Postmenopausal Hormone Therapy and Change in Mammographic Density

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Background: **Mammographic density is an independent risk factor for breast cancer. Postmenopausal hormone use is associated with an increase in mammographic density, but the magnitude of the density increase is unknown.** ***Methods:*** **Baseline and 12-month mammograms were obtained for 571 (65%) of the 875 women, aged 45–64 years, who were enrolled in the Postmenopausal Estrogen/Progestin Interventions Trial and randomly assigned to receive placebo, daily conjugated equine estrogens at 0.625 mg/day (CEE), daily CEE and medroxyprogesterone acetate (MPA) at 10 mg/day on days 1–12 (CEE+MPA-cyclic), daily CEE and MPA at 2.5 mg/day (CEE+MPA-continuous), or daily CEE and micronized progesterone (MP) at 200 mg/day on days 1–12 (CEE+MP). We analyzed digitized mammograms to determine the percentage of the left breast that was composed of dense tissue (i.e., mammographic percent density). Linear regression analysis was used to examine the effects of treatments on the change in mammographic percent density between baseline and 12 months, before and after adjustment for possible confounders. All statistical tests were two-sided.** ***Results:*** **The adjusted absolute mean changes in mammographic percent density over 12 months were 4.76% (95% confidence interval [CI] = 3.29% to 6.23%), 4.58% (95% CI = 3.19% to 5.97%), and 3.08% (95% CI = 1.65% to 4.51%) for women in the CEE+MPA-cyclic, CEE+MPA-continuous, and CEE-MP groups, respectively. Each of those absolute mean changes was statistically significantly different from the adjusted absolute mean change in mammographic percent density for women in the placebo group, which was –0.07% (95% CI = –1.50% to 1.38%).** ***Conclusion:*** **Greater mammographic density was associated with the use of estrogen/progestin combination therapy, regardless of how the progestin was given, but not with the use of estrogen only. [J Natl Cancer Inst 2003;95:30–7]**

Mammographic density is an independent risk factor for breast cancer; the degree of risk that is associated with mammographic density is greater than the degree of risk that is associated with almost all other known breast cancer risk factors *(1–4).* Mammographic density is determined by the relative amounts of epithelial tissue, connective tissue, and fat in the breast. On mammographic x-rays, connective and epithelial tissues appear white (i.e., "dense"), whereas fat appears dark (i.e., "non-dense").

The amount of dense tissue in the breast can be gauged either categorically or continuously. Wolfe *(5)* was the first to describe four categorical patterns of mammographic density: the N1 pattern corresponds to breast that is almost completely fat; the P1 and P2 patterns correspond to breasts in which the ducts are increasingly prominent; and the DY pattern corresponds to breasts that show diffuse and extensive nodular density. The Breast Imaging Reporting and Data System (BI-RADS), a classification system that is used in clinical radiology practice in the United States, also divides mammograms into four categories of increasing density: grade 1, entirely fatty breast; grade 2, breast containing scattered fibroglandular tissue; grade 3, heterogeneously dense breast; and grade 4, extremely dense breast *(6).* In contrast to these categorical rating systems, percent-density methods of evaluating mammographic density provide a continuous estimate of the proportion of the breast area that is made up of dense tissue. Mammographic percent density can be measured with the use of a planimeter (outlining tool) *(7)* or by using computer-assisted techniques *(8,9).*

The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial is the only published randomized, placebo-controlled study of the effects of postmenopausal hormone therapy (estrogen alone or estrogen plus one of three progestin regimens) on mammographic density. In a previous study, we used the BI-RADS grading system to assess the effects of the postmenopausal hormone regimens used in PEPI on change in mammographic density *(10).* Between 16% and 26% of estrogen/progestin users experienced categorical breast density increases, while only 3.5% of those using estrogen-only advanced in BI-RADS grade, a rate not significantly different from that of placebo-treated women *(10).* However, the magnitude of the increase in breast density was not addressed in that study. The magnitude of breast density increase may be important because numerous studies show that higher endogenous (i.e., naturally occurring mammographic density) mammographic percent density is associated, in a graded manner, with higher breast cancer risk *(3,4).* Using a computer-assisted method to continuously grade mammographic percent density, we therefore conducted a second mammographic density study among women who were enrolled in the PEPI trial. Here we describe the effects of placebo, conjugated equine estrogens alone, and conjugated equine estrogens com-

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bined with three different progestin regimens on the 12-month change in mammographic percent density.

Subjects and Methods

Parent Study

Between 1989 and 1991, the PEPI trial enrolled 875 postmenopausal women, aged 45–64 years, at seven clinical centers and one coordinating center in the United States: George Washington University, Washington, DC; The Johns Hopkins University, Baltimore, MD; Stanford University, Stanford, CA; The University of California at Los Angeles; The University of California at San Diego; The University of Iowa, Iowa City; The University of Texas Health Science Center, San Antonio; and the Coordinating Center at Wake Forest University School of Medicine, Winston-Salem, NC. The eligibility criteria and study design for this trial have been reported elsewhere in detail *(11)*. Briefly, exclusion criteria for the PEPI study were 1) current use of estrogens or progestins (postmenopausal hormones could be stopped at least 2 months prior to the first screening visit), 2) any major contraindication for use of estrogen or progestin treatment (including a diagnosis of breast cancer), or 3) a diagnosis of any cancer other than basal cell skin cancer within the previous 5 years. Gynecologic exclusion criteria were 1) last menses occurred less than 12 months or more than 10 years prior to randomization; 2) last menstrual period occurred prior to age 44 years; 3) hysterectomy less than 2 months prior to first screening visit; 4) bilateral oophorectomy before age 44 years or more than 10 years prior to randomization; or 5) serum levels of follicle-stimulating hormone (FSH) less than 40 mIU/mL. Participants were randomly assigned to receive placebo, daily conjugated equine estrogens at 0.625 mg/day (CEE), daily CEE and medroxyprogesterone acetate (MPA) at 10 mg/day on days 1–12 (CEE+MPA-cyclic), daily CEE and MPA at 2.5 mg/day (CEE+MPA-continuous), or daily CEE and micronized progesterone (MP) at 200 mg/day on days 1–12 (CEE+MP).

Measurements

All women enrolled in the PEPI trial for whom we were able to obtain technically adequate baseline and 12-month mammograms and who did not have breast implants were eligible for our study. Demographics, medical history, physical activity, and lifetime use of cigarettes, contraceptive and noncontraceptive estrogens, and alcohol were assessed using standardized questionnaires *(12–14)*. The height and weight of each participant were measured, with the participant wearing light clothing and no shoes. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Medications and placebos were dispensed in blister packs, and adherence was evaluated by pill counts at each visit. Adherence was defined as having taken at least 80% of the study pills at each 6-month study visit *(15)*. Written informed consent was obtained from each participant. The PEPI protocol was approved by the institutional review boards at each participating center.

Mammograms that were taken between 1989 and 1994 as part of the original PEPI protocol were retrieved from the radiology centers at the seven clinical sites that participated in PEPI. Baseline mammograms were acquired prior to randomization. These conventional craniocaudal mammogram films of the left breast were scanned to create an 8-bit image with 256 shades of gray that was linear in the optical density range of 0–2.8. Mammograms were scanned at a resolution of 150 pixels per inch (i.e., 59 dots per centimeter) with the use of a COBRASCAN CX-312T scanner (Radiographic Digital Imaging, Inc., Compton, CA) and Adobe Photoshop software (Adobe Systems, Inc., San Jose, CA) with ScanWizard 3.0.9, a specially designed plug-in program (Microtek International, Inc., Carson, CA). We studied only the left breast because we *(10)* and others *(16)* have shown virtually complete concordance between density readings from the right and left breasts.

All density assessments were performed by one individual (G. Ursin) who used a computer-assisted, quantitative method *(9)* and was blinded to treatment and study visit. Films were read one at a time, and the reader did not know whether the film represented a baseline or a follow-up mammogram. In brief, on the digitized mammographic image displayed on the computer screen, the reader first outlined the entire breast while excluding artifacts such as the pectoralis muscle. The reader then applied a yellow tint to dense pixels above some threshold, X, searching for the best threshold where all pixels greater than X (and <255, the whitest value in the image) were considered to represent mammographic densities. (In all computer-assisted reading methods such as this one, on each mammogram, X is chosen by the expert reader as the least dense appearing "white tissue". Thus, the absolute density value of X varies among mammograms.) The software then counted the total number of pixels and the number of tinted pixels and calculated the mammographic percent density, which is the ratio of the tinted area to the total area of the breast. This method gives percent-density results similar to those of the expert outlining method used in planimetry-based studies *(7,17,18)*.

A reliability study of readings of breast density, percent density, and total breast area was conducted on a random sample of 10% of the baseline and follow-up mammograms (N = 120). The same mammogram was read twice during one session by the study reader, who was unaware of treatment assignment or whether the film was a baseline or follow-up mammogram. Each mammogram was also rated by the reader according to whether the mammogram was not difficult to read, slightly difficult, difficult, or very difficult. Difficulties in reading mammograms could arise from technical limitations (e.g., how the mammogram was originally obtained) and/or from characteristics of the breast parenchyma. The test–retest reliability for breast density, percent density, and total breast area was high (intra-class correlation coefficients [ICCs] were >0.95) for the mammograms that were rated not difficult or slightly difficult to read (N = 104). ICCs for the difficult or very-difficult-to-read mammograms (N = 16) were 0.93 for breast density, 0.91 for percent density, and greater than 0.95 for total breast area.

Statistical Analysis

The primary outcome was the change in mammographic percent density from baseline to 12 months and was analyzed on an intention-to-treat basis. Linear regression models were adjusted for mammographic percent density at baseline, BMI (kg/m^2), daily grams of alcohol consumed (in tertiles), cigarette smoking (current versus former/never), level of physical activity (in tertiles), 12-month change in BMI, and the randomization and blocking variables (i.e., clinic site and hysterectomy status). Interaction terms were specified *a priori* and were based on published reports and plausible biologic hypotheses *(10)*. Interactions between treatment and age, BMI, change in BMI, alcohol

use, smoking, level of physical activity, and baseline mammographic density were considered. Sensitivity analyses were performed to assess the effects of including women who did not adhere to their treatment assignment or women whose menopausal status was uncertain *(19,20).* Regression diagnostic statistics were calculated to identify outliers in the data *(21),* and mammograms were then reread for verification. Two sets of mammograms were excluded from the regression models because the verification reading revealed extreme projection differences between the baseline and 12-month mammograms, which precluded the ability to accurately assess change in percent density for these two women. Statistical significance of interaction terms was tested using likelihood ratio chi-square tests. All analyses were performed using the Statistical Analysis System (version 6.2; SAS Institute, Cary, NC). All statistical tests were two-sided.

RESULTS

We were able to retrieve baseline mammograms for 603 of the original 875 PEPI participants. Baseline mammograms for 272 women were unavailable or missing. Seven of the baseline films were excluded from analysis because the breasts they imaged had implants, and two of the baseline films were excluded because of inadequate mammographic technique, resulting in a baseline mammographic density study sample of 594 (68% of the original participants). We were able to retrieve 1-year follow-up mammograms for 571 of these women (65% of the original participants). The availability of mammograms was unrelated to treatment assignment ($P = .73$).

The characteristics of the women who participated in our mammographic density study were similar to those of the PEPI study participants who were not included in our study (Table 1). Women whose baseline films were included in our mammographic density study had a higher mean BMI ($P = .06$) and were more likely to be adherent to treatment assignment than the original PEPI study participants not included in our study ($P = .03$). Adherence rates for the women in our study were 81%, 85%, 90%, 90%, and 91% for those assigned to receive placebo, CEE, CEE+MPA-cyclic, CEE+MPA-continuous, and CEE+MP, respectively. At baseline, 31 (5.2%) mammograms had a percent-density reading of 0%. As was expected for postmenopausal women, most of the baseline mammograms were in the lower density range; 64% of baseline mammograms had percent-density values of 30% or lower (Fig. 1).

The mean unadjusted differences in percent density between the baseline and 12-month mammograms were 5.1% (95% confidence interval [CI] = 3.5% to 6.7%), 4.5% (95% CI = 2.7% to 6.3%), and 3.2% (95% CI = 1.4% to 5.0%) for women in the CEE+MPA-cyclic, CEE+MPA-continuous, and CEE-MP treatment groups, respectively (Fig. 2). Thus, the unadjusted mean 12-month change in percent density was statistically significantly different from 0% for women in each progestin-containing treatment group, but there were no statistically significant differences in 12-month change scores among the progestin-containing treatments ($P>.1$ for each pair-wise comparison among combination treatments). By contrast, the unadjusted mean 12-month change in mammographic percent density was 1.34% (95% CI = −0.05% to 2.63%) for women in the CEE-only group and −0.50% (95% CI = −1.4% to 0.50%) for women in the placebo group; neither change was statistically different from 0%, nor did these 12-month change values differ statistically significantly from each other. We

Table 1. Characteristics of PEPI mammographic density study participants compared to those of the remainder of PEPI participants*

Characteristics	Mammographic density study sample n = 594	Remainder of PEPI participants n = 281	*P* value†
Mean age, y (SD)	56.0 (4.3)	56.2 (4.3)	.59
Mean years since menopause (SD)‡	5.2 (2.7)	5.3 (2.8)	.75
Mean BMI, kg/m^2 (SD)	26.2 (4.5)	25.6 (4.3)	.06
Prior use of HRT, No. (%)	329 (55.4)	165 (58.7)	.35
Mean recency of HRT use, months (SD)§	25.0 (46.2)	27.4 (56.8)	.66
Smoking status, No. (%)			.72
Current	77 (13.0)	41 (14.6)	
Former	225 (37.9)	100 (35.6)	
Never	292 (49.2)	140 (49.8)	
Mean alcohol use, g/day (SD)‖	6.4 (12.4)	6.9 (11.5)	.52
Level of physical activity, No. (%)¶			.24
Low	400 (67.3)	175 (62.3)	
Medium	189 (31.8)	105 (37.4)	
High	5 (0.8)	1 (0.4)	
Non-white, No. (%)	72 (12.1)	28 (10.0)	.35
Adherent to treatment assignment, No. (%)	519 (87.4)	230 (81.9)	.03
Uncertain menopause, No. (%)#	142 (23.9)	78 (27.8)	.22

*PEPI = The Postmenopausal Estrogen/Progestin Interventions Study; SD = standard deviation; BMI = body mass index; HRT = hormone replacement therapy.

†*P* value for *t* test of means or chi-square test of proportions.

‡Sample sizes for years since menopause were 417 (mammographic density sample) and 184 (remainder of PEPI). Women with hysterectomy but one ovary remaining could not answer this question.

§Recency of use refers to the number of months since the participant last used postmenopausal hormone therapy. We calculated this interval based on the date of the baseline visit and the self-reported date of last use of postmenopausal hormones. Sample sizes for recency of use were 301 (mammographic density sample) and 139 (remainder of PEPI). This question did not apply to never-users of HRT and was missing in 28 former-users.

‖One woman in remainder of PEPI sample was missing data for this variable.

¶Tertiles, based on PEPI physical activity scale *(12).*

#Uncertain menopause status defined as women with hysterectomy and at least one intact ovary. All women had follicle-stimulating hormone levels ≥40 mIU/mL.

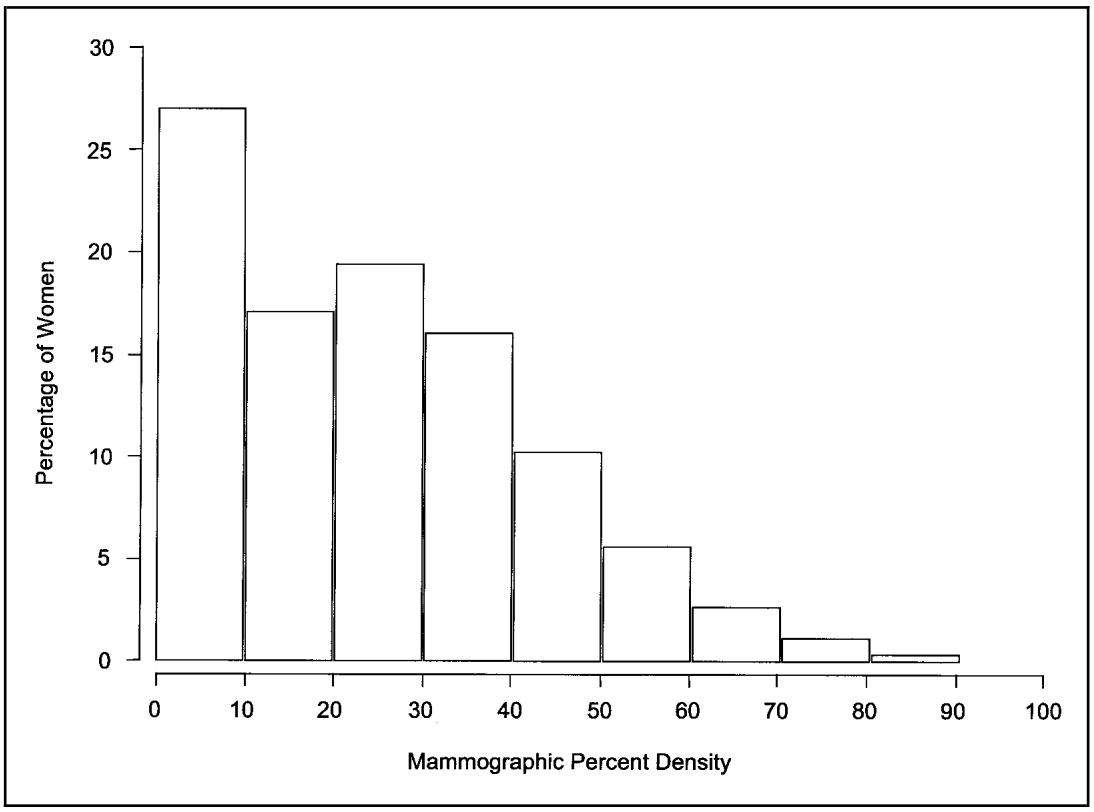

Fig. 1. Distribution of the percent density of baseline mammograms (N = 594).

obtained similar results when we restricted our analyses to the study participants who were adherent to their treatment assignments (Fig. 2, B).

Table 2 shows the results of the multivariable model that compared the 12-month change in mammographic percent density of women who received each of the active treatments with that of women who received placebo. These values were adjusted for baseline percent density, BMI, alcohol use, cigarette smoking, level of physical activity, 12-month change in BMI, and the randomization blocking variables, clinic site and hysterectomy status. The adjusted absolute mean change in mammographic percent density was 4.76% (95% CI = 3.29% to 6.23%), 4.58% (95% CI = 3.19% to 5.97%), and 3.08% (95% CI = 1.65% to 4.51%) for women in the CEE+MPA-cyclic, CEE+MPA-continuous, and CEE-MP groups, respectively. Each change was statistically significantly different from the adjusted absolute mean change in percent density for women in the placebo group, which was –0.07% (95% CI = –1.50% to 1.38%). None of the interactions, specified *a priori* (and listed in the "Subjects and Methods" section), were statistically significant. Results of a multivariable analysis that was restricted to the 504 women who were adherent to their treatment assignments were not different from those of the intention-to-treat analysis (data not shown).

The PEPI entry criterion that study participants have an FSH value greater than or equal to 40 mIU/mL did not guarantee that women who had undergone a simple hysterectomy but still had at least one ovary intact had undergone menopause. Therefore, inclusion of such possibly non-postmenopausal women in the intention-to-treat analysis could potentially result in negatively biased estimates of the effects of treatments on mammographic density *(19,20).* We found that analyses that excluded the 142 mammographic study participants who had had a hysterectomy but had one intact ovary gave results similar to those of analyses that included all of the mammographic study participants (data not shown).

To understand the relation between BI-RADS density readings and percent-density readings, we conducted an analysis of the 243 PEPI participants who were part of both the first PEPI mammographic density study (which used BI-RADS to grade density) and this study (which used percent-density readings). We found that there was an ordered correspondence between the BI-RADS grades and the percent-density readings. For example, baseline BI-RADS grades 1, 2, 3, and 4 corresponded to mean percent-density values (standard deviation) of 2.4% (4.2%), 20.5% (12.2%), 44.3% (12.5%), and 61.3% (19.2%), respectively (data not shown). On average, mammograms that changed from a BI-RADS grade 1 to a BI-RADS grade 2 displayed a mean increase in percent density of 18.19% (range = 0.47%–38.29%), whereas mammograms that changed from a BI-RADS grade 2 to a BI-RADS grade 3 displayed a mean increase in percent density of 14.41% (range = 6.54%–26.32%) (Table 3).

DISCUSSION

We found that 12 months of treatment with CEE alone did not affect mean mammographic percent density of women enrolled in the randomized, placebo-controlled PEPI trial. However, women who were randomly assigned to any of the three CEE/progestin combination treatment arms had mean increases in density that ranged from 3% to 5%. In each of these combination treatment arms, the change in percent density from baseline to follow-up differed statistically significantly from 0%. The average increases in mammographic percent density produced by each of the three combination hormone treatments did not differ statistically significantly from each other. Results of the inten-

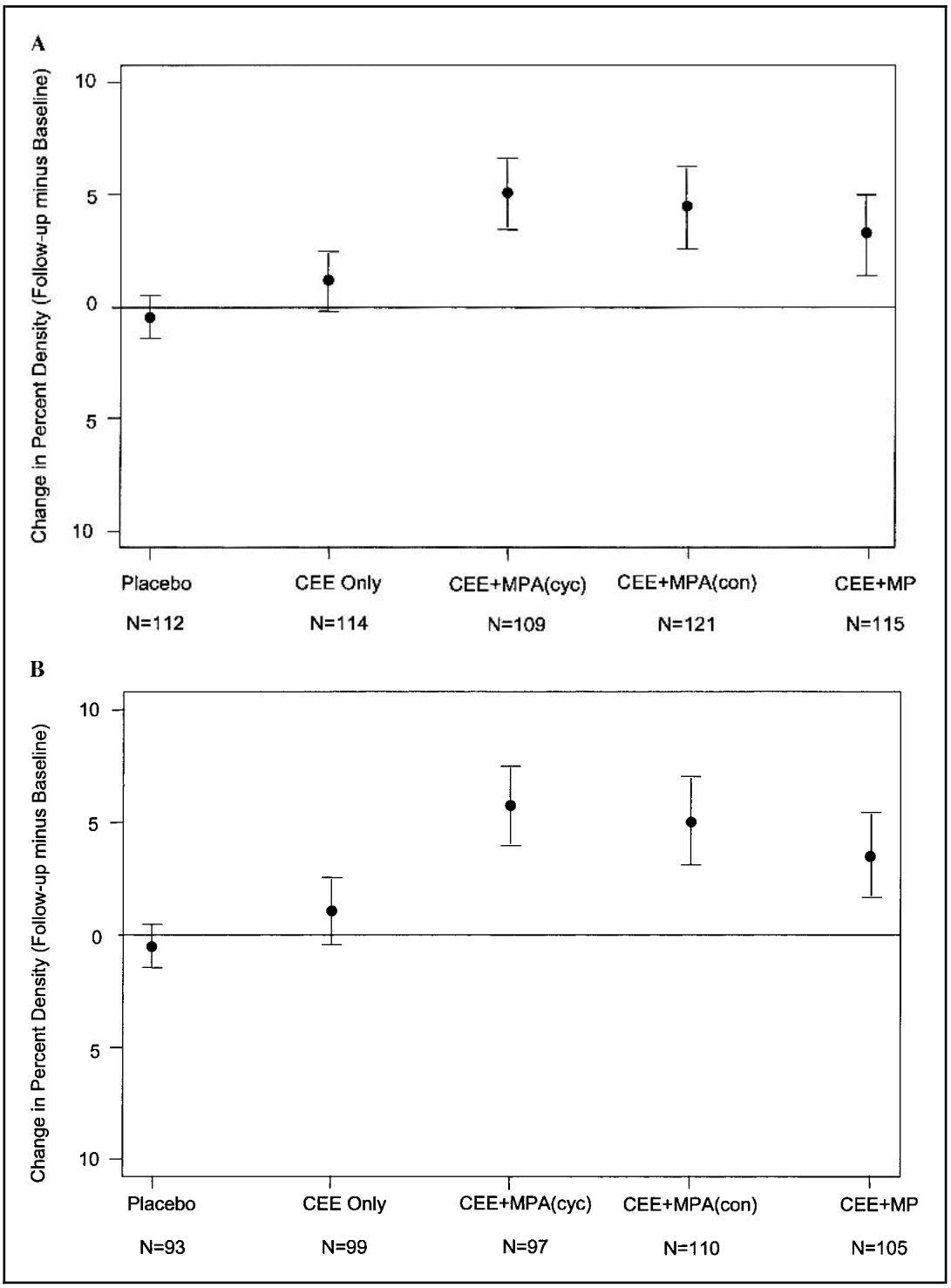

Fig. 2. Change in percent density from baseline to 12-month follow-up within individual treatment groups. The mean change in percent density is plotted as a point (**A**) for the entire mammographic density study sample (N = 571) and (**B**) for the participants who adhered to their treatment assignments (N = 504). CEE = conjugated equine estrogens, 0.625 mg/day; CEE+MPA(cyc) = CEE, 0.625 mg/day, and medroxyprogesterone acetate, 10 mg/day on days 1–12; CEE+MPA(con) = CEE, 0.625 mg/day, and MPA 2.5 mg/day; CEE+MP = CEE, 0.625 mg/day, and micronized progesterone, 200 mg/day, on days 1–12. **Error bars** represent 95% confidence intervals.

tion-to-treat analyses and analyses that were restricted to the women who had adhered to their treatment assignment were similar.

Most information about the effects of hormones on mammographic density comes from nonrandomized studies of postmenopausal hormone use performed within mammography screening cohorts *(22–25).* In those studies, postmenopausal women who began using hormone therapy between mammography screening visits were more likely to experience breast density increases, measured categorically by Wolfe or BI-RADS classifications, than were postmenopausal women who did not begin using hormones during the same interval. Two studies *(24,25)* compared the effects of estrogens alone with the effects of combination treatments on changes in breast density and found that, overall, starting estrogen-only treatment was not associated with breast density increases, whereas starting continuous combined estrogen/progestin therapy was related to increases in breast density. However, these two studies differed in their conclusions about effects of cyclical estrogen/progestin therapy regimens on changes in breast density. One study *(24)* reported that women who started estradiol-only therapy (i.e., oral estradiol valerate at 2 mg/day or transcutaneous 17-β es-

Table 2. Adjusted absolute mean change and 95% confidence intervals (CIs) in percent density from baseline to follow-up by treatment assignment (N = 569)*

Treatment assignment	N	Adjusted absolute mean change (follow-up minus baseline) in mammographic percent density (95% CI)	*P* value†‡
Placebo	112	–0.07% (–1.50% to 1.38%)	NA
CEE§	113	1.17% (–0.28% to 2.62%)	.241
CEE + MPA-cyclic‖	109	4.76% (3.29% to 6.23%)	<.001
CEE + MPA-continuous¶	121	4.58% (3.19% to 5.97%)	<.001
CEE + MP#	114	3.08% (1.65% to 4.51%)	.002

*CEE = conjugated equine estrogens; MPA = medroxyprogesterone acetate; MP = micronized progesterone; NA = not applicable.

†Model adjusted for baseline percent density, age, body mass index, alcohol use (tertiles), smoking (current versus former/never), level of physical activity (tertiles), 12-month change in body mass index, clinic site, and hysterectomy status (yes/no). Two women with baseline and 12-month mammograms were excluded from the final model after outlier analysis (see "Subjects and Methods" section for details).

‡*P* value for *t* test of null hypothesis that mean change in percent density was not different from that of placebo.

§CEE = conjugated equine estrogens, 0.625 mg/day.

‖CEE + MPA-cyclic = CEE, 0.625 mg/day, and medroxyprogesterone acetate, 10 mg/day, on days 1–12.

¶CEE + MPA-continuous = CEE, 0.625 mg/day, and medroxyprogesterone acetate, 2.5 mg/day.

#CEE + MP = CEE, 0.625 mg/day, and micronized progesterone, 200 mg/day, on days 1–12.

Table 3. Relation between absolute change in percent density and change in the Breast Imaging Reporting and Data System (BI-RADS) grade between baseline and 12-month mammograms (N = 242)*

Baseline BI-RADS grade	12-month BI-RADS grade	Absolute change in percent density (follow-up minus baseline) Mean (SD)	Minimum	Maximum	Sample size
1	1	0.11% (4.26%)	–12.14%	17.45%	33
1	2	18.19% (12.48%)	0.47%	38.29%	9
2	2	2.64% (7.92%)	–24.86%	27.50%	130
2	3	14.41% (8.04%)	6.54%	26.32%	21
3	3	0.93% (5.97%)	–13.52%	10.33%	46
4	4	7.8% (4.90%)	4.14%	13.37%	3

*Two hundred forty-three women in the mammographic density study sample were included in the BI-RADS study, but one woman did not have a follow-up percent-density mammogram *(10)*. SD = standard deviation.

tradiol at 50 μg/day) did not have a statistically significantly elevated relative risk (RR) of developing greater breast density, using Wolfe's classification patterns, compared with nonusers (RR = 1.5, 95% CI = 0.7 to 3.5). Women who used either cyclic estrogen/progestin therapies (oral estradiol valerate at 2 mg/day with cyclic levonorgestrel at 250 μg/day or oral 17-β estradiol at 2 mg/day with cyclic norethisterone acetate at 1 mg/day) or continuous estrogen/progestin therapies (17-β estradiol at 2 mg/day with norethisterone acetate at 1 mg/day) had greater mammographic density compared with women who did not use postmenopausal hormones. That study also found that women who began a cyclic regimen of hormone therapy were 3.4 times (95% CI = 1.6 to 7.2) more likely to advance to a denser Wolfe classification than women who did not use hormones and that initiation of oral continuous combined therapy was associated with an 11.4-fold increased risk (95% CI = 5.9-fold to 28.1-fold) of increasing breast density *(24)*. Results from the second study *(25)* also showed that unopposed estrogen therapy (i.e., oral estradiol valerate at 2 mg/day or conjugated equine estrogens at 0.625 mg/day) was not associated with increases in mammographic density: only one of 50 women who took estrogen-only advanced in Wolfe grade. Continuous combined hormone treatment (i.e., oral 17-β estradiol at 2 mg/day with norethisterone at 1 mg/day) was also associated with density increases: 15 of 50 women exposed to this regimen advanced in Wolfe grade. However, none of the mammograms of 75 women who began using sequential cyclic combined hormones displayed an increase in mammographic density. The two sequential combined regimens used in the second study were oral estradiol valerate at 2 mg/day for 11 days, followed by estradiol valerate at 2 mg/day with levonorgestrel at 250 μg/day for 10 days, or estradiol valerate at 2 mg/day for 12 days, followed by estradiol valerate at 2 mg/day with norethisterone acetate at 1 mg/day for 10 days, followed by estradiol valerate at 1 mg/day for 6 days *(25)*.

Although our results are generally concordant with those from the observational studies, they disagree in part with the outcome of one small randomized study that compared the effects of CEE, raloxifene, and placebo on mammographic density. Freedman and colleagues *(26)* reported results of a mammographic density substudy that included 168 (27%) of the 619 participants who were enrolled in a randomized controlled trial that compared the effects of two doses of raloxifene to those of CEE (at 0.625 mg/day) and placebo on the preservation of bone mineral density. That study was similar to our study in that a percent-density threshold-based reading of digitized conventional films was performed. However, that study differed from our study in three ways: the individual who read the films knew the order in which they were obtained, a weighted average of the mammographic density of both breasts was used, and the interval between the mammograms was 2 years. The CEE group in that study *(26)* manifested a 2-year change in mammographic density of 1.2% (95% CI = –0.6% to 3.0%), which was strikingly similar to the 1-year results from our study. By contrast, the placebo group exhibited a decrease in mammographic density of –1.3% (95% CI = –2.2 to –0.4), which was roughly twice the decrease that we observed at 1 year. In the study by Freedman et al. *(26)*, the change in percent density among women in the CEE group was statistically significantly different from the change in percent density among women in the placebo group, whereas in our study, it was not. We suspect that these discrepant findings are related to the larger 2-year decrease in mammographic density that occurred among the women in the placebo group in the Freeman et al. study.

With the exception of the study reviewed above *(26)*, results from other observational *(10,24,25)* and intervention *(10)* studies, as well as those from our study, suggest that the use of a wide variety of unopposed estrogen formulations is not associated with statistically significant increases in mammographic density. However, most of those studies *(10,24,25)* used categorical rather than continuous measurements of mammographic density. Thus, it is possible that the categorical methods used to measure mammographic density in those studies were not sensitive enough to detect small changes in the breast densities of women who received estrogens only. Our results illus-

trate that mammographic density changes can be quite small (e.g., the adjusted mean change was only 1.17% among the women in the CEE group in our study) and may not differ from the changes associated with placebo. Thus, although categorical density assessments would likely miss small changes in density, insensitive measurements were not the probable cause of the negative findings in previous studies. Evidenced by our study, the precisely measured percent-density change resulting from CEE treatment is indeed small.

There is limited and conflicting information about whether the effects of progestin therapy on mammographic density differ according to whether the progestin is given cyclically or continuously *(24,25).* We observed a modest 3%–5% increase in mammographic percent density among the women who were treated with combination hormone therapies, and those increases did not differ by progestin formulation or schedule. These results agree with those of other studies *(24,25)* and suggest that combinations of estrogen and progestin have a greater effect on the breast than does estrogen alone, but do not corroborate a difference between cyclical and continuous administration.

Our use of the percent-density method to evaluate mammographic density permits us to assess the degree of density increase caused by postmenopausal hormone use and to compare the magnitude of this effect to cohort studies of endogenous mammographic density. Greater endogenous mammographic density is a strong, independent risk factor for breast cancer *(1–4,7,18),* and results of several studies support a dose–response relation between percent density and breast cancer risk *(3,8,15).* For example, two large nested case–control studies reported that the relative risks of breast cancer associated with percent density of 75% or greater, compared with those associated with 0%, were between 4.4 *(15)* and 5.3 *(8).* Moreover, a linear model *(4)* predicted a 2% increase in the relative risk of breast cancer for each 1% increment in endogenous percent density.

As the evidence linking endogenous mammographic density to breast cancer risk continues to mount, so does interest in adding mammographic density to clinical risk assessment *(27).* It is also possible that a change in mammographic density could serve as a surrogate marker for a change in breast cancer risk; some intervention trials have measured change in mammographic density as an outcome *(28–32).* Although the inter-rater reliability of BI-RADS and Wolfe's patterns is high in some research settings *(8,10,15),* one study of BI-RADS readings by practicing mammographers reported poor agreement between readers *(33).* The results of our comparison of baseline percent density with baseline BI-RADS grade demonstrated that the latter method of evaluating mammographic density was an ordered, but imprecise, scale. The insensitivity of BI-RADS grades to change in density was also identified: a one-step increase in BI-RADS grade translated into a 14%–18% increase in density.

A potential limitation of our study was that we were unable to retrieve 35% of the original PEPI trial mammograms. However, it is unlikely that the absence of the nonretrievable films from our analyses introduced a systematic bias, because there is no reason to suspect that the reasons that those mammograms were nonlocatable were related to the effects of the various hormonal therapies on breast density.

In conclusion, combination CEE/progestin use, but not the use of CEE only, was associated with increases in mammographic percent density. These results parallel those of cohort *(34)* and case–control *(35)* studies that found that combinations of estrogen and progestin were associated with greater breast cancer risk than estrogen alone and suggest that increasing mammographic density may be a marker for elevated breast cancer risk in postmenopausal women who use postmenopausal estrogen/progestin therapies. However, the link between *change* in breast density resulting from hormone use and *change* in breast cancer risk remains uncertain.

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NOTES

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