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# Mammographic Density Change With Estrogen and Progestin Therapy and Breast Cancer Risk

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## Abstract

**Background:** Estrogen plus progestin therapy increases both mammographic density and breast cancer incidence. Whether mammographic density change associated with estrogen plus progestin initiation predicts breast cancer risk is unknown.
**Methods:** We conducted an ancillary nested case-control study within the Women's Health Initiative trial that randomly assigned postmenopausal women to daily conjugated equine estrogen 0.625 mg plus medroxyprogesterone acetate 2.5 mg or placebo. Mammographic density was assessed from mammograms taken prior to and one year after random assignment for 174 women who later developed breast cancer (cases) and 733 healthy women (controls). Logistic regression analyses included adjustment for confounders and baseline mammographic density when appropriate.
**Results:** Among women in the estrogen plus progestin arm (97 cases/378 controls), each 1% positive change in percent mammographic density increased breast cancer risk 3% (odds ratio [OR] = 1.03, 95% confidence interval [CI] = 1.01 to 1.06). For women in the highest quintile of mammographic density change (>19.3% increase), breast cancer risk increased 3.6-fold (95% CI = 1.52 to 8.56). The effect of estrogen plus progestin use on breast cancer risk (OR = 1.28, 95% CI = 0.90 to 1.82) was eliminated in this study, after adjusting for change in mammographic density (OR = 1.00, 95% CI = 0.66 to 1.51).
**Conclusions:** We found the one-year change in mammographic density after estrogen plus progestin initiation predicted subsequent increase in breast cancer risk. All of the increased risk from estrogen plus progestin use was mediated through mammographic density change. Doctors should evaluate changes in mammographic density with women who initiate estrogen plus progestin therapy and discuss the breast cancer risk implications.

After 5.6 years (mean) of intervention, the Women's Health Initiative (WHI) randomized clinical trial identified net harm for estrogen plus progestin users including 24% increased breast cancer incidence (1,2). Subsequently, estrogen plus progestin use declined in the United States and elsewhere (3–7), followed by lower invasive breast cancer incidence in most Western countries, largely attributed to reduced estrogen plus progestin usage (6–9). Clinical trials confirmed the observational studies' reports that percent mammographic density, the proportion of total breast area appearing dense on a mammogram (10–12),

increased with estrogen plus progestin use (13–15). Mammographic density increased more with combined estrogen plus progestin use than estrogens alone (13,14,16–18). After one year, estrogen plus progestin use increased mammographic density by 6.9% (95% confidence interval [CI] = 5.3% to 8.5%) compared with placebo in the WHI (15).

Mammographic density strongly predicted breast cancer risk in numerous studies (10–12,19). Risk increased four- to sixfold comparing women with high mammographic density with women with low mammographic breast density (10–12). Until now, only insufficient indirect evidence has suggested that mammographic density change, occurring with estrogen plus progestin initiation, predict increased breast cancer risk. The extent that mammographic density change following estrogen plus progestin initiation increased breast cancer incidence remains unclear. We conducted an ancillary nested case-control study within the WHI randomized, placebo-controlled clinical trial of estrogen plus progestin, which addressed this important question.

## Methods

### Study Population

All study sites' institutional review boards approved this ancillary study and all participants provided written informed consent.

Between 1993 and 1998, the WHI randomly assigned 16 608 postmenopausal women without prior hysterectomy to estrogen plus progestin (0.625 mg daily conjugated equine estrogen and 2.5 mg medroxyprogesterone acetatein, single tablet; Prempro, Wyeth Ayerst, Philadelphia, PA) or an identically appearing placebo (20). Eligibility criteria, recruitment, and implementation details were published (20). In brief, eligible postmenopausal women age 50 to 79 years had no prior hysterectomy or breast cancer and estimated survival of three or more years (20). Prior use of menopausal hormones required three-month washout before baseline. WHI participation required baseline mammogram and clinical breast exam without suggestion of cancer. Study continuation required annual mammogram and breast exam without suspicions of cancer. Centrally trained local adjudicators evaluated pathology reports and medical records for all self-reported breast cancers. WHI Clinical Coordinating Center coders performed final adjudication blinded to random assignment status. Follow-up for clinical outcomes among consenting women lasted 7.9 years (mean) (21). Compliance to the treatment protocol was greater than 90% in both arms of the WHI study through year 1 (1,2). Compliance decreased throughout the WHI, so by the end of follow-up, 42% assigned estrogen plus progestin and 38% assigned placebo stopped the study medications for at least some time (1,2). Furthermore, 6.2% of women assigned estrogen plus progestin and 10.7% assigned placebo arm stopped the trial medication and obtained hormones outside of the WHI (1,2).

This ancillary study sought baseline and one-year follow-up mammograms for women who subsequently developed invasive breast cancer (cases) and a stratified (stratum based on age—within two years, clinical center, and race/ethnicity) random sample of three unaffected women (controls) per case. In total, 399 breast cancer cases were identified (n = 224 assigned estrogen plus progestin and n = 175 assigned placebos), and 1197 cancer-free controls were identified (n = 618 assigned estrogen plus progestin and n = 579 assigned placebos). Controls

were free of breast cancer when the matched case was diagnosed. We contacted and consented eligible participants requesting mammograms for this ancillary study. Procedures to obtain the relevant mammograms followed those from an earlier WHI mammography study (15). Thirty-six of the 40 WHI clinical centers participated, and 61% of the mammograms requested were obtained. A smaller percentage of mammograms were obtained for cases (43.6%) than for controls (61.2%); however, no substantial difference by treatment arm for either cases (43.3% for estrogen plus progestin and 44% for placebo) or for controls (61.2% for estrogen plus progestin and 61.3% for placebo) existed. The major reasons for recovery failure included consent not obtained (42%), films no longer available (33%), incomplete image sets provided (13%), patient deceased (8%), and patient ineligible (4%).

All baseline and follow-up film mammograms sent to the University of North Carolina were digitized using a Kodak Lumisys 85 laser-scanning digitizer (Kodak, Rochester, NY). Standard data averaging methods reduced the image, digitized at 50 micron/pixel spatial resolution and 12-bit depth, to 675 × 925 pixels. Mammographic density assessment used craniocaudal views from the contralateral, cancer-free breast side for all cases and a random side for the controls. All batches included approximately 5% repeated images for within- and between-batch reproducibility assessments. All mammographic images randomly sorted for a given participant were read within the same batch (22). Mammograms for 973 participants were obtained and digitized. However, 66 participants had no baseline mammograms, leaving 907 participants with complete baseline and follow-up mammogram sets, comprising 174 breast cancer cases (97 from the estrogen plus progestin arm and 77 from the placebo arm) and 733 controls (378 from the estrogen plus progestin arm and 355 from the placebo arm).

### Assessment of Mammographic Density

Using two different but comparable validated interactive software tools, four readers (CB, GU, CM, and JP), blinded to participant information, viewed all mammograms on high-resolution monitors for breast density assessment. Three readers (CB, CM, and JP) used Cumulus software developed at the University of Toronto (23,24). The fourth reader (GU) used Madena software developed at the University of Southern California (25). Both software tools calculated the number of pixels defined by the reader as dense within the breast and the number of pixels in the total breast area. The ratio of dense pixels to total pixel in the breast, expressed as a percentage, determined percent mammographic density.

### Statistical Analyses

We used SAS 9.0 (Cary, NC) for data analyses and calculations of 95% confidence intervals (CIs) and P values based on the Wald test. All statistical tests were two-sided, and results were considered statistically significant at a P value of less than .05. Univariate and bivariate analyses presented the primary variable distributions. A woman's original randomized treatment arm (estrogen plus progestin or placebo) defined exposure, regardless of subsequent compliance. Initial logistic regression models evaluated the percent density and percent density change effects on breast cancer risk separately by reader. Although dense area values differed by reader, the comparison of the dense area measures between readers correlated highly

**Table 1.** Baseline percent mammographic density and change in percent mammographic density and univariate odds ratios for the association with breast cancer risk by case status and treatment arm for each of four readers and the average of the four readers

| | Reader | | | | |
|---|---|---|---|---|---|
| Comparisons/subgroups | 1 | 2 | 3 | 4 | Average of all readers |
| Reliability coefficient | 0.876 | 0.899 | 0.938 | 0.965 | |
| Baseline percent Mammographic density | | | | | |
| Placebo (n = 432) | | | | | |
| Controls (n = 355) Mean (SD) | 20.42 (18.50) | 9.50 (10.60) | 12.84 (15.38) | 14.66 (17.00) | 14.36 (14.65) |
| Cases (n = 77) Mean (SD) | 17.28 (13.91) | 8.45 (8.99) | 10.07 (11.41) | 13.21 (14.50) | 12.25 (11.60) |
| OR* (95% CI) | 0.99 (0.98 to 1.00) | 0.99 (0.97 to 1.02) | 0.99 (0.97 to 1.01) | 1.00 (0.98 to 1.01) | 0.99 (0.97 to 1.01) |
| Estrogen plus progestin (n = 475) | | | | | |
| Controls (n = 378) Mean (SD) | 18.84 (15.88) | 9.96 (10.29) | 11.38 (13.02) | 12.66 (14.18) | 13.55 (13.01) |
| Cases (n = 97) Mean (SD) | 22.20 (18.62) | 12.96 (12.02) | 15.89 (16.93) | 14.59 (16.59) | 17.54 (16/09) |
| OR* (95% CI) | 1.01 (0.999 to 1.03) | 1.02 (1.01 to 1.04) | 1.02 (1.01 to 1.04) | 1.02 (1.01 to 1.03) | 1.02 (1.00 to 1.04) |
| Change in percent Mammographic density | | | | | |
| Placebo (n = 432) | | | | | |
| Controls (n = 355) Mean (SD) | to 0.68 (9.85) | to 0.06 (3.40) | to 0.22 (5.44) | 0.08 (4.36) | to 0.22 (4.49) |
| Cases (n = 77) Mean (SD) | 0.98 (10.45) | to 0.12 (2.92) | 0.74 (5.81) | 1.32 (5.53) | 0.73 (5.03) |
| OR* (95% CI) | 1.02 (0.99 to 1.04) | 0.99 (0.92 to 1.07) | 1.03 (0.99 to 1.08) | 1.06 (1.00 to 1.11) | 1.05 (0.99 to 1.10) |
| Estrogen plus progestin (n = 475) | | | | | |
| Controls (n = 378) Mean (SD) | 12.66 (14.18) | 4.45 (6.53) | 4.17 (7.84) | 10.57 (12.71) | 9.49 (10.50) |
| Cases (n = 97) Mean (SD) | 14.59 (16.59) | 10.29 (12.64) | 10.67 (14.85) | 13.18 (15.82) | 10.65 (12.63) |
| OR* (95% CI) | 1.01 (0.99 to 1.02) | 0.99 (0.96 to 1.03) | 1.00 (0.99 to 1.02) | 1.01 (1.00 to 1.03) | 1.01 0.99 to 1.03 |

*Note that these are univariate results and the odds ratios for breast cancer correspond with having 1% more mammographic density at baseline and a 1% mean change in mammographic density. CI = confidence interval; OR = odds ratio.

(Pearson correlations of R = 0.90–0.97), and breast area assessment also correlated highly between readers (Pearson correlations of R = 0.94–0.99). Because univariate odds ratios (ORs) for breast cancer associated with mammographic density across the four readers were quite consistent, subsequent analyses used the mean mammographic density of the four readers (Table 1). The continuous variable of mammographic density compared a 1% difference in baseline mammographic density percent or 1% change in mammographic density percent.

## Analytic Model

Because earlier WHI analyses established that estrogen plus progestin treatment increased breast cancer risk, our initial analyses determined if mammographic density change was associated with increased breast cancer risk. To adjust for potential confounders, logistic regression models included breast cancer risk factors associated with mammographic density change. The initial model contained center, age at baseline (continuous), ethnicity (non-Hispanic white, African American, and other) baseline BMI (continuous), BMI change (continuous), age at first birth (<20, 20–24, 25–29, 30–34, and 35+ years/missing), parity (0, 1, 2, 3, missing), length of follow-up (continuous), personal history of menopausal hormone therapy use (never, previous, current user prior to three-month washout), age at last birth (<20, 20–24, 25–29, 30+ years, and missing), time since menopause (continuous), first-degree family history of breast cancer (yes, no, and missing), and alcohol consumption at baseline (yes, no, and missing). Individually, factors least associated with cancer risk were considered for removal. Retained factors changed the beta estimate in the final model by 10% or more. The final model included age, baseline BMI, center, age at first birth, and parity. We evaluated effects of baseline mammographic density and mammographic density change stratified by treatment group, as well as potential interactions between these measures. Separate logistic regression models adjusted for covariates included interaction terms for baseline density and treatment arm, baseline density and mammographic density change, and treatment arm and mammographic density change. Additional analyses of quintiles of mammographic density change evaluated potential nonlinear effects. Mammographic density change quintile cut-points were 0.6%, 4.6%, 10.7%, and 19.3% in the estrogen plus progestin group, and -2.8%, -0.51%, 0.40%, and 2.2% in the placebo group. Separate logistic regression models evaluated mammographic density change associations across three levels of baseline mammographic density (<10%, 10%–25%, and >25%). We calculated the attributable risk for increased mammographic density after estrogen plus progestin initiation with 95% confidence intervals using bootstrap methods.

To evaluate how well this study represented the WHI clinical trial cohort, we determined estrogen plus progestin effects on breast cancer risk in this mammography substudy with logistic regression. We evaluated if the odds ratio differed after controlling for mammographic density change, hypothesizing that a reduced or null odds ratio for breast cancer associated with estrogen plus progestin would indicate that mammographic density change explained the estrogen plus progestin influence on breast cancer risk. We conducted mediation analyses of the estrogen plus progestin total effect on breast cancer risk, with mammographic density change as the mediating variable, to assess the proportion-mediated measure (indirect effect/total effect) (26).

## Results

Women assigned placebos exhibited minimal mammographic density change over one year (mean change = -0.05%) (Figure 1). In contrast, those assigned estrogen plus progestin had a larger and a broad distribution of mammographic density change
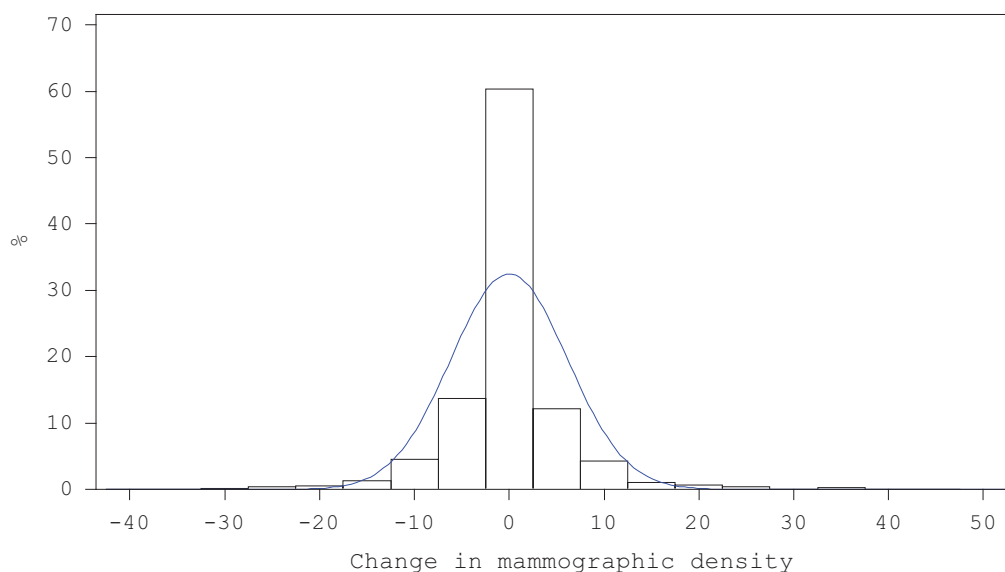
**Figure 1.** Distribution of change in mammographic density from baseline to at least one year after random assignment within participants from the placebo arm of the Women's Health Initiative. The mean change in percent mammographic density declined by 0.05%, with a median change in mammographic density of 0.0% and a standard deviation of 6.36.
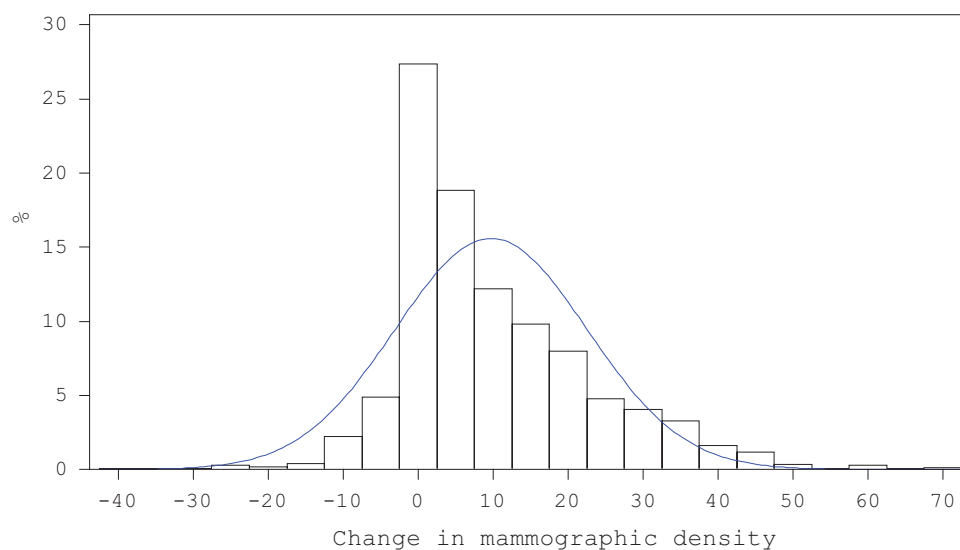


**Figure 2.** Distribution of change in mammographic density from baseline to at least one year after random assignment within participants from the estrogen plus progestin arm of the Women's Health Initiative. The mean change in percent mammographic density increased by 9.73%, with a median change in mammographic density of 6.02% and a standard deviation of 12.80.

(mean change = 9.7%) (Figure 2). Of the 432 placebo-assigned women, 202 (46.8%) increased in mammographic density while 230 (53.2%) decreased. In contrast, of 475 estrogen plus progestin–assigned women, 399 (84.0%) increased in mammographic density while only 76 (16.0%) decreased. After adjusting for covariates including baseline density, the difference in mean change in mammographic density between the placebo (-0.65%, 95% CI = -1.86 to 0.55) and the estrogen plus progestin (9.49%, 95% CI = 8.25 to 10.72) treatment arms was statistically significant (P < .001).

Overall, after adjustment, each 1% difference in baseline mammographic density increased breast cancer risk by 1% (OR = 1.01, 95% CI = 1.00 to 1.02). Additional adjustment for

treatment arm did not change this association. In treatment-stratified analyses, baseline mammographic density did not increase breast cancer risk among women assigned placebos, but breast cancer risk increased 3% among women assigned estrogen plus progestin (OR = 1.03, 95% CI = 1.01 to 1.05) with each 1% baseline density difference (Table 2). The interaction between baseline mammographic density with treatment ($\beta = -0.032$, P = .02) was statistically significant. Controlling for baseline mammographic density (Table 2), a 1% change in mammographic density increased breast cancer risk 4%, but not statistically significantly, in women assigned placebos. In contrast, the increased breast cancer risk in women assigned estrogen plus progestin of 3% (OR = 1.03, 95% CI = 1.01 to 1.06) with a

**Table 2.** Adjusted odds ratios and 95% confidence intervals for breast cancer associated with continuous measures of baseline mammographic breast density and change in percent mammographic density

| Analysis categories | OR* (95% CI) |
| --- | --- |
| Placebo (n = 432) | |
| Baseline percent mammographic density† | 1.00 (0.98 to 1.02) |
| Change in percent mammographic density† | 1.04 (0.98 to 1.11) |
| Estrogen plus progestin (n = 475) | |
| Baseline percent mammographic density† | 1.03 (1.01 to 1.05) |
| Change in percent mammographic density† | 1.03 (1.01 to 1.06) |

*All logistic regression models adjusted for baseline body mass index, age, clinical center, age at first birth, and parity, and the odds ratio is the increase in breast cancer risk with a 1% increase in mammographic density. These analyses were mutually adjusted for baseline and change in percent mammographic density. CI = confidence interval; OR = odds ratio.
†A mean value based on the average of readers 1–4.

**Table 3.** The adjusted odds ratios and 95% confidence intervals associated with change of 1% mammographic breast density from baseline to one year by treatment group within strata of baseline density

| Analysis category | No. of cases/controls | OR* (95% CI) |
| --- | --- | --- |
| Placebo (n = 432) | | |
| Baseline density, % | | |
| <10 | 48/189 | 1.08 (0.98 to 1.18) |
| 10–25 | 16/97 | 1.01 (0.89 to 1.15) |
| >25 | 13/69 | 1.01 (0.90 to 1.13) |
| Estrogen plus progestin (n = 475) | | |
| Baseline density, % | | |
| <10 | 45/207 | 1.04 (1.00 to 1.07) |
| 10–25 | 27/99 | 1.08 (1.02 to 1.14) |
| >25 | 25/72 | 0.94 (0.87 to 1.01) |

*All odds ratio adjusted for age, baseline body mass index, clinical center, age at first birth, and parity and are the increase in breast cancer risk with a 1% change in mammographic density. CI = confidence interval; OR = odds ratio.

1% change in mammographic density was statistically significant. Controlling for baseline mammographic density, the interaction between mammographic density change and treatment group was not statistically significant (P = .34). Table 3 shows the mammographic density change effects within strata of baseline density. In the placebo and the estrogen plus progestin arms, having more than 25% density at baseline reduced the magnitude of the association between mammographic density change and breast cancer risk (Table 3). Controlling for treatment arm and other covariates, baseline mammographic density negatively interacted with mammographic density change ($\beta$ = -0.0013, P = .03).

In addition to linear effects, we evaluated quintiles of mammographic density change based on the distributions within each randomized arm (Table 4). The cut-points for the upper quintile of mammographic density change were 2.2% or greater for women using placebo and 19.3% or greater for women using estrogen plus progestin. In the placebo arm, the increase in breast cancer risk was not statistically significant (OR = 1.20, 95% CI = 0.48 to 2.97) comparing the highest to the lowest quintile of mammographic density change. In contrast, there was a statistically significant 3.6-fold increased risk in breast cancer in the estrogen plus progestin arm comparing the highest with

the lowest quintile of mammographic density change (OR = 3.61, 95% CI = 1.52 to 8.56).

Women in the estrogen plus progestin arm had increased breast cancer risk compared with those in the placebo arm (OR = 1.28, 95% CI = 0.90 to 1.82), a finding comparable with the overall randomized clinical trial where a 24% increase was reported (hazard ratio [HR] =1.24, 95% CI = 1.01 to 1.54) (2,27). Including mammographic density change in the analytic model, no residual effect of estrogen plus progestin on breast cancer risk remained (OR = 1.00, 95% CI = 0.66 to 1.51). In an unadjusted mediation model, 97.4% of the estrogen plus progestin use effect was mediated through density change, while in a model adjusted for covariates and interaction, 100% of the estrogen plus progestin effect was mediated through density change. To determine if estrogen plus progestin only increased breast cancer risk when women had a very large (≥20%) mammographic density change, we removed from the analysis those with a 20% or greater increase in mammographic density and a 13% increased breast cancer risk with hormone use persisted (OR = 1.13, 95% CI = 0.77 to 1.65). Further adjustment for mammographic density change eliminated that association (OR = 0.97, 95% CI = 0.63 to 1.51). The attributable fraction comparing a 20% or greater increase to a less than 1% increase in density identified that 17% of the breast cancer cases were potentially explained by increased breast density after estrogen plus progestin initiation.

## Discussion

In this case-control study, nested in the WHI trial, breast cancer risk increased with increasing mammographic density following estrogen plus progestin initiation. For every 1% increase in mammographic density among those assigned estrogen plus progestin, breast cancer risk increased 3.4%. Adjustment for mammographic density change left no residual effect of combined estrogen plus progestin use on breast cancer risk. Our results suggested that increased mammographic density with estrogen plus progestin initiation should raise concern and warrant consideration of stopping therapy. In the WHI randomized trial, in addition to increasing breast cancer incidence (2), estrogen plus progestin also statistically significantly interfered with breast cancer detection (28), leading to more advanced stage at diagnosis (2,29), increased breast cancer mortality (21), and other adverse health outcomes (30).

These results pertained to postmenopausal women initiating estrogen plus progestin therapy and not to use of estrogen alone. This nested study's strengths included the assessment of serial mammograms using validated quantitative measures of density and the WHI strengths of the randomized placebo-controlled trial design, large participant numbers, comprehensive breast cancer risk assessment, central breast cancer adjudication, and requirement for annual mammography and clinical breast exam. Using the average measure of mammographic density from four readers provided a more conservative estimate of assessing mammographic density where no established gold standard for assessment exists. Retrospectively collecting mammograms required participant re-consent, limiting this study's numbers. Although we obtained 61% of requested mammograms with differences in recovery by case-control status, no meaningful differences in mammogram recovery existed between estrogen plus progestin and placebo-assigned participants overall, reducing concerns of bias. We conducted an intent-to-treat analysis based on WHI random assignment. Our study measure, mammographic density change, would only be

**Table 4.** Breast cancer risk by quintile of change in percent mammographic density

| Analysis categories | Q1* | Q2 | Q3 | Q4 | Q5 |
|---|---|---|---|---|---|
| Placebo (n = 432) | | | | | |
| Cut-points† | <-2.77 | <-0.51 | <0.40 | <2.2 | ≥2.2 |
| OR (95% CI) | 1.00 (ref) | 0.94 (0.38 to 2.30) | 1.38 (0.54 to 3.48) | 0.96 (0.38 to 2.43) | 1.20 (0.48 to 2.97) |
| Estrogen+progestin (n = 475) | | | | | |
| Cut-points† | <0.64 | <4.61 | <10.68 | <19.32 | ≥19.32 |
| OR (95% CI) | 1.00 (ref) | 1.77 (0.76 to 4.12) | 1.04 (0.45 to 2.43) | 1.72 (0.74 to 4.02) | 3.61 (1.52 to 8.56) |

*Q1 is the reference category for comparisons within the treatment arm. All odds ratios adjusted for age, baseline body mass index, clinical center, age at first birth, and parity. CI = confidence interval; OR = odds ratio.
†Quintile cut-points of change in percent mammographic density.

minimally impacted by noncompliance (<10% at year 1). Given higher noncompliance with years of follow-up, in the WHI the true effect of mammographic density change on breast cancer risk was likely underestimated given the noncompliance in both arms of the study.

This study was not without limitations. The number of cases detected in the WHI clinical trial before the trial stopped and our ability to obtain mammograms limited this study's size. The number of breast cancer cases in the placebo arm with both mammograms obtained (n = 77) could have limited detectability of statistically significant risk with a 1% increase in baseline breast density. We found statistically significant and slightly negative interactions between baseline mammographic density and treatment arm, and baseline mammographic density and mammographic density change with respect to breast cancer risk. Given this negative interaction and as the women who became cases in the estrogen plus progestin arm had higher baseline density despite random assignment, all analyses controlled for baseline density. The negative interaction with mammographic density change suggested that already high breast cancer risk in women with high baseline mammographic density did not increase as much with density change from estrogen plus progestin use as did risk for women who started with a low baseline mammographic density. However, this interaction should be interpreted with caution, as it may have reflected the limitations in measuring density change among women with dense breasts.

The current findings support emerging evidence that physiologic responses in breast tissue to hormone-based intervention may foreshadow subsequent breast cancer risk. For women assigned estrogen plus progestin, in the highest 20th percentile of increased mammographic density, 3.6-fold increased breast cancer risk was statistically significant. Cuzick and colleagues (31) reported a breast cancer risk reduction among women with the greatest mammographic density reduction from a case-control study nested in the first International Breast Cancer Intervention Study (IBIS-I), a randomized prevention trial comparing tamoxifen with placebo. In women receiving tamoxifen, the categorical mammographic density reduction was linked with subsequent breast cancer incidence reduction. However, analyses adjusting for tamoxifen-associated mammographic density change were not presented.

Measuring mammographic density at only one time point for each woman, Boyd and colleagues (32) provided indirect evidence that mammographic density was a biomarker of breast cancer risk. Combining data from three nested case-control studies, they found that estrogen plus progestin users who developed breast cancer had higher mammographic density. Crandall and colleagues reported on breast tenderness onset in WHI estrogen plus progestin trial participants (33,34). After one

year, women using estrogen plus progestin compared with placebo experienced breast tenderness more often (36% vs 12%, $P < .001$) and breast cancer risk increased ($P = .02$) for those taking estrogen plus progestin who had breast tenderness. Mammographic breast density was associated with breast tenderness, yet adjusting for breast tenderness onset did not remove but lowered the estrogen plus progestin breast cancer risk association (HR = 1.19, 95% CI = 0.94 to 1.76, after adjustment) (33,35). In contrast, in our study, adjustment for mammographic density change eliminated the increased risk among estrogen plus progestin users, suggesting that mammographic density change better predicted breast cancer risk from estrogen plus progestin use than did breast tenderness.

In our analyses, after adjustment for mammographic density change, estrogen plus progestin use no longer increased breast cancer risk (OR = 1.00, 95% CI = 0.66 to 1.51). Mediation analyses found no residual direct effect of estrogen plus progestin use on breast cancer risk after including the indirect mammographic density change effect. These findings clarify the estrogen plus progestin use, mammographic density change, and breast cancer risk associations. In this study, mammographic density change with estrogen plus progestin use was an intermediate surrogate marker of breast cancer risk. Based on this study, doctors should evaluate changes in mammographic density in women who initiate estrogen plus progestin therapy and discuss the breast cancer risk implications.

## Funding

## Notes

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