VMS on breast cancer risk by race/ethnicity, but it is unclear if this is due to true physiological differences, a lack of statistical power after stratification, or racial/ethnic variation in the self-reporting of VMS.

We anticipated that the association between VMS and breast cancer risk may be related to common hormonal etiologies; specifically, we hypothesized that any association would be attenuated after adjustment for endogenous sex hormones. We observed a significant reduction in breast cancer risk for women who experienced VMS, but contrary to our expectation, our results were unchanged after adjustment for endogenous sex hormones. Sex hormone levels were not statistically significantly associated with breast cancer risk in our study sample, although a positive relationship between E2 and breast cancer, and inverse relationships between FSH and SHBG and breast cancer are generally supported in the literature (92). The direction of our observed associations was consistent with the literature, and our results could be explained by low statistical power due to the small number of breast cancer cases. Additionally, 37% of pre- and perimenopausal hormone data in our study were collected outside the first 2-5 days of menses (EFP), although considerable effort was made to collect blood samples during the EFP window

(86). Excess variation in hormone levels may have still further reduced our statistical power, despite controlling for cycle day and repeating analyses without observations collected outside the EFP window.

On the other hand, our finding of minimal change in risk with hormone adjustment could imply that the mechanism linking VMS to breast cancer is not strongly dependent on endogenous hormone levels. While VMS have been associated with estrogen fluctuations during the menopausal transition (42), these alone cannot account for the onset of symptoms. All women experience a decline in estrogen during menopause, but not all women experience VMS (47). Non-hormonal mechanisms such as changes in skin vasculature and central nervous system regulation have been hypothesized to trigger VMS (45, 93). It is possible that non-hormonal mechanisms that are important in both the onset of VMS and breast carcinogenesis could explain our results. Future analyses in larger cohorts are needed to definitively determine the role of endogenous hormones in mediating the observed relationship between VMS and breast cancer.

Our study was strengthened by the large, prospective, multi-racial/ethnic SWAN cohort with annual assessment of demographic, reproductive, and clinical factors over up to 13 years of follow-up. The results of our study must be interpreted within the context of its limitations, however. Both VMS and breast cancer information were self-reported in our analysis. Previous analyses in the SWAN cohort using our definition of presence and frequency of VMS have found significant associations between VMS and lower bone mineral density, insulin resistance, and subclinical cardiovascular disease (55-57). Self-report of breast cancer has been shown to be valid in large epidemiologic studies (94, 95), but, along with the relatively small number of incident breast cancer cases, did limit our capacity to look separately at invasive versus in situ disease and at molecular subtypes.

Establishing VMS as a risk marker for incident breast cancer has considerable public health implications. VMS are easily reportable without invasive or expensive procedures. An

association between self-reported VMS and breast cancer risk could provide additional insight to clinicians on the individual breast cancer risk of their patients. However, a better understanding of the mechanisms underlying this association is needed before VMS may be suggested as an enhancement to current risk prediction tools. Our findings may imply that hormonal pathways have a lesser role in the association between VMS and breast cancer risk than previously hypothesized. This result strongly justifies the need for future investigations to examine the biological mechanisms linking the onset of VMS to breast cancer etiology.

Table 8: Selected characteristics measured at baseline by breast cancer diagnosis status (N=3,098); SWAN 1996-2013

	No breast cancer diagnosis N = 2969	Breast cancer diagnosis N = 129	Relative risk [a] OR	95% CI
General characteristics				
Age, years: mean (std)	46.3 (2.69)	46.7 (2.73)	1.14	1.02, 1.28
BMI, kg/m^2: mean (std)	28.2 (7.18)	28.8 (8.24)	1.01	0.96, 1.06
Underweight/normal: < 25	1,201 (40.5)	51 (39.5)	Ref	Ref
Overweight: 25 - < 30	796 (26.8)	24 (18.6)	0.60	0.24, 1.49
Obese: 30+	939 (31.6)	52 (40.3)	1.39	0.64, 3.03
Race/ethnicity				
Caucasian	1,398 (47.1)	70 (54.3)	Ref	Ref
African-American	850 (28.6)	32 (24.8)	0.91	0.43, 1.96
Asian	494 (16.6)	26 (20.2)	0.76	0.31, 1.87
Hispanic	227 (7.7)	1 (0.7)	0.35	0.04, 2.97
Education level				
< High school	195 (6.6)	4 (3.1)	0.72	0.08, 6.63
High school	517 (17.4)	14 (10.8)	Ref	Ref
College graduate	957 (32.2)	40 (31.0)	1.56	0.60, 4.02
Post-college	598 (20.2)	33 (25.6)	2.63	1.01, 6.84
Family history of breast cancer (mother or sister)	295 (9.9)	22 (17.1)	1.46	0.66, 3.26
Physical activity score (tertiles)				
<7.1	1,083 (36.5)	49 (38.0)	Ref	Ref
7.1 - <8.5	859 (28.9)	38 (29.5)	0.61	0.28, 1.31
8.5+	906 (30.5)	37 (28.7)	0.78	0.38, 1.62
Alcohol consumption (past year)				
Non-drinkers	1,475 (49.7)	55 (42.6)	Ref	Ref
Drinkers	1,484 (50.0)	74 (57.4)	1.20	0.65, 2.23
Smoking status				
Never smoked / no passive exposure	855 (28.8)	37 (28.7)	Ref	Ref
Never smoked / passive exposure	841 (28.3)	41 (31.8)	0.65	0.30, 1.40
Former smoker	759 (25.5)	29 (22.5)	0.46	0.20, 1.07
Current smoker	488 (16.4)	20 (15.5)	0.58	0.22, 1.56
Reproductive history				
Menopausal status at baseline				
Premenopausal	1,555 (52.4)	67 (51.9)	Ref	Ref
Early perimenopausal	1,345 (45.3)	61 (47.3)	0.66	0.35, 1.22
Age at menarche (years)				
< 12	698 (23.5)	39 (30.2)	Ref	Ref
12	777 (26.2)	29 (22.5)	0.68	0.29, 1.62
13	798 (26.9)	31 (24.0)	0.63	0.26, 1.53
14+	668 (22.5)	30 (23.3)	1.49	0.67, 3.34
Age at first birth (years)				
No children	503 (16.9)	29 (22.5)	Ref	Ref
< 20	492 (16.6)	12 (9.3)	0.42	0.11, 1.56
20 - 29	1,392 (46.9)	57 (44.2)	0.79	0.33, 1.89
30+	575 (19.4)	31 (24.0)	1.29	0.51, 3.26
Number of live births				
0	501 (16.9)	29 (22.5)	Ref	Ref
1	504 (17.0)	23 (17.8)	1.05	0.40, 2.78
2	1,002 (33.7)	45 (34.9)	0.94	0.39, 2.28
3+	955 (32.2)	32 (24.8)	0.62	0.24, 1.65
Hormone therapy use before baseline (ever)	198 (6.7)	12 (9.3)	0.85	0.25, 2.85
Oral contraceptive use before baseline (ever)	2,167 (73.0)	95 (73.6)	1.08	0.54, 2.19

Percentages may not sum to 100% due to missing values

[a] Relative risks adjusted for all selected baseline characteristics

Table 9: Odds ratio (OR) and 95% confidence intervals (CI) for the risk of breast cancer diagnosis; SWAN 1996-2013

	N (events)	Fully adjusted [a] OR	95% CI
All women (N=3,098)	**129**		
Non-symptomatic (no VMS)	32	Ref	Ref
Symptomatic (any VMS)	97	0.62	0.39, 0.99
Ever, infrequent (1-5 times in past two weeks)	69	0.59	0.36, 0.97
Ever, frequent (6+ times in past two weeks)	28	0.70	0.39, 1.27
White women (N=1,468)	**70**		
Non-symptomatic (no VMS)	22	Ref	Ref
Symptomatic (any VMS)	48	0.41	0.22, 0.79
Ever, infrequent (1-5 times in past two weeks)	36	0.41	0.21, 0.81
Ever, frequent (6+ times in past two weeks)	12	0.42	0.18, 0.97

[a] Adjusted for age, SWAN visit number, age at first birth, BMI, first degree family history of breast cancer, and hormone use during the study period

Table 10: Odds ratio (OR) and 95% confidence intervals (CI) for the risk of postmenopausal breast cancer diagnosis among women who naturally transitioned to postmenopausal during the study period; SWAN 1996-2013

	N (events)	Fully adjusted [a] OR	95% CI
All women (N=2,468)	**80**		
Non-symptomatic (no VMS)	24	Ref	Ref
Symptomatic (any VMS)	56	0.45	0.26, 0.77
Ever, infrequent (1-5 times in past two weeks)	21	0.39	0.20, 0.75
Ever, frequent (6+ times in past two weeks)	35	0.50	0.28, 0 90
No HT use during the study period (N=1,348)	**40**		
Non-symptomatic (no VMS)	13	Ref	Ref
Symptomatic (any VMS)	27	0.50	0.23, 1.10
Ever, infrequent (1-5 times in past two weeks)	12	0.47	0.19, 1.21
Ever, frequent (6+ times in past two weeks)	15	0.52	0.22, 1.23

HT, hormone therapy

[a] Adjusted for age at enrollment, SWAN site, age at first birth, BMI at baseline, family history of breast cancer, and hormone use during study period

Table 11: Odds ratio (OR) and 95% confidence intervals (CI) for the risk of breast cancer diagnosis, adjusted for endogenous hormone levels; SWAN 1996-2013

		Original Model							
	N	Fully adjusted [a]		Adj. for E2		Adj. for FSH		Adj. for SHBG	
	(events)	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
All women (N=3,096)	**88**								
Non-symptomatic (no VMS)	26	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Symptomatic (any VMS)	62	0.65	0.39, 1.09	0.68	0.40, 1.13	0.68	0.41, 1.14	0.66	0.40, 1.10
Ever, infrequent (1-5 times in past two weeks)	39	0.57	0.33, 0.97	0.59	0.34, 1.01	0.59	0.34, 1.02	0.57	0.33, 0.99
Ever, frequent (6+ times in past two weeks)	23	0.92	0.50, 1.69	0.98	0.52, 1.82	0.97	0.52, 1.83	0.94	0.50, 1.75
No HT use during the study period (N=2,049)	61								
Non-symptomatic (no VMS)	21	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Symptomatic (any VMS)	40	0.71	0.40, 1.26	0.75	0.42, 1.34	0.74	0.41, 1.34	0.72	0.40, 1.30
Ever, infrequent (1-5 times in past two weeks)	28	0.66	0.36, 1.21	0.69	0.37, 1.28	0.69	0.37, 1.28	0.67	0.36, 1.23
Ever, frequent (6+ times in past two weeks)	12	0.87	0.41, 1.86	0.94	0.44, 2.05	0.92	0.42, 2.01	0.92	0.42, 1.97

E2, estradiol; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin; HT, hormone therapy

[a] Adjusted for age, SWAN visit number, age at first birth, BMI, first degree family history of breast cancer, hormone therapy use during the study period, and cycle day of blood draw (day 2-5 or other), and baseline hormone level

REFERENCES

1. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: Results from the Canadian national breast screening study. J Natl Cancer Inst. 1995;87(9):670-5.

2. Boyd NF, Lockwood GA, Martin LJ, et al. Mammographic densities and breast cancer risk. Breast disease. 1998;10(3-4):113-26.

3. Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: Effects with time, age, and menopause status. J Natl Cancer Inst. 1995;87(21):1622-9.

4. Warner E, Lockwood G, Tritchler D, Boyd NF. The risk of breast cancer associated with mammographic parenchymal patterns: A meta-analysis of the published literature to examine the effect of method of classification. Cancer Detect Prev. 1992;16(1):67-72.

5. Boyd NF, Lockwood GA, Martin LJ, Byng JW, Yaffe MJ, Trichter DL. Mammographic density as a marker of susceptibility to breast cancer; a hypothesis. In: Miller AB, Bartsch H, Boffetta P, Dragsted L, Vainio H, editors. Biomarkers in cancer chemoprevention. International Agency for Research on Cancer; 2001. p. 163-9.

6. Endogenous Hormones and Breast Cancer Collaborative Group1, Key TJ, Appleby PN, Reeves GK, Roddam AW, Helzlsouer KJ, Alberg AJ, Rollison DE, Dorgan JF, Brinton LA, Overvad K, Kaaks R, Trichopoulou A, Clavel-Chapelon F, Panico S, Duell EJ, Peeters PH, Rinaldi S, Fentiman IS, Dowsett M, Manjer J, Lenner P, Hallmans G, Baglietto L, English DR, Giles GG, Hopper JL, Severi G, Morris HA, Hankinson SE, Tworoger SS, Koenig K, Zeleniuch-Jacquotte A, Arslan AA, Toniolo P, Shore RE, Krogh V, Micheli A, Berrino F, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Lui LY, Cummings SR, Gunter MJ, Rohan TE, Strickler HD. Circulating sex hormones and breast cancer risk factors in postmenopausal women: Reanalysis of 13 studies.. Br J Cancer. 2011 Aug 23;105(5):709-22.

7. Boyd N, Martin L, Bronskill M, Yaffe M, Duric N, Minkin S. Breast tissue composition and susceptibility to breast cancer. J Natl Cancer Inst. 2010;102(16):1224-37.

8. Boyd N, Martin L, Sun L, et al. Body size, mammographic density, and breast cancer risk. Cancer epidemiology, biomarkers prevention. 2006;15(11):2086-92.

9. Jeffreys M, Warren R, Highnam R, Davey Smith G. Breast cancer risk factors and a novel measure of volumetric breast density: Cross-sectional study. Br J Cancer. 2008;98(1):210-6.

10. Lokate M, Kallenberg MGJ, Karssemeijer N, Van den Bosch,Maurice A A J., Peeters PHM, Van Gils C. Volumetric breast density from full-field digital mammograms and its association with breast cancer risk factors: A comparison with a threshold method. Cancer epidemiology, biomarkers prevention. 2010;19(12):3096-105.

11. Aitken Z, McCormack V, Highnam R, et al. Screen-film mammographic density and breast cancer risk: A comparison of the volumetric standard mammogram form and the interactive

threshold measurement methods. Cancer epidemiology, biomarkers prevention. 2010;19(2):418-28.

12. Gierach G, Geller B, Shepherd J, et al. Comparison of mammographic density assessed as volumes and areas among women undergoing diagnostic image-guided breast biopsy. Cancer epidemiology, biomarkers & prevention. 2014.

13. Ding J, Warren R, Warsi I, et al. Evaluating the effectiveness of using standard mammogram form to predict breast cancer risk: Case-control study. Cancer epidemiology, biomarkers prevention. 2008;17(5):1074-81.

14. Boyd N, Martin L, Gunasekara A, et al. Mammographic density and breast cancer risk: Evaluation of a novel method of measuring breast tissue volumes. Cancer epidemiology, biomarkers prevention. 2009;18(6):1754-62.

15. Shepherd J, Kerlikowske K, Ma L, et al. Volume of mammographic density and risk of breast cancer. Cancer epidemiology, biomarkers prevention. 2011;20(7):1473-82.

16. Harris H, Tamimi R, Willett W, Hankinson S, Michels K. Body size across the life course, mammographic density, and risk of breast cancer. Am J Epidemiol. 2011;174(8):909-18.

17. Baglietto L, Krishnan K, Stone J, et al. Associations of mammographic dense and nondense areas and body mass index with risk of breast cancer. Am J Epidemiol. 2014;179(4):475-83.

18. Kelemen L, Pankratz VS, Sellers T, et al. Age-specific trends in mammographic density: The minnesota breast cancer family study. Am J Epidemiol. 2008;167(9):1027-36.

19. Maskarinec G, Pagano I, Lurie G, Kolonel L. A longitudinal investigation of mammographic density: The multiethnic cohort. Cancer epidemiology, biomarkers prevention. 2006;15(4):732-9.

20. Guthrie J, Milne R, Hopper J, Cawson J, Dennerstein L, Burger H. Mammographic densities during the menopausal transition: A longitudinal study of australian-born women. Menopause. 2007;14(2):208-15.

21. Work, Meghan Reimers, Laura Quante, Anne Crew, Katherine Whiffen,Amy Beth Terry, Mary. Changes in mammographic density over time in breast cancer cases and women at high risk for breast cancer. International journal of cancer. 2014.

22. Reeves K, Stone R, Modugno F, et al. Longitudinal association of anthropometry with mammographic breast density in the study of women's health across the nation. International journal of cancer. 2009;124(5):1169-77.

23. Boyd NF, Greenberg C, Lockwood G, et al. Effects at two years of a low-fat, high-carbohydrate diet on radiologic features of the breast: Results from a randomized trial. canadian diet and breast cancer prevention study group. J Natl Cancer Inst. 1997;89(7):488-96.

24. Breast cancer surveillance consortium: Fact sheet [homepage on the Internet]. Bethesda, MD: National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human

Services. February 2013. Available from: Available at: http://breastscreening.cancer.gov/about/bcsc_fact_sheet.pdf.

25. Shepherd J, Herve L, Landau J, Fan B, Kerlikowske K, Cummings S. Novel use of single X-ray absorptiometry for measuring breast density. Technology in cancer research treatment. 2005;4(2):173-82.

26. Malkov S, Wang J, Kerlikowske K, Cummings S, Shepherd J. Single x-ray absorptiometry method for the quantitative mammographic measure of fibroglandular tissue volume. Med Phys. 2009;36(12):5525-36.

27. Diggle P, Heagerty P, Liang K, Zeger S. Analysis of longitudinal data. 2nd ed. Oxford, UK: Oxford University Press; 2004.

28. Amadou A, Hainaut P, Romieu I. Role of obesity in the risk of breast cancer: Lessons from anthropometry. Journal of Oncology. 2013;2013:906495-.

29. Key TJ, Appleby PN, Reeves GK, et al. Sex hormones and risk of breast cancer in premenopausal women: A collaborative reanalysis of individual participant data from seven prospective studies. Lancet oncology. 2013;14(10):1009-19.

30. Aitken Z, McCormack V, Highnam R, et al. Screen-film mammographic density and breast cancer risk: A comparison of the volumetric standard mammogram form and the interactive threshold measurement methods. Cancer epidemiology, biomarkers prevention. 2010;19(2):418-28.

31. Haars G, van Noord PAH, van Gils C, Grobbee D, Peeters PHM. Measurements of breast density: No ratio for a ratio. Cancer epidemiology, biomarkers prevention. 2005;14(11):2634-40.

32. Harris H, Tamimi R, Willett W, Hankinson S, Michels K. Body size across the life course, mammographic density, and risk of breast cancer. Am J Epidemiol. 2011;174(8):909-18.

33. Habel L, Capra A, Oestreicher N, et al. Mammographic density in a multiethnic cohort. Menopause. 2007;14(5):891-9.

34. Ursin G, Ma H, Wu A, et al. Mammographic density and breast cancer in three ethnic groups. Cancer epidemiology, biomarkers prevention. 2003;12(4):332-8.

35. Avis NE, Crawford SL, McKinlay SM. Psychosocial, behavioral, and health factors related to menopause symptomatology. Womens Health. 1997;3(2):103-20.

36. Freeman, Ellen Sammel, Mary Lin, Hui Gracia, Clarisa Kapoor,Shiv Ferdousi, Tahmina. The role of anxiety and hormonal changes in menopausal hot flashes. Menopause. 2005;12(3):258-66.

37. Gold EB, Sternfeld B, Kelsey JL, et al. Relation of demographic and lifestyle factors to symptoms in a multi-racial/ethnic population of women 40-55 years of age. Am J Epidemiol. 2000;152(5):463-73.

38. Gold E, Colvin A, Avis N, et al. Longitudinal analysis of the association between vasomotor symptoms and race/ethnicity across the menopausal transition: Study of women's health across the nation. Am J Public Health. 2006;96(7):1226-35.

39. Huang Y, Malone K, Cushing Haugen K, Daling J, Li C. Relationship between menopausal symptoms and risk of postmenopausal breast cancer. Cancer epidemiology, biomarkers prevention. 2011;20(2):379-88.

40. Fei C, Deroo L, Sandler D, Weinberg C. Menopausal symptoms and the risk of young-onset breast cancer. Eur J Cancer. 2012.

41. Key T. Endogenous oestrogens and breast cancer risk in premenopausal and postmenopausal women. Steroids. 2011;76(8):812-5.

42. Freedman R, Blacker C. Estrogen raises the sweating threshold in postmenopausal women with hot flashes. Fertil Steril. 2002;77(3):487-90.

43. Sassarini J, Fox H, Ferrell W, Sattar N, Lumsden MA. Vascular function and cardiovascular risk factors in women with severe flushing. Clin Endocrinol (Oxf). 2011;74(1):97-103.

44. Berendsen HH. The role of serotonin in hot flushes. Maturitas. 2000;36(3):155-64.

45. Freedman R. Pathophysiology and treatment of menopausal hot flashes. Semin Reprod Med. 2005;23(2):117-25.

46. MacLennan AH. Evidence-based review of therapies at the menopause. Int J Evid Based Healthc. 2009 Jun;7(2):112-23.

47. Schindler AE, Müller D, Keller E, Göser R, Runkel F. Studies with clonidine (dixarit) in menopausal women. Arch Gynecol. 1979;227(4):341-7.

48. Freedman RR, Woodward S, Sabharwal SC. Alpha 2-adrenergic mechanism in menopausal hot flushes. Obstet Gynecol. 1990;76(4):573-8.

49. McCormack V, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: A meta-analysis. Cancer epidemiology, biomarkers prevention. 2006;15(6):1159-69.

50. Boyd N, Martin L, Yaffe M, Minkin S. Mammographic density and breast cancer risk: Current understanding and future prospects. Breast cancer research. 2011;13(6):223-.

51. Boyd NF, Melnichouk O, Martin LJ, et al. Mammographic density. response to hormones, and breast cancer risk. Journal of Clinical Oncology. 2011 Aug;29(22):2985-92.

52. Greendale G, Palla S, Ursin G, et al. The association of endogenous sex steroids and sex steroid binding proteins with mammographic density: Results from the postmenopausal estrogen/progestin interventions mammographic density study. Am J Epidemiol. 2005;162(9):826-34.

53. Johansson H, Gandini S, Bonanni B, et al. Relationships between circulating hormone levels, mammographic percent density and breast cancer risk factors in postmenopausal women. Breast Cancer Res Treat. 2008;108(1):57-67.

54. Sowers M, Crawford S, Sternfeld B, et al. SWAN: A multicenter, multiethnic, community-based cohort study of women and the menopausal transition. In: Lobo R, Kelsey J, Marcus R, Lobo AR, editors. Menopause: biology and pathology. New York: Academic Press; 2000. p. 175-188.

55. Crandall C, Zheng Y, Crawford S, et al. Presence of vasomotor symptoms is associated with lower bone mineral density: A longitudinal analysis. Menopause. 2009;16(2):239-46.

56. Thurston R, Sutton Tyrrell K, Everson Rose S, Hess R, Matthews K. Hot flashes and subclinical cardiovascular disease: Findings from the study of women's health across the nation heart study. Circulation. 2008;118(12):1234-40.

57. Thurston R, El Khoudary S, Sutton Tyrrell K, et al. Vasomotor symptoms and insulin resistance in the study of women's health across the nation. J Clin Endocrinol Metab. 2012;97(10):3487-94.

58. Butler L, Gold E, Greendale G, et al. Menstrual and reproductive factors in relation to mammographic density: The study of women's health across the nation (SWAN). Breast Cancer Res Treat. 2008;112(1):165-74.

59. Reeves K, Stone R, Modugno F, et al. A method to estimate off-schedule observations in a longitudinal study. Ann Epidemiol. 2011;21(4):297-303.

60. Conroy S, Butler L, Harvey D, et al. Metabolic syndrome and mammographic density: The study of women's health across the nation. International journal of cancer. 2011;129(7):1699-707.

61. Conroy S, Butler L, Harvey D, et al. Physical activity and change in mammographic density: The study of women's health across the nation. Am J Epidemiol. 2010;171(9):960-8.

62. Butler L, Gold E, Conroy S, et al. Active, but not passive cigarette smoking was inversely associated with mammographic density. CCC.Cancer causes & control. 2010;21(2):301-11.

63. van den Berg J, Mishra G, van der Schouw Y, Herber-Gast G. Vasomotor menopausal symptoms are not associated with incidence of breast cancer in a population-based cohort of mid-aged women. Eur J Cancer. 2014;50(4):824-30.

64. Tamimi R, Hankinson S, Colditz G, Byrne C. Endogenous sex hormone levels and mammographic density among postmenopausal women. Cancer epidemiology, biomarkers & prevention. 2005;14(11):2641-7.

65. Warren R, Skinner J, Sala E, et al. Associations among mammographic density, circulating sex hormones, and polymorphisms in sex hormone metabolism genes in postmenopausal women. Cancer epidemiology, biomarkers & prevention. 2006;15(8):1502-8.

66. McCormack V, Dowsett M, Folkerd E, et al. Sex steroids, growth factors and mammographic density: A cross-sectional study of UK postmenopausal caucasian and afro-caribbean women. Breast cancer research. 2009;11(3):R38-.

67. Sprague B, Trentham Dietz A, Gangnon R, et al. Circulating sex hormones and mammographic breast density among postmenopausal women. Hormones & cancer. 2011;2(1):62-72.

68. Tamimi R, Byrne C, Colditz G, Hankinson S. Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 2007;99(15):1178-87.

69. Schoemaker MJ, Folkerd EJ, Jones ME, et al. Combined effects of endogenous sex hormone levels and mammographic density on postmenopausal breast cancer risk: Results from the breakthrough generations study. Br J Cancer. 2014;110(7):1898-907.

70. Marchesoni D, Driul L, Ianni A, et al. Postmenopausal hormone therapy and mammographic breast density. Maturitas. 2006;53(1):59-64.

71. Carmona Sánchez E, Cuadros López J, Cuadros Celorrio ÁM, Pérez-Roncero G, González Ramírez A, Fernández Alonso A. Assessment of mammographic density in postmenopausal women during long term hormone replacement therapy. Gynecological endocrinology. 2013;29(12):1067-70.

72. Colacurci N, Fornaro F, De Franciscis P, Palermo M, del Vecchio W. Effects of different types of hormone replacement therapy on mammographic density. Maturitas. 2001;40(2):159-64.

73. Anderson G, Chlebowski R, Aragaki A, et al. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: Extended follow-up of the women's health initiative randomised placebo-controlled trial. Lancet oncology. 2012;13(5):476-86.

74. Gold E, Lasley B, Crawford S, McConnell D, Joffe H, Greendale G. Relation of daily urinary hormone patterns to vasomotor symptoms in a racially/ethnically diverse sample of midlife women: Study of women's health across the nation. Reproductive sciences. 2007;14(8):786-97.

75. Boyd N, Guo H, Martin L, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med. 2007;356(3):227-36.

76. Lokate M, Stellato R, Veldhuis W, Peeters PHM, van Gils C. Age-related changes in mammographic density and breast cancer risk. Am J Epidemiol. 2013;178(1):101-9.

77. Grisso JA, Freeman EW, Maurin E, Garcia Espana B, Berlin JA. Racial differences in menopause information and the experience of hot flashes. Journal of general internal medicine. 1999;14(2):98-103.

78. Avis NE, Stellato R, Crawford S, et al. Is there a menopausal syndrome? menopausal status and symptoms across racial/ethnic groups. Social science medicine. 2001;52(3):345-56.

79. Brown D, Sievert L, Morrison L, Reza A, Mills P. Do japanese american women really have fewer hot flashes than european americans? the hilo women's health study. Menopause. 2009;16(5):870-6.

80. Hankinson S. Endogenous hormones and risk of breast cancer in postmenopausal women. Breast disease;24:3-15.

81. Menarche, menopause, and breast cancer risk: Individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet oncology. 2012;13(11):1141-51.

82. Gompel A, Santen RJ. Hormone therapy and breast cancer risk 10 years after the WHI. Climacteric. 2012;15(3):241-9.

83. Al Azzawi FP, Santiago. Hormonal changes during menopause. Maturitas. 2009;63(2):135-7.

84. Alexander C, Cochran C, Gallicchio L, Miller S, Flaws J, Zacur H. Serum leptin levels, hormone levels, and hot flashes in midlife women. Fertil Steril. 2010;94(3):1037-43.

85. Luetters C, Huang M, Seeman T, et al. Menopause transition stage and endogenous estradiol and follicle-stimulating hormone levels are not related to cognitive performance: Cross-sectional results from the study of women's health across the nation (SWAN). Journal of women's health. 2007;16(3):331-44.

86. Randolph J, Sowers M, Bondarenko I, Harlow S, Luborsky J, Little R. Change in estradiol and follicle-stimulating hormone across the early menopausal transition: Effects of ethnicity and age. J Clin Endocrinol Metab. 2004;89(4):1555-61.

87. Crandall C, Tseng C, Karlamangla A, et al. Serum sex steroid levels and longitudinal changes in bone density in relation to the final menstrual period. J Clin Endocrinol Metab. 2013;98(4):E654-63.

88. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986;124(3):453-69.

89. Allison PD. Survival analysis using SAS: A practical guide. Second Edition ed. Cary, NC: SAS Institute Inc.; 2010.

90. Schwingl PJ, Hulka BS, Harlow SD. Risk factors for menopausal hot flashes. Obstet Gynecol. 1994;84(1):29-34.

91. Miller S, Gallicchio L, Lewis L, et al. Association between race and hot flashes in midlife women. Maturitas. 2006;54(3):260-9.

92. Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: Reanalysis of nine prospective studies. J Natl Cancer Inst. 2002;94(8):606-16.

93. Berendsen HH. The role of serotonin in hot flushes. Maturitas. 2000;36(3):155-64.

94. Stavrou E, Vajdic C, Loxton D, Pearson S. The validity of self-reported cancer diagnoses and factors associated with accurate reporting in a cohort of older australian women. Cancer Epidemiology. 2011;35(6):e75-80.

95. Bergmann MM, Calle EE, Mervis CA, Miracle McMahill HL, Thun MJ, Heath CW. Validity of self-reported cancers in a prospective cohort study in comparison with data from state cancer registries. Am J Epidemiol. 1998;147(6):556-62.