Association of New-Onset Breast Discomfort With an Increase in Mammographic Density During Hormone Therapy

Carolyn J. Crandall, MD, MS; Arun Karlamangla, MD, PhD; Mei-Hua Huang, DrPH; Giske Ursin, PhD; Min Guan, MS; Gail A. Greendale, MD

Background: Postmenopausal use of estrogen and progestin therapy increases breast density and breast discomfort. Whether this increase in breast density is heralded by new-onset breast discomfort is unknown.

Methods: We used data from the Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study, which retrieved and examined baseline and 12-month mammograms for 594 (67.9%) of 875 women aged 45 to 64 years enrolled in the randomized controlled trial. Treatments included placebo, 0.625 mg/d of conjugated equine estrogens, 0.625 mg/d of conjugated equine estrogens and medroxyprogesterone acetate (10 mg/d for 12 d/mo or 2.5 mg/d continuously), or 0.625 mg/d of conjugated equine estrogens and 200 mg/d of micronized progesterin for 12 d/mo. Breast density (the percent of the breast composed of dense tissue) was calculated from digitized mammograms obtained at baseline and at 12-month follow-up. Breast discomfort was ascertained at baseline and at follow-up using standardized self-report questionnaires. In bivariate analysis, and then in multivariable linear regression models, we assessed the association between change in percent breast density from baseline to 12-month follow-up and new-onset breast discomfort in participants who had no breast discomfort at baseline (N=533).

Results: After adjustment for age, treatment assignment (placebo, conjugated equine estrogens, or progestin-containing regimen), and other potential confounders, women with new-onset breast discomfort had a 3.9% increase in percent breast density compared with a 0.6% increase in percent breast density among women without new-onset breast discomfort (β=.033, P<.001). The association between incident breast discomfort and increased percent breast density was similar in all active treatment arms.

Conclusion: In postmenopausal women randomly assigned to menopausal hormone therapy vs placebo, new-onset breast discomfort is associated with increased mammographic density.

Arch Intern Med. 2006;166:1578-1584
METHODS

PARTICIPANTS

Data for this analysis came from the PEPI-MDS, an independent study initiated after the PEPI trial was completed. The PEPI trial design has been described in detail. In brief, the PEPI trial was a double-blind, randomized, controlled trial designed to compare the effects of placebo and several regimens of orally administered menopausal HT on cardiovascular disease risk factors in postmenopausal women recruited between December 27, 1989, and February 8, 1991, by the following 7 participating centers: George Washington University, Washington, DC; The Johns Hopkins University, Baltimore, Md; Stanford University, Stanford, Calif; University of California, Los Angeles; University of California, San Diego; University of Iowa, Iowa City; and University of Texas Health Science Center, San Antonio. The treatment regimens were placebo (placebo arm), 0.625 mg/d of conjugated equine estrogens (CEE arm), 0.625 mg/d of conjugated equine estrogens and 10 mg/d of medroxyprogesterone acetate for 12 d/mo (CEE and cyclical MPA arm), 0.625 mg/d of conjugated equine estrogens and 2.5 mg/d of medroxyprogesterone acetate continuously (CEE and continuous MPA arm), or 0.625 mg/d of conjugated equine estrogens and 200 mg/d of micronized progesterin for 12 d/mo (CEE and MP arm). The 875 PEPI trial participants (of 1557 women screened) had to be between 45 and 64 years old and had been menopausal for at least 1 year at enrollment into the trial. Women with any major contraindication to the use of combined estrogen or progestin therapy (eg, breast cancer) were excluded. 7

The PEPI-MDS was designed to elucidate hormonal, genetic, and lifestyle determinants of breast density. Of 875 PEPI trial participants, 603 had retrievable baseline mammograms. Of these, 7 women had breast implants and 2 women’s mammograms were technically inadequate. As a result of these exclusions, mammograms from 594 women (67.9% of 875 women in the PEPI trial) were examined in the PEPI-MDS. 3-14-16

Fifty-two of 594 women in the PEPI-MDS who had reported breast discomfort at baseline and were excluded from the present study. An additional 9 women were excluded because breast discomfort information was unavailable for them at 12-month follow-up, leaving us with an analytic sample size of 533.

MEASUREMENTS

Information about age, ethnicity, cigarette smoking, alcohol use, physical activity level, parity, ethnicity, prior hysterectomy, years since menopause, and presence of breast discomfort came from standardized self-report questionnaires. The following specific questions regarding breast discomfort were asked at baseline and at 12-month follow-up: “During the past week including today, did any of these symptoms bother you or interfere with your life? Breast sensitivity/tenderness (yes or no), painful breasts (yes or no).” We classified women who reported breast sensitivity/tenderness (yes or no) and/or painful breasts (yes or no) as having breast discomfort. Weight and height were measured at baseline. Compliance with treatment was defined as having taken at least 80% of study medication at 6-month and 12-month follow-up visits, verified by pill count.

The serum estrone level was measured from fasting blood samples drawn at baseline and at 12-month follow-up and was analyzed using radioimmunoassay as previously described. Interassay and intra-assay coefficients of variation for serum estrone were 15% and 16%, respectively. The lower limit of detection of the estrone assay was 3 pg/mL.

PRerandomization conventional craniocaudal mammograms obtained between 1989 and 1994 during the original PEPI study were retrieved from the 7 participating PEPI trial clinical centers. Percent breast density was quantified by a single reader (G.U.) as the percent of the breast image that was composed of dense tissue using methods detailed previously. In brief, mammograms were digitized at a resolution of 150 pixels per square inch. Breast tissue was outlined on the digitized mammogram image by the reader; next, the reader established for each image a density threshold above which breast tissue was to be considered dense and a density threshold below which breast tissue was to be considered nondense. The software calculated percent breast density as the ratio of the total area of dense breast tissue in the image to the total area of the breast tissue.

STATISTICAL ANALYSIS

We compared key demographic and lifestyle-related characteristics of the analytic sample with those of the remainder of the PEPI trial participants using χ² test (continuous variables) or χ² test (categorical variables). Because baseline percent breast density was not normally distributed, we compared baseline percent breast density in women who had baseline breast discomfort vs those who did not have baseline breast discomfort using Wilcoxon rank sum test.

In the longitudinal sample of 533 women without baseline breast discomfort, we compared the mean 12-month change in percent breast density in women who had new-onset breast discomfort vs those who did not have new-onset breast discomfort using t test. We then adjusted for potential confounders using a multivariable linear regression model with change in percent breast density as the outcome and incident breast discomfort (yes or no) as the main predictor. The incidence of new-onset breast discomfort and the 12-month change in percent breast density were similar among women assigned to the 3 progestin-containing regimens. Therefore, we grouped the progestin arms and included treatment arm as a 3-level covariate composed of placebo, CEE alone, and any progestin-containing regimens. We also included pill compliance as a covariate. Other covariates for the regression models, chosen on the basis of prior publications, biological plausibility, and statistical considerations, included current smoking (yes or no), ethnicity (white vs nonwhite), number of pregnancies resulting in live births (continuous), age at first pregnancy (continuous), body mass index (continuous), and alcohol consumption (continuous) and log-transformed baseline percent breast density.

We considered potential interactions by adding interaction terms to the linear regression models containing these covariates. To assess the statistical significance of each interaction term, we evaluated the P values for the interaction term in the linear regression model. Interaction terms were dropped from the model if P > .10 for tests of significance. Four poten-

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therapy. Therefore, in secondary analyses, we added baseline level during menopausal combined estrogen and progestin breast density was related to the magnitude of change in estrone containing the covariates. PEPI trial, we added an interaction term (recent hormone use–treatment assignment–new-onset breast discomfort interaction. Third, we tested for compliance–treatment assignment interaction. Fourth, to determine whether the association between new-onset breast discomfort and change in breast density was modified by recent HT use (ie, quitting hormones to join the PEPI trial), we added an interaction term (recent hormone use–new-onset breast discomfort) to the linear regression model containing the covariates.

The PEPI-MDS previously reported that change in percent breast density was related to the magnitude of change in estrone level during menopausal combined estrogen and progestin therapy. Therefore, in secondary analyses, we added baseline estrone level and change in estrone level to the model to assess whether the new-onset breast discomfort–change in percent breast density link was mediated by change in estrone level. All statistical tests were 2-sided. \( P<.05 \) for main terms or \( P<.10 \) for interaction terms was considered statistically significant. Statistical analyses were performed using SAS version 9.0 (SAS Institute Inc, Cary, NC). The protocol was approved by the institutional review boards at each participating center.

RESULTS

The 533 participants included in the present analysis were similar to the rest of the PEPI trial participants for variables of interest (Table 1). Participants in the analytic sample were on average 56.1 years old and had been menopausal for 5.6 years. Most were white and nonsmokers. These women were also similar to the 61 PEPI-MDS participants who were excluded on the basis of baseline breast discomfort (n=52) or lack of follow-up discomfort information (n=9). A higher proportion of the 61 excluded participants were compliant with treatment assignment, had prior use of hormone therapy, and had lower body mass index than participants excluded because of baseline breast discomfort. In addition, these women were more likely to have had bilateral oophorectomy and to be nonwhite than those included in the present analysis. These women were also similar to the rest of the PEPI trial participants for variables of interest (Table 1). Participants in the analytic sample were on average 56.1 years old and had been menopausal for 5.6 years. Most were white and nonsmokers. These women were also similar to the 61 PEPI-MDS participants who were excluded on the basis of baseline breast discomfort (n=52) or lack of follow-up discomfort information (n=9). A higher proportion of the 61 excluded participants were compliant with treatment assignment, had prior use of hormone therapy, and had lower body mass index than participants excluded because of baseline breast discomfort. In addition, these women were more likely to have had bilateral oophorectomy and to be nonwhite than those included in the present analysis.

### Table 1. Baseline Characteristics of the Analytic Sample Compared With Those of the Excluded PEPI-MDS Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Remainder of PEPI-MDS Participants</th>
<th>Excluded Because of Baseline Breast Discomfort</th>
<th>Remainder of Excluded Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 61)</td>
<td>(n = 61)</td>
<td>(n = 281)</td>
</tr>
<tr>
<td>Age, y</td>
<td>56.1 ± 4.3</td>
<td>55.0 ± 4.3</td>
<td>56.2 ± 4.3</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>5.8 ± 2.7</td>
<td>5.6 ± 2.8</td>
<td>5.7 ± 2.8</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.0 ± 4.5</td>
<td>27.9 ± 4.3</td>
<td>25.6 ± 4.3</td>
</tr>
<tr>
<td>Prior use of hormone therapy</td>
<td>295 (55.3)</td>
<td>34 (55.7)</td>
<td>163 (58.0)</td>
</tr>
<tr>
<td>Recency of hormone therapy use</td>
<td>25.4 ± 46.9</td>
<td>18.7 ± 31.5</td>
<td>26.3 ± 55.0</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>69 (12.9)</td>
<td>8 (13.1)</td>
<td>41 (14.6)</td>
</tr>
<tr>
<td>Former</td>
<td>202 (37.9)</td>
<td>23 (37.7)</td>
<td>100 (35.6)</td>
</tr>
<tr>
<td>Never</td>
<td>262 (49.2)</td>
<td>30 (49.2)</td>
<td>140 (49.8)</td>
</tr>
<tr>
<td>Alcohol use, g/d</td>
<td>6.5 ± 12.2</td>
<td>5.3 ± 14.1</td>
<td>6.9 ± 11.5</td>
</tr>
<tr>
<td>Level of physical activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>362 (67.9)</td>
<td>38 (62.3)</td>
<td>175 (62.3)</td>
</tr>
<tr>
<td>Medium</td>
<td>166 (31.1)</td>
<td>23 (37.7)</td>
<td>105 (37.4)</td>
</tr>
<tr>
<td>High</td>
<td>9 (0.9)</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Compliance with treatment assignment</td>
<td>467 (87.6)</td>
<td>52 (85.2)</td>
<td>230 (81.9)</td>
</tr>
<tr>
<td>Uncertain menopause</td>
<td>123 (23.1)</td>
<td>19 (31.1)</td>
<td>78 (27.8)</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>154 (28.9)</td>
<td>25 (41.0)</td>
<td>100 (35.6)</td>
</tr>
<tr>
<td>Baseline % breast density, median (IQR)</td>
<td>23.8 (28.0)</td>
<td>19.0 (30.4)</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; PEPI-MDS, Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study.

aMost (52 of 61 participants) had baseline breast discomfort.

bData are given as mean ± SD or as number (percent) unless otherwise indicated.

The last menstrual period for women who had a hysterectomy with bilateral oophorectomy was considered to be the date of the hysterectomy. Because of some missing answers, sample sizes for years since menopause were 36 (women with baseline breast discomfort), 417 (women without baseline breast discomfort), and 204 (remainder of PEPI-MDS participants).

cCalculated as weight in kilograms divided by the square of height in meters.

dPrior use of hormone therapy was determined by self-report at the baseline visit. The protocol was approved by the institutional review boards at each participating center.

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women had prior hysterectomy compared with participants included in our analytic sample. The median baseline percent breast density did not differ between the 2 groups (19.0% in the excluded group vs 23.8% in the analytic sample; P=.42, Wilcoxon rank sum test).

The overall group median percent breast density at baseline was 23.6% (interquartile range, 28.1%). The baseline percent breast density was similar by treatment arm (P=.97, analysis of variance test of difference by treatment arm). The prevalence of breast discomfort at baseline was also similar among treatment arms (range, 7.6%-11.4%).

In the 533 women in our analytic sample, the incidence of new-onset breast discomfort at 12-month follow-up was 21.2%. The incidence differed statistically among treatment arms: 12.5%, 14.8%, 28.0%, 23.2%, and 27.5%, respectively, in the placebo arm, CEE arm, CEE and cyclical MPA arm, CEE and continuous MPA arm, and CEE and MP arm (P=.01, overall χ² test) (Figure 1).

Compared with the placebo and CEE-only arms, in which the risks of new-onset breast discomfort were 12.5% and 14.8%, respectively, the risks in the 3 progestin-containing arms were about double (range, 23.2%-28.0%) (Figure 1) (range, P=.04 to P=.006 for comparison of the 3 progestin-containing arms with the placebo arm and P=.02 to P=.11 for comparison of the 3 progestin-containing arms with the CEE alone arm). The occurrence of new-onset breast discomfort did not differ significantly among the 3 progestin-containing arms (range, P=.42 to P=.96 for pairwise comparisons). The occurrence of new-onset breast discomfort was 26.2% in the aggregated progestin-containing arms, significantly higher than that in the CEE-only arm (P=.02) and in the placebo arm (P<.01), as demonstrated in a prior PEPI study. The incidence of new-onset breast discomfort in the CEE-only arm was not statistically different from that in the placebo arm (P=.62).

The mean±SD 12-month increase in percent breast density (12-month percent breast density minus baseline percent breast density) was 2.7%±8.7%. Figure 2 shows that at 12 months the percent breast density had decreased 0.4% in the placebo arm and had increased in the CEE arm (0.9%), CEE and cyclical MPA arm (4.6%), CEE and continuous MPA arm (4.4%), and CEE and MP arm (3.1%). The mean change in percent breast density was not different among the 3 progestin-containing arms (P=.68). Taken as a single group, the change in percent breast density was 4.0% in the progestin-containing arms, and this was significantly higher than that in the CEE-only arm (P<.001) and in the placebo arm (P<.001). The change in percent breast density in the CEE-only arm was not significantly higher than that in the placebo arm (P=.25).

**UNADJUSTED AND ADJUSTED PRIMARY ASSOCIATION**

The mean change in percent breast density was higher in women who had new-onset breast discomfort vs those who did not have new-onset breast discomfort (5.4% vs 1.8%, P<.001). After adjustment for parity, age at baseline, age at first pregnancy, body mass index, alcohol intake, smoking, ethnicity, baseline percent breast density, compliance with treatment in a linear regression model, and treatment arm assignment (placebo, CEE-only, or progestin-containing regimen), women with new-onset breast discomfort had a 3.5% increase in percent breast density compared with a 0.2% increase in women without new-onset breast discomfort (β=.033, P<.001).
TESTS OF EFFECT MODIFICATION

We next added a treatment assignment–×-compliance interaction term to the linear regression model. After inclusion of the treatment assignment–×-compliance interaction term in the model, women with new-onset breast discomfort had a 3.9% increase in percent breast density compared with a 0.6% increase in percent breast density among women without new-onset breast discomfort (β=.033, P<.001) (Table 2).

The compliance–×–new-onset breast discomfort interaction was statistically nonsignificant (P=.32), indicating that compliance with treatment did not affect the association between new-onset breast discomfort and change in percent breast density. Therefore, this interaction term was omitted from the final model. Recent use of HT before joining the PEPI trial also did not affect the association between new-onset breast discomfort and change in percent breast density (P=.42 for the interaction term, β=.033 without the interaction term and β=.036 with the interaction term).

The treatment assignment–×–new-onset breast discomfort interaction term was of borderline statistical significance (P=.15) with reference to our predesignated interaction term cutoff (P<.1). The inability to detect effect modification by treatment arm assignment might reflect the small treatment arm sizes and not a true uniformity of the association between new-onset breast discomfort and increase in percent breast density across treatment arms.

Figure 3 shows the mean change in percent breast density in women who had new-onset breast discomfort vs those who did not have new-onset breast discomfort before and after stratification by treatment arm. Although the effect of new-onset breast discomfort on increase in percent breast density seemed to be markedly greater in women assigned to progestin-containing therapies, this trend was statistically nonsignificant (P=.15, test of effect modification by treatment arm).
SECONDARY EXPLORATORY ANALYSIS

In secondary analyses designed to explore whether the relation between incident breast discomfort and change in percent breast density was mediated by the increase in estrone level during postmenopausal HT, we added baseline estrone level and change in estrone level to the final linear regression model. Although change in estrone level significantly predicted change in percent breast density (P < .01), new-onset breast discomfort remained a significant predictor of change in percent breast density (β = .030, P < .01).

The primary research question of this study was “Does new-onset breast discomfort herald increased percent breast density?” Our findings suggest that the answer is yes. The results of 2 previous studies suggest a link between HT-induced breast discomfort and increased breast density; however, these studies were nonrandomized and unblinded. The lack of blinding is a particularly important limitation because breast discomfort data are self-reported.

If increased mammographic density during HT is indicative of increased breast cancer risk, new-onset breast discomfort during HT may be a marker of increased risk of breast cancer. While the PEPI-MDS showed that estrogen and progestin therapy increases mammographic density, the present analysis shows that new-onset breast discomfort provides additional independent information that distinguishes women who experience greater increases in percent breast density during HT.

Neither the magnitude of the association between incident breast discomfort and increase in percent breast density, nor the statistical significance of this association, was altered by adjustment for baseline estrone level and change in estrone level. This observation underscores the importance of considering the possible roles of growth factors, sex hormone–binding globulin, and other estrogens, estrone metabolites, or sex steroids as mediators of this association between new-onset breast discomfort and change in breast density.

The limitations of our study are that it could not capture any breast discomfort that appeared and resolved between the baseline and 12-month assessments and that we did not assess changes in the severity of breast discomfort. Our study also did not allow us to evaluate the chronological sequence (ie, we cannot determine whether the mammographic density change preceded or followed the onset of breast discomfort). The association between new-onset breast discomfort and increase in percent breast density may not be the same for other estrogens and progestins compared with those used in the PEPI trial. Finally, the link between increased breast density in the context of HT use and possible increased risk of breast cancer remains unproven. The strengths of the PEPI-MDS include the high level of participant compliance with assigned therapy, a detailed standardized questionnaire that supplied information about multiple characteristics linked with mammographic density, the best available means of assessing mammo-graphic density, and a prospective, placebo-controlled, randomized, blinded design.

In postmenopausal women without breast discomfort before the initiation of CEE or CEE and a progestin, new-onset breast discomfort signals increased mammographic density independent of the actual therapy regimen. If increased mammographic density is indicative of increased breast cancer risk during HT, our findings suggest that new-onset breast discomfort during therapy might be a useful clinical marker for increased risk of breast cancer. We expect to test this hypothesis in future studies with breast cancer outcomes. The mechanisms behind HT-associated increases in breast discomfort and mammographic density, and the potential utility of surveillance strategies for women who develop new-onset breast discomfort during menopausal HT, deserve further investigation.

Accepted for Publication: May 11, 2006.

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Author Contributions: Dr Crandall had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: None reported.

Funding/Support: This study was supported by grant 8KB-0035 from the California Breast Cancer Research Program, by grant 5K12AG01004 from the National Institute on Aging, and by grant 2R01 CA077708 (to Dr Greendale) from the National Institutes of Health.

Role of the Sponsors: The funding sources did not participate in the design or conduct of the study; collec-
...tion, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. **Acknowledgment:** We thank the women who generously participated in the PEPI trial.

**REFERENCES**


**Correction**

Incorrect Total Sample Size Reported in Table I. In the Original Investigation titled “Association of New-Onset Breast Discomfort With an Increase in Mammographic Density During Hormone Therapy,” by Crandall et al, published in the August 14/28 issue of the ARCHIVES (2006;166:1578-1584), the total sample size was incorrectly reported in Table 1 on page 1580. In the column labeled “Remainder of PEPI-MDS Participants,” the value should have been (N=533).