Conjugated Equine Estrogen Influence on Mammographic Density in Postmenopausal Women in a Substudy of the Women's Health Initiative Randomized Trial

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ABSTRACT

Purpose

Increased mammographic density is associated with increased breast cancer risk and reduced sensitivity of screening mammography and is related to hormone exposure. However, the effects of conjugated equine estrogens (CEEs) alone on mammographic density in diverse racial/ethnic populations are not established. We examined the effect of CEE alone on mammographic density in a subsample of the Women's Health Initiative (WHI) clinical trial participants.

Patients and Methods

In the WHI trial, women were randomly assigned to daily CEE 0.625 mg or placebo. The effect of CEE on mammographic percent density was determined over 1 and 2 years in a stratified random sample of 435 racially and ethnically diverse participants from 15 of 40 WHI clinics.

Results

Use of CEE resulted in mean increase in mammographic percent density of 1.6 percentage points (95% CI, 0.8 to 2.4) at year 1 compared with a mean decrease of 1.0 percentage point (95% CI, -1.7 to -0.4) in the placebo group ($P < .001$). The effect persisted for 2 years, with a mean increase of 1.7 percentage points (95% CI, 0.7 to 2.7) versus a mean decrease of 1.2 percentage points (95% CI, -1.8 to -0.5; $P < .001$) in the hormone and placebo groups, respectively. These effects were greater in women age 60 to 79 years ($P = .03$ for interaction across age).

Conclusion

Use of CEE results in a modest but statistically significant increase in mammographic density that is sustained over at least a 2-year period. The clinical significance of the CEE effect on mammographic density remains to be determined.

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INTRODUCTION

Mammographic density is positively associated with breast cancer risk.¹⁻⁵ The decrease in mammographic density after menopause⁶ suggests a hormonal influence, and observational and short-term clinical trials have shown an association between menopausal hormone therapy use and increased mammographic density.⁷⁻¹¹ A relationship between an intervention's influence on breast density and its influence on breast cancer risk has been proposed.¹² However, the effects of menopausal hormone therapy on breast density in racial/ethnic populations and duration effects are not established.

The Women's Health Initiative (WHI) reported that combined conjugated equine estrogens (CEEs) plus medroxyprogesterone acetate (MPA) doubled mammographic density⁹ and significantly

increased breast cancer incidence in postmenopausal women with no prior hysterectomy.¹³ However, CEE alone did not increase breast cancer incidence but suggested a decrease in incidence.¹⁴ We evaluated the effect of CEE alone on mammographic density over 2 years in a randomly identified subsample of WHI CEE clinical trial participants, with over-sampling of women from racial/ethnic minorities.

PATIENTS AND METHODS

WHI Study Design

The WHI CEE-alone trial enrolled 10,739 postmenopausal women from 1993 to 1998 at 40 clinical centers.^{15,16} The study was approved by human subjects committees at each institution. Briefly, women recruited by multiple methods¹⁷ were eligible if they were between

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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age 50 and 79 years at entry and were postmenopausal, had prior hysterectomy, and provided written informed consent. Women with prior breast cancer or those with medical conditions likely to result in death within 3 years were excluded. Prior menopausal hormone use required a 3-month washout period before baseline testing (mammographic density has been shown to decrease within a few weeks of stopping menopausal hormone use).¹⁸ Before random assignment, 46% had used estrogen alone and 5% had used estrogen plus progesterone. Baseline mammogram and clinical breast examination within 6 months before random assignment were required. Women were randomly assigned to daily CEE 0.625 mg in a single tablet (Premarin; Wyeth Ayerst, Philadelphia, PA), or an identical-appearing placebo. Participants had follow-up clinic visits every 6 months, where pill adherence was obtained from pill counts, potential adverse effects were determined, and exposure covariates were updated.¹⁴ During follow-up, women received annual clinical breast examinations and screening mammograms. After a mean of 6.8 years of follow-up, the trial was stopped early on the basis of an increased risk of stroke and no reduction in coronary heart disease risk.¹⁵

Mammogram Density Ancillary Study

Fifteen of 40 WHI clinical centers participated in the separately funded Mammogram Density Ancillary Study. The Clinical Coordinating Center identified a stratified random sample of women in the WHI CEE trial to be approached for participation, with the goal of sampling 150 women within each of the following groups: African Americans, Asian/Pacific Islanders, Hispanics, and non-Hispanic whites. A 10% over-sample was selected to allow for those electing not to participate. Inclusion criteria included availability of a prerandomization (baseline) mammogram plus at least one follow-up mammogram after 1 or 2 years. Sample size was based on the primary study aim—an anticipated mammographic density difference of 8% (standard deviation [SD], 10%) between CEE and placebo groups after 1 year of treatment, with the assumption of 33% loss to follow-up/nonadherence. A secondary aim of the study was to determine whether a hormone effect exists within different race/ethnic groups; therefore, the study was powered to assess effects on density within the four race/ethnic groups. Two years of follow-up was the time frame chosen to determine the longer-term effects of CEE on density.

Data Collection

Our mammographic density measurement methods have been previously published⁹ and have been validated across all mammographic density

types (fatty to dense) and all age groups, including older women.¹⁹ Briefly, films were digitized on a Lumisys 85 laser digitizer (Eastman Kodak Company, Rochester, NY), and the files were converted to bitmap format suitable for display and density measurement. Mammographic density was assessed with a previously validated²⁰ computer-assisted interactive thresholding technique using Imaging Research Program software (Sunnybrook Health Science Centre, Toronto, Ontario, Canada). After identifying thresholds, the software was used to calculate the total breast area, the total area of density, and percent mammographic density (ratio of dense breast area to total breast area). The right craniocaudal view was measured, unless it was unavailable, in which case the left craniocaudal view was used. Measurements were done in batches of 30 to 40 films selected without regard to whether they were before random assignment or follow-up films, and the films were sorted in random order. Baseline and follow-up films from the same participant were not necessarily included in the same batch, and batches for each observer were generated independently.

Two trained investigators (C.M. and J.D.P.), blind to treatment arm and film time sequence, performed density measurements on all films, with high reliability (intraclass correlation coefficients > 0.92). We calculated percent density for each film as the mean of both investigators' measures for that film.

Statistical Methods

All primary analyses focused on changes in mean percent density at baseline compared with years 1 and 2 by CEE random assignment. Mixed-effects (repeated measures) models were used to formally test whether treatment affected longitudinal density change, whether this relationship depended on race/ethnicity, mammogram sequence (baseline or follow-up), or baseline characteristics. Log-transformed percent density values were used in these models to reduce skewness of the percent density data. We also present differences over time (eg, year 1 – baseline) that are not skewed. Change in weight and physical activity were determined at each follow-up interval chosen because of their potential associations with mammographic density.²¹ We assessed several baseline characteristics as potential effect modifiers, including age (50 to 59, 60 to 69, and 70 to 79 years), race/ethnicity (non-Hispanic white, African American, white), body mass index (BMI; < 25.0, 25.0 to 29.9, ≥ 30 kg/m²), smoking status (past, never, current), and parity (0, 1 to 2, 3 to 4, ≥ 5), and Gail risk score²² (5-year risk, < 1.7% v ≥ 1.7% [the cutoff used in the

Fig 1. Flow diagram of the Mammogram Density Ancillary Study of the Conjugated Equine Estrogen (CEE) Component of the Women's Health Initiative Study.

Table 1. Baseline Characteristics of the 435 Women in the Women's Health Initiative Mammogram Density Ancillary Study, CEE Trial Subsample, by Random Assignment

Characteristic	CEE (n = 209)		CEE Placebo (n = 226)		P
	No.	%	No.	%	
Age group at screening, years					.36
50-59	74	35.4	94	41.6	
60-69	87	41.6	89	39.4	
70-79	48	23.0	43	19.0	
Ethnicity					.21
White	91	43.5	84	37.2	
Black	80	38.3	106	46.9	
Hispanic	37	17.7	33	14.6	
Asian/Pacific Islander	1	0.5	3	1.3	
Education					.69
High school diploma/GED or less	66	32.0	79	35.4	
School after high school	93	45.1	92	41.3	
College or higher	47	22.8	52	23.3	
Income, \$					.73
< 35,000	119	60.7	131	62.4	
≥ 35,000	77	39.3	79	37.6	
Years since menopause					.14
< 5	24	13.7	18	9.7	
5-< 10	23	13.1	20	10.8	
10-< 15	27	15.4	19	10.2	
≥ 15	101	57.7	129	69.4	
Years since hysterectomy					.06
< 18	66	31.9	61	27.2	
18-< 25	60	29.0	89	39.7	
≥ 25	81	39.1	74	33.0	
Age at menarche, years					.97
≤ 12	103	49.3	110	49.1	
> 12	106	50.7	114	50.9	
No. of term pregnancies					.22
Never pregnant/never had term pregnancy	20	9.8	22	9.8	
1-2	53	25.9	72	32.1	
3-4	88	42.9	75	33.5	
5+	44	21.5	55	24.6	
Duration of oral contraceptive use, years					.65
Nonuser	132	63.2	132	58.4	
< 5	48	23.0	55	24.3	
5-< 10	15	7.2	23	10.2	
10+	14	6.7	16	7.1	
Lifetime HRT duration, years					.84
Never used	128	61.2	137	60.6	
< 5	41	19.6	49	21.7	
≥ 5	40	19.1	40	17.7	
Years since last HRT use					.55
Never used HRT	128	61.2	137	60.6	
Current user/≤ 1	30	14.4	26	11.5	
> 1	51	24.4	63	27.9	
Family history of breast cancer (female)	31	16.1	28	13.1	.39
Prior bilateral oophorectomy	69	35.9	76	38.0	.67
BMI, kg/m ²					.59
Normal (< 25.0)	36	17.2	36	16.1	
Overweight (25.0-29.9)	74	35.4	71	31.7	
Obese (≥ 30.0)	99	47.4	117	52.2	

(continued in next column)

Table 1. Baseline Characteristics of the 435 Women in the Women's Health Initiative Mammogram Density Ancillary Study, CEE Trial Subsample, by Random Assignment (continued)

Characteristic	CEE (n = 209)		CEE Placebo (n = 226)		P
	No.	%	No.	%	
Physical activity (METs/wk)					.83
Lower tertile	98	51.9	109	52.4	
Middle tertile	54	28.6	63	30.3	
Upper tertile	37	19.6	36	17.3	
Smoking					.06
Never smoked	116	55.5	128	57.4	
Past smoker	73	34.9	60	26.9	
Current smoker	20	9.6	35	15.7	
Alcohol intake, g/d					.64
Nondrinker	125	62.8	128	59.8	
< 2.7	38	19.1	49	22.9	
≥ 2.7	36	18.1	37	17.3	

NOTE. There were no statistically significant differences between treatment arms. Categories were chosen on the basis of distribution of the variable in the entire Women's Health Initiative data set.

Abbreviations: CEE, conjugated equine estrogen; GED, General Equivalency Diploma; HRT, hormone replacement therapy; BMI, body mass index; MET, metabolic equivalent tasks.

breast cancer chemoprevention trials^{23,24}). Statistical significance of interactions between random assignment and baseline characteristics was judged by a Bonferroni-corrected alpha to account for 19 tests (.05/19 = .003). CIs, based on the *t* distribution, are presented for changes in mammographic percent density. Comparisons of baseline characteristics by random assignment were made by χ^2 tests of association. All primary analyses were based on the intention-to-treat principle, and all statistical tests were two-sided.

RESULTS

Demographics

A total of 498 women in the WHI CEE-alone clinical trial were randomly selected for participation in the Mammogram Density Ancillary Study. Forty declined participation, 458 were eligible and consented, and mammograms were received and digitized for 438. Three women developed invasive breast cancer during the study period and were excluded, resulting in 435 (209 CEE, 226 placebo) participants (Fig 1). Baseline mammograms were available within 6 months before random assignment for all except three women whose baseline mammograms occurred more than 6 months before random assignment (mean time from baseline mammogram to random assignment was 367.3 days; SD, 187.1 days). Two of the women reported never using hormone therapy before entering the trial, and one reported prior use. These women were included in the analysis. Including these three women in the analysis, the mean time between baseline mammogram and random assignment was 45.4 days (SD, 35.9 days), and excluding them it was 43.2 days (SD, 20.2 days).

The mean age of participants was 62.2 years (SD, 8.0 years). Ancillary study participants were younger, more likely to be black or Hispanic (by design), fewer years past menopause, less likely to have used hormone therapy, less physically active, reporting less alcohol consumption, and more likely to have never smoked (all *P* < .01; data

not shown), compared with the overall trial participants. Most ancillary study participants were non-Hispanic white (40.2%) or African American (42.8%), 16.1% were Hispanic, and 0.9% were Asian/Pacific Islander (Table 1). There were no statistically significant baseline differences ($P < .05$) between CEE and placebo participants on demographic and health factors. However, several factors differed between treatment groups by $\geq 5\%$ including age, ethnicity, years since menopause, years since hysterectomy, parity, oral contraception use, BMI, and smoking. Therefore, these factors were evaluated as potential confounders in analyses.

All participants had at least one, and 93% had two, follow-up mammograms. Mean time was 1.2 years (SD, 0.4 years) between the baseline and first follow-up mammogram, and 1.1 years (SD, 0.3 years) between the first and second follow-up mammograms. Eleven women developed breast cancer from the end of the mammogram density measurement period to the end of the trial.

Baseline Mammographic Percent Density

Baseline mammographic percent density ranged between 0% and 69.4% with a mean of 7.3% (95% CI, 6.4% to 8.2%) and similar distributions within each treatment group (mean, 6.8%; 95% CI, 5.6% to 8.0% in CEE; and mean, 7.7%; 95% CI, 6.3% to 9.0% in placebo; $P = .80$; Table 2). BMI was inversely related to baseline mammographic percent density, with overweight women having lower baseline percent density ($P < .001$; data not shown). Age had modest inverse relationships with percent density ($P = .02$; data not shown). Baseline percent density differed slightly, but not statistically significantly, among the four ethnic groups ($P = .11$). Mean percent density was 7.7% (median, 6.8%) for the four Asian-American women, 9.7% (median, 5.5%) for the 70 Hispanics, 6.2% (median, 2.8%) for the 175 non-Hispanic whites, and 7.3% (median, 3.7%) for the 186 African Americans. After adjusting for age and BMI, these slight differences remained ($P = .09$). Women who had used menopausal hormone therapy within 6 months before random assignment (eg, just before the required 3-month washout period; $n = 38$) had a mean percent density of 7.2% (95% CI, 4.4% to 10.0%) compared with 7.3% (95%

CI, 6.3% to 8.2%) for nonusers. Adjustment for age and BMI did not change these results.

Effect of CEEs on Mammographic Percent Density

After 1 year, women in the CEE group had a mean percent density increase of 1.6 percentage points (95% CI, 0.8 to 2.4) compared with a mean 1.0 percentage point decrease (95% CI, -1.7 to -0.4) in the placebo group ($P < .001$; Table 2). The increase in density with CEE use persisted to year 2, with a mean increase of 1.7 percentage points (95% CI, 0.7 to 2.7) from baseline compared with a mean decrease of 1.2 percentage points (95% CI, -1.8 to -0.5) in the placebo group ($P < .001$). The increase in density from year 1 to year 2 among CEE users was not statistically significant ($P = .44$). Relative to baseline, 56% and 55% of CEE-treated participants experienced an increase in percent density at year 1 and year 2, respectively (data not shown).

Effect of Adherence to Study Medications

To adjust for study medication adherence, percent density measurements at follow-up were censored when a participant became nonadherent (stopped taking study drugs, used $< 80\%$ of study drugs over a 6-month interval or, if in the placebo group, started nonprotocol hormone therapy). The compliance effect of CEE was similar to that shown in the intention-to-treat analyses. Among women who were adherent to treatment, the CEE group had a mean of 1.6 percentage points (95% CI, 0.6 to 2.5) increase at year 1 versus a 0.9 percentage points (95% CI, -1.6 to -0.2) decrease in placebo-treated women (Table 2). While percent density decreased by a mean 0.4 percentage points (95% CI, -1.2 to 2.1) from year 1 to year 2 among women on CEE who became nonadherent within the first year of the trial, it was not statistically significant.

Subgroup Analyses

CEE-related increase in percent density was consistent between non-Hispanic white and African American women, while no increase in percent density with CEE versus placebo was observed in Hispanic

Table 2. MPD at Baseline, Year 1, and Year 2, by Treatment Assignment

Measurement	CEE MPD				Placebo MPD				P*	P†
	No.	Median	Mean	95% CI	No.	Median	Mean	95% CI		
ITT										
Baseline	209	3.4	6.8	5.6 to 8.0	226	3.4	7.7	6.3 to 9.0	.80	
Year 1	209	4.6	8.4	7.2 to 9.7	226	3.1	6.6	5.5 to 7.8		
Year 2	193	5.2	8.8	7.5 to 10.1	213	2.9	6.6	5.4 to 7.8		
Year 1–baseline	209	0.2	1.6	0.8 to 2.4	226	−0.2	−1.0	−1.7 to −0.4	< .001	< .001
Year 2–baseline	193	0.3	1.7	0.7 to 2.7	213	−0.3	−1.2	−1.8 to −0.5	< .001	
Adjusted for adherence										
Baseline	209	3.4	6.8	5.6 to 8.0	226	3.4	7.7	6.3 to 9.0		
Year 1	149	5.3	8.8	7.3 to 10.4	184	3.0	6.3	5.1 to 7.6		
Year 2	113	5.9	9.1	7.3 to 10.8	138	2.9	6.4	5.0 to 7.8		
Year 1–baseline	149	0.2	1.6	0.6 to 2.5	184	−0.2	−0.9	−1.6 to −0.2	.002	< .001
Year 2–baseline	113	0.5	1.8	0.6 to 3.1	138	−0.3	−0.7	−1.4 to −0.1	< .001	

Abbreviations: MPD, mammographic percent density; CEE, conjugated equine estrogen; ITT, intention to treat.

* P value of main effect of CEE, by visit, is based on a two-sided t test from a repeated measures model with log (percent density + 0.001) as the response. All statistical tests were two-sided.

† P value of overall main effect of CEE at follow-up is based on an F test from a repeated measures model with log (percent density + 0.001) as the response.

Conjugated Equine Estrogen Influence on Mammographic Density

Table 3. MPD at Baseline, Year 1, and Year 2, by Baseline Characteristics and Treatment Assignment

Characteristic	CEE MPD				Placebo MPD				P*
	No.	Median	Mean	95% CI	No.	Median	Mean	95% CI	
Race									.09
Non-Hispanic white									
Baseline	91	2.8	5.3	4.0 to 6.5	84	2.6	7.2	5.1 to 9.2	
Year 1–baseline	91	0.6	2.3	1.2 to 3.5	84	–0.2	–1.0	–1.8 to –0.3	
Year 2–baseline	88	0.6	2.7	1.3 to 4.1	82	–0.3	–0.9	–1.7 to –0.1	
African American									
Baseline	80	3.9	7.2	5.3 to 9.1	106	3.4	7.4	5.6 to 9.3	
Year 1–baseline	80	0.1	1.7	0.2 to 3.2	106	–0.1	–0.4	–1.5 to 0.6	
Year 2–baseline	75	0.2	1.3	–0.3 to 2.9	102	0.0	–0.7	–1.6 to 0.2	
Hispanic									
Baseline	37	4.3	9.5	5.3 to 13.7	33	5.6	10.0	5.0 to 14.9	
Year 1–baseline	37	0.1	–0.2	–2.0 to 1.5	33	–1.4	–3.2	–5.3 to –1.1	
Year 2–baseline	29	–0.2	–0.5	–3.4 to 2.3	26	–2.1	–3.7	–6.3 to –1.1	
Age, years									.03
50–59									
Baseline	74	5.0	9.0	6.6 to 11.5	94	4.6	7.9	6.0 to 9.9	
Year 1–baseline	74	–0.1	0.4	–1.0 to 1.9	94	–0.6	–1.4	–2.3 to –0.5	
Year 2–baseline	69	–0.5	–0.2	–1.8 to 1.5	91	–0.7	–2.0	–3.0 to –0.9	
60–69									
Baseline	87	2.8	5.9	4.3 to 7.5	89	2.5	7.5	5.2 to 9.8	
Year 1–baseline	87	0.3	1.9	0.8 to 3.0	89	–0.1	–0.6	–1.7 to 0.6	
Year 2–baseline	79	0.5	2.5	1.0 to 4.1	82	–0.2	–0.7	–1.6 to 0.2	
70–79									
Baseline	48	1.9	5.0	3.2 to 6.8	43	3.3	7.4	4.2 to 10.6	
Year 1–baseline	48	0.4	2.9	1.0 to 4.8	43	–0.1	–1.2	–2.4 to –0.1	
Year 2–baseline	45	1.2	3.0	1.1 to 5.0	40	–0.0	–0.2	–1.4 to 1.0	
BMI, kg/m²									.003
Normal (< 25)									
Baseline	36	5.6	9.4	5.9 to 13.0	36	7.1	11.8	6.9 to 16.6	
Year 1–baseline	36	2.2	3.2	0.5 to 5.8	36	–1.0	–1.0	–3.2 to 1.2	
Year 2–baseline	35	1.8	3.4	0.0 to 6.7	35	–0.6	–0.3	–2.3 to 1.7	
Overweight (25–30)									
Baseline	74	3.8	7.7	5.7 to 9.7	71	5.2	9.7	7.0 to 12.4	
Year 1–baseline	74	0.9	2.8	1.5 to 4.2	71	–0.6	–2.1	–3.2 to –0.9	
Year 2–baseline	67	0.8	2.7	1.0 to 4.3	67	–1.0	–2.6	–3.9 to –1.2	
Obese (> 30)									
Baseline	99	2.5	5.2	3.7 to 6.7	117	2.4	5.3	4.0 to 6.5	
Year 1–baseline	99	0.0	0.2	–0.8 to 1.1	117	–0.1	–0.4	–1.2 to 0.3	
Year 2–baseline	91	0.2	0.3	–0.9 to 1.5	109	0.0	–0.6	–1.2 to 0.0	
Smoking									< .001
Never									
Baseline	116	3.6	7.0	5.4 to 8.6	128	3.5	7.1	5.6 to 8.7	
Year 1–baseline	116	0.1	1.4	0.3 to 2.4	128	–0.1	–0.8	–1.5 to –0.1	
Year 2–baseline	108	0.5	2.0	0.7 to 3.2	123	–0.2	–1.0	–1.8 to –0.2	
Past									
Baseline	73	2.0	6.0	4.1 to 7.9	60	5.5	8.4	5.8 to 11.0	
Year 1–baseline	73	0.4	2.4	0.8 to 4.0	60	–1.1	–1.8	–2.9 to –0.7	
Year 2–baseline	68	0.3	2.1	0.3 to 3.9	56	–0.7	–1.2	–2.4 to –0.0	
Current									
Baseline	20	5.6	8.7	4.4 to 13.1	35	1.7	8.4	3.4 to 13.5	
Year 1–baseline	20	–0.0	0.5	–1.1 to 2.0	35	–0.2	–0.5	–3.1 to 2.1	
Year 2–baseline	17	–1.3	–1.7	–4.8 to 1.4	31	0.1	–1.5	–3.5 to 0.5	

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Table 3. MPD at Baseline, Year 1, and Year 2, by Baseline Characteristics and Treatment Assignment (Continued)

Characteristic	CEE MPD				Placebo MPD				P*
	No.	Median	Mean	95% CI	No.	Median	Mean	95% CI	
Parity									.04
Never									
Baseline	20	10.6	10.8	7.0 to 14.7	22	8.4	12.6	7.2 to 18.1	
Year 1—baseline	20	−0.3	−0.2	−1.9 to 1.6	22	0.4	−0.8	−3.3 to 1.6	
Year 2—baseline	20	0.5	−0.5	−3.0 to 2.0	22	−0.4	−0.9	−3.0 to 1.1	
1 to 2									
Baseline	53	3.8	7.6	4.8 to 10.4	72	2.7	6.6	4.4 to 8.8	
Year 1—baseline	53	−0.0	0.4	−1.1 to 1.9	72	−0.3	−0.7	−1.9 to 0.5	
Year 2—baseline	50	−0.2	−0.1	−1.8 to 1.7	67	−0.4	−1.6	−2.6 to −0.6	
3 to 4									
Baseline	88	3.2	6.7	4.9 to 8.6	75	3.7	8.8	6.0 to 11.6	
Year 1—baseline	88	0.3	2.2	0.8 to 3.7	75	−0.1	−1.4	−2.6 to −0.2	
Year 2—baseline	78	0.6	2.4	0.6 to 4.2	72	−0.6	−1.5	−2.7 to −0.3	
≥ 5									
Baseline	44	2.0	4.3	2.6 to 5.9	55	3.1	5.8	4.1 to 7.5	
Year 1—baseline	44	0.3	2.2	0.9 to 3.6	55	−0.8	−1.2	−2.2 to −0.2	
Year 2—baseline	41	0.5	2.9	1.0 to 4.8	50	−0.2	−0.4	−1.6 to 0.9	

NOTE. *P* values for interactions between CEE and subgroups are based on an *F* test from a repeated measures model with log (percent density + 0.001) as the response.

Abbreviations: MPD, mammographic percent density; BMI, body mass index; CEE, conjugated equine estrogen.

women ($P = .09$ for test of heterogeneity; Table 3). This association of race/ethnicity with change in density by CEE versus placebo remained after censoring for nonadherence (data not shown). The greatest increase in percent density with CEE use was seen in women age 70 to 79 years ($P = .03$ for interaction). The increase in mammographic density in CEE versus placebo participants was greater in normal-weight women (BMI < 25.0 kg/m²) compared with heavier participants ($P = .003$ for interaction), and results were similar when weight was used as a measure of adiposity (data not shown). The increase in percent density in CEE versus placebo participants was greater in women who were not currently smokers compared with current or previous smokers ($P < .001$), and in those with three or more births compared with women of lower parity ($P = .04$). We assessed the

effect of prior hormone use on trial results by stratifying participants into three groups—those who had never used hormone therapy, those who used hormone therapy ≤ 1 year before random assignment, and those with last use more than 1 year before random assignment—and assessed change in percent density in CEE versus placebo participants in these three groups. The test for interaction was not statistically significant ($P = .87$).

Women with a Gail risk score $\geq 1.7\%$ experienced a greater increase in percent density if they were randomly assigned to CEE versus placebo compared with women with Gail risk score less than 1.7% ($P = .02$ for interaction; Table 4). When we investigated effects of individual components of the Gail risk score as potential effect modifiers (age, age at menarche, age at first full-term pregnancy,

Table 4. MPD at Baseline, Year 1, and Year 2, by Treatment Assignment and Gail Risk

Group	CEE MPD				Placebo MPD			
	No.	Median	Mean	95% CI	No.	Median	Mean	95% CI
Low (< 1.7%)								
Baseline	166	3.2	6.9	5.5 to 8.3	189	2.9	7.4	5.9 to 8.9
Year 1	166	3.8	8.2	6.7 to 9.7	189	2.9	6.4	5.1 to 7.7
Year 2	151	4.7	8.6	7.1 to 10.0	177	2.7	6.5	5.2 to 7.9
Year 1—baseline	166	0.1	1.3	0.4 to 2.1	189	−0.2	−1.0	−1.7 to −0.3
Year 2—baseline	151	0.3	1.2	0.1 to 2.3	177	−0.2	−1.1	−1.8 to −0.4
High								
Baseline	43	3.8	6.3	4.4 to 8.2	37	5.5	9.1	5.8 to 12.3
Year 1	43	7.7	9.3	6.9 to 11.8	37	3.9	7.9	5.2 to 10.5
Year 2	42	6.7	9.6	6.7 to 12.4	36	4.8	6.8	4.4 to 9.3
Year 1—baseline	43	1.3	3.0	1.0 to 5.1	37	−0.3	−1.2	−2.8 to 0.4
Year 2—baseline	42	0.9	3.4	1.0 to 5.8	36	−0.5	−1.5	−2.7 to −0.3

NOTE. $P = .02$ for interaction between estrogen and menopausal hormone therapy is based on a two-sided *F* test from a repeated measures model with log (percent density + 0.001) as the response.

Abbreviations: MPD, mammographic percent density; CEE, conjugated equine estrogen.

Table 5. MPD at Baseline, Year 1, and Year 2, by Treatment Assignment and Percent Density at Baseline

Group	CEE MPD				Placebo MPD			
	No.	Median	Mean	95% CI	No.	Median	Mean	95% CI
MPD first tertile < 1.5%								
Baseline	66	0.5	0.6	0.5 to 0.7	77	0.5	0.6	0.5 to 0.7
Year 1	66	0.7	2.5	1.3 to 3.8	77	0.7	1.0	0.8 to 1.3
Year 2	60	0.9	2.5	1.4 to 3.5	71	0.7	1.1	0.9 to 1.4
Year 1—baseline	66	0.2	2.0	0.7 to 3.2	77	0.2	0.5	0.3 to 0.6
Year 2—baseline	60	0.5	1.9	0.9 to 2.9	71	0.3	0.6	0.3 to 0.9
MPD second tertile 1.5%-7.35%								
Baseline	80	3.5	3.8	3.4 to 4.2	68	3.2	3.7	3.3 to 4.1
Year 1	80	3.3	5.9	4.5 to 7.2	68	3.0	3.8	2.7 to 4.8
Year 2	72	4.1	6.8	5.1 to 8.4	64	2.1	3.3	2.5 to 4.0
Year 1—baseline	80	-0.0	2.0	0.8 to 3.3	68	-0.8	0.1	-1.0 to 1.1
Year 2—baseline	72	0.5	2.9	1.4 to 4.5	64	-1.0	-0.5	-1.1 to 0.2
MPD third tertile ≥ 7.35%								
Baseline	63	14.5	17.1	14.9 to 19.4	81	13.9	17.7	15.2 to 20.2
Year 1	63	16.8	17.9	15.7 to 20.1	81	11.2	14.4	12.1 to 16.6
Year 2	61	16.7	17.3	15.0 to 19.7	78	11.5	14.2	11.8 to 16.6
Year 1—baseline	63	0.2	0.8	-1.0 to 2.6	81	-3.4	-3.4	-4.8 to -2.0
Year 2—baseline	61	-1.0	0.0	-2.3 to 2.4	78	-3.6	-3.3	-4.8 to -1.9

NOTE. $P = .38$ for the interaction between CEE and baseline percent density is based on an F test from a repeated measures model with log (percent density + 0.001) as the response.

Abbreviations: MPD, mammographic percent density; CEE, conjugated equine estrogen.

history of breast biopsy, and history of atypical hyperplasia), only age modified the CEE effect on mammographic density (for age, see Table 3; other data not shown). None of the other baseline characteristics, including baseline percent density (Tables 3 and 5), were statistically significant effect modifiers of the CEE effect (at the Bonferroni adjusted or unadjusted 0.05 level).

DISCUSSION

In an ancillary study of the WHI randomized clinical trial, CEE resulted in a higher mammographic percent density compared with placebo after use for 1 year (2.6 percentage point difference; 95% CI, 1.0 to 2.7) and 2 years (2.9 percentage point difference; 95% CI, 0.6 to 3.1). Similar effects were observed in African American and non-Hispanic white women.

These results, suggesting that the effect of CEE on percent density is maintained over a 2-year period, extend the findings from the Postmenopausal Estrogen and Progesterone Intervention (PEPI) trial in which using CEE 0.625 mg/d for 1 year increased mammographic density by an absolute mean of 1.2% versus a decrease of 0.1% in placebo-treated women.⁸ In a small trial, postmenopausal women randomly assigned to CEE 0.625 mg/d ($n = 36$) had an absolute mean increase in mammographic density over 2 years of 1.2% versus a 1.3% decrease in women randomly assigned to placebo ($n = 45$; $P < .01$).⁷ A report that included women from two trials found that postmenopausal women randomly assigned to 1 mg/d of micronized 17 β -estradiol ($n = 104$) had an adjusted absolute mean increase in mammographic density of 4.6% after 12 months versus a 0.02% increase in women assigned to placebo ($n = 93$).²⁵

Our finding of a 2.6 percentage point difference in percent density in women randomly assigned to CEE versus placebo is lower than

the 6.9 percentage point difference observed for CEE plus MPA 2.5 mg/d in WHI, where CEE plus MPA increased percent density by a mean of 6.0 percentage points (95% CI, 4.6 to 7.5) at year 1 compared with a mean decrease of 0.9 percentage points (95% CI, -0.2 to -1.5) in the placebo group ($P < .001$), with differences largely persisting after 2 years. These results suggest a sizeable role for progestin on mammographic density, but our results suggest that CEE alone also can influence mammographic density.

Unlike a prior report by Ursin et al⁴ of substantially lower density in African American women, mammographic breast density was not substantially or significantly lower in African Americans relative to Hispanic and non-Hispanic whites in our study population. This inconsistency could be attributed to racial/ethnic variations by age or menopausal status, given that the Ursin et al study was in younger women (ages 35 to 64 years) and included 44% premenopausal women. The effect of CEE in our study on increasing percent density was consistent between non-Hispanic whites and African American women but was not observed in Hispanic women.

Breast cancer risk increases by $\approx 15\%$ with each 10% increase in percent density,³ and a meta-analysis including more than 14,000 patients confirmed a strong relationship between percent density and breast cancer risk.²⁶ Too few breast cancer cases ($n = 11$) occurred in this study to assess the relationship between the small hormone-associated increase in mammographic density and individual breast cancer risk.

One hypothesis is that mammographic density change may predict an intervention's effect on breast cancer risk.^{9,12} Combined hormone therapy increases mammographic density⁹ and breast cancer risk,¹³ while tamoxifen reduces mammographic density^{27,28} and breast cancer risk.^{24,29} However, raloxifene, which reduces invasive breast cancer risk²³ has not consistently been reported to decrease breast density.^{7,30} Similarly, data currently do not support an

effect of aromatase inhibitors on breast density,³¹ although these medications reduce new contralateral breast cancers.²⁴ In the WHI hormone therapy trials, use of CEE plus MPA increased breast density⁹ and breast cancer risk,¹² while CEE alone, which increased breast density, did not increase breast cancer incidence but rather showed a trend toward decreased risk.¹⁴ It remains to be determined whether an individual's change in mammographic density represents a breast cancer risk factor for that woman.

Strengths of this report include the double-blind randomized design; the quantitative, quality-controlled mammographic density assessment; the 2-year follow-up period, which provides new information on duration and the cumulative nature of CEE effects; and the over-representation of women from several race and ethnic minority groups. Study limitations include the evaluation of a single formulation, dose and schedule of hormone therapy, and a population that is older than women typically beginning hormone therapy.

In conclusion, use of CEE alone results in a modest but statistically significant increase in mammographic percent density which is sustained over at least a 2-year period. The clinical significance of the CEE effect on mammographic density remains to be determined.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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