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Research Article

Vitamin D and Calcium Supplementation and One-Year Change in Mammographic Density in the Women's Health Initiative Calcium and Vitamin D Trial

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Abstract

Background: Calcium and vitamin D may be inversely related to breast cancer risk, in part by affecting mammographic density. However, results from previous, mostly cross-sectional studies have been mixed, and there have been few randomized clinical trials of the effect of calcium and vitamin D supplementation on change in mammographic density.

Methods: We assessed the effect of one year of supplementation on mammographic density in 330 postmenopausal women enrolled in the Women's Health Initiative hormone therapy (HT) and calcium and vitamin D (CaD) trials. Women were randomized to receive 1,000 mg/d of elemental calcium carbonate plus 400 IU/d of vitamin D_3 or placebo.

Results: After approximately one year, mammographic density decreased 2% in the CaD supplementation group and increased 1% in the placebo group (ratio of means = 0.97; 95% CI = 0.81–1.17). Results suggested potential interaction by HT use ($P = 0.08$). Among women randomized to HT placebo, the ratio of mean density comparing CaD supplementation and placebo groups was 0.82 (95% CI = 0.61–1.11) vs. 1.16 (95% CI = 0.92–1.45) in women randomized to active HT. In sensitivity analyses limited to women taking ≥80% of study supplements, ratios were 0.67 (95% CI = 0.41–1.07) in women not assigned to HT and 1.07 (95% CI = 0.79–1.47) women assigned to HT.

Conclusions: We observed no overall effect of vitamin D and calcium supplementation on mammographic density after one year.

Impact: Potential interaction between these nutrients and estrogen as related to mammographic density warrants further study. *Cancer Epidemiol Biomarkers Prev; 21(3); 462–73. ©2012 AACR.*

Introduction

Numerous studies have assessed whether vitamin D and calcium may be related to the risk of breast cancer (1, 2). Results from prospective studies of dietary intake of these nutrients and/or blood levels of the main circulating vitamin D metabolite, 25-hydroxyvitaminD (25OHD), and breast cancer risk have been inconsistent. Whereas some have observed inverse associations (3–5), others including the Women's Health Initiative (WHI) Calcium and Vitamin D Supplementation Trial have reported null findings (6–8), or suggest that the association may vary by menopausal status, age, tumor hormone receptor status, and other hormone-related factors (9, 10). Vitamin D and calcium may affect breast cancer risk in part by reducing mammographic density, a strong predictor of breast cancer risk (11, 12). Numerous *in vitro* studies have indicated that 1,25-dihydroxyvitamin D, the biologically active vitamin D metabolite, can inhibit cellular proliferation and promote differentiation in normal breast tissue as well as tumor tissue (13). Vitamin D status may thus be associated with lower mammographic density and consequently, lower breast cancer risk. In addition, calcium intake may also influence

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mammographic density by regulating cell differentiation and proliferation independently of vitamin D (14).

Previous studies evaluating the relation between calcium, vitamin D, and mammographic density have been largely cross-sectional and results have been mixed (15–28). Few prospective studies of these association have been conducted (29, 30) and have not supported strong associations between vitamin D and calcium intake in childhood (30) or adulthood (29, 30) and mammographic density at midlife. Because results from these observational studies may be subject to residual confounding by other dietary and lifestyle factors affecting density that are also correlated with vitamin D intake, it is important to test these associations in randomized clinical trials. We thus examined the effect of 1 year of supplementation with 400 IU/d of vitamin D along with 1,000 mg/d of elemental calcium carbonate compared with placebo on breast density in a subset of postmenopausal women enrolled in the WHI Calcium and Vitamin D trial who were concurrently enrolled in the hormone therapy (HT) trials.

Methods

The WHI hormone therapy trials

Establishment of the WHI HT trials has been described previously (31–33). Briefly, between 1993 and 1998, postmenopausal women 50 to 79 years of age were recruited through direct mailing campaigns and media awareness programs. Recruitment was conducted at 40 clinical centers throughout the United States. Major ineligibility criteria included previous history of breast cancer, history of other cancers (other than nonmelanoma skin cancer) within the previous 10 years, medical conditions likely to result in death within 3 years, and conditions likely to interfere with retention in the study. Ultimately, 16,608 women who had not had a hysterectomy were randomized to 0.625 mg conjugated equine estrogen plus 2.5 mg medroxyprogesterone acetate per day in a single table or a similar placebo (E+P trial; Fig. 1). An additional 10,739 women without a uterus were randomized to 0.625 mg conjugated equine estrogen daily or a similar placebo (E-alone trial; Fig. 1). At baseline enrollment visits prior to randomization, participants completed questionnaires that assessed a variety of demographic, reproductive, behavioral, and health factors. Participants were required to have had a screening mammogram within 6 months prior to randomization or were referred for a screening mammogram before they were randomized.

WHI calcium and vitamin D trial

The WHI calcium and vitamin D (CaD) trial included 36,282 women previously enrolled in the HT and/or dietary modification (DM) HT trials and has been described in detail previously (34). Eligible HT and DM trial participants were invited to join the CaD trial at their first or second annual follow-up clinic visit. More than 95% of HT participants joining the CaD trial did so at their first HT follow-up visit.

As part of the CaD trial, participants were randomized to receive a combined calcium plus vitamin D supplement or an identical appearing placebo using a permuted block algorithm (Fig. 1). Participants were asked to take 2 pills per day (each containing 500 mg of elemental calcium as calcium carbonate and 200 IU of vitamin D_3), for a total daily dose of 1,000 mg of elemental calcium and 400 IU of vitamin D_3. Women were allowed to continue personal use of calcium and vitamin D supplements, with initial cutoffs of 1,000 mg/d for calcium and 600 IU/d for vitamin D (later increased to 1,000 IU/d during the trial). Supplementation was terminated if women reported kidney stones, kidney dialysis, hypercalcemia, calcitriol use, or personal use of vitamin D supplements at dosages higher than 600 IU/d (later 1,000 IU/d). Participants were contacted 4 weeks post-CaD randomization and then twice per year to assess safety, adherence, and clinical outcomes. Adherence in the trials was defined as taking 80% or more of study medication. In the first 3 years of follow-up, adherence ranged from 60% to 63% and an additional 13% to 21% of participants took at least half of their medications (1 of 2 pills per day on average; ref. 6). In a substudy conducted among 448 CaD trial participants, after 2 years of supplementation 25OHD levels among women assigned to calcium and vitamin D were 28% higher than those assigned to placebo (35). Of note, no biologic correlate of calcium intake is available for similar analyses of calcium status.

The study protocol was approved by Institutional Review Boards at each participating institution and registered at clinicaltrials.gov (NCT00000611). An independent data and safety monitoring board reviewed all clinical outcomes for the study.

Study population

The present analysis includes members of the HT and CaD trials who also enrolled in the Mammogram Density Ancillary Study of the HT trials. This ancillary study was designed to evaluate the effect of postmenopausal hormones on mammographic density and has been described in detail previously (36, 37). Briefly, women who had a mammogram taken prior to HT randomization and at least one follow-up mammogram 1 to 2 years after HT randomization were considered eligible and were selected for inclusion in this substudy using a stratified random sampling protocol.

Among E+P trial participants selected for the random sample, 214 of the 233 women assigned to E+P and 223 of the 240 women assigned to placebo agreed to join in the Mammogram Density Ancillary Study. Complete mammogram data showing no evidence of invasive breast cancer were received from 202 women assigned to E+P and 211 assigned to placebo. Among E-alone trial participants selected for the random sample, 220 of the 234 women assigned to E-alone and 238 of the 264 women assigned to placebo agreed to join the Mammogram

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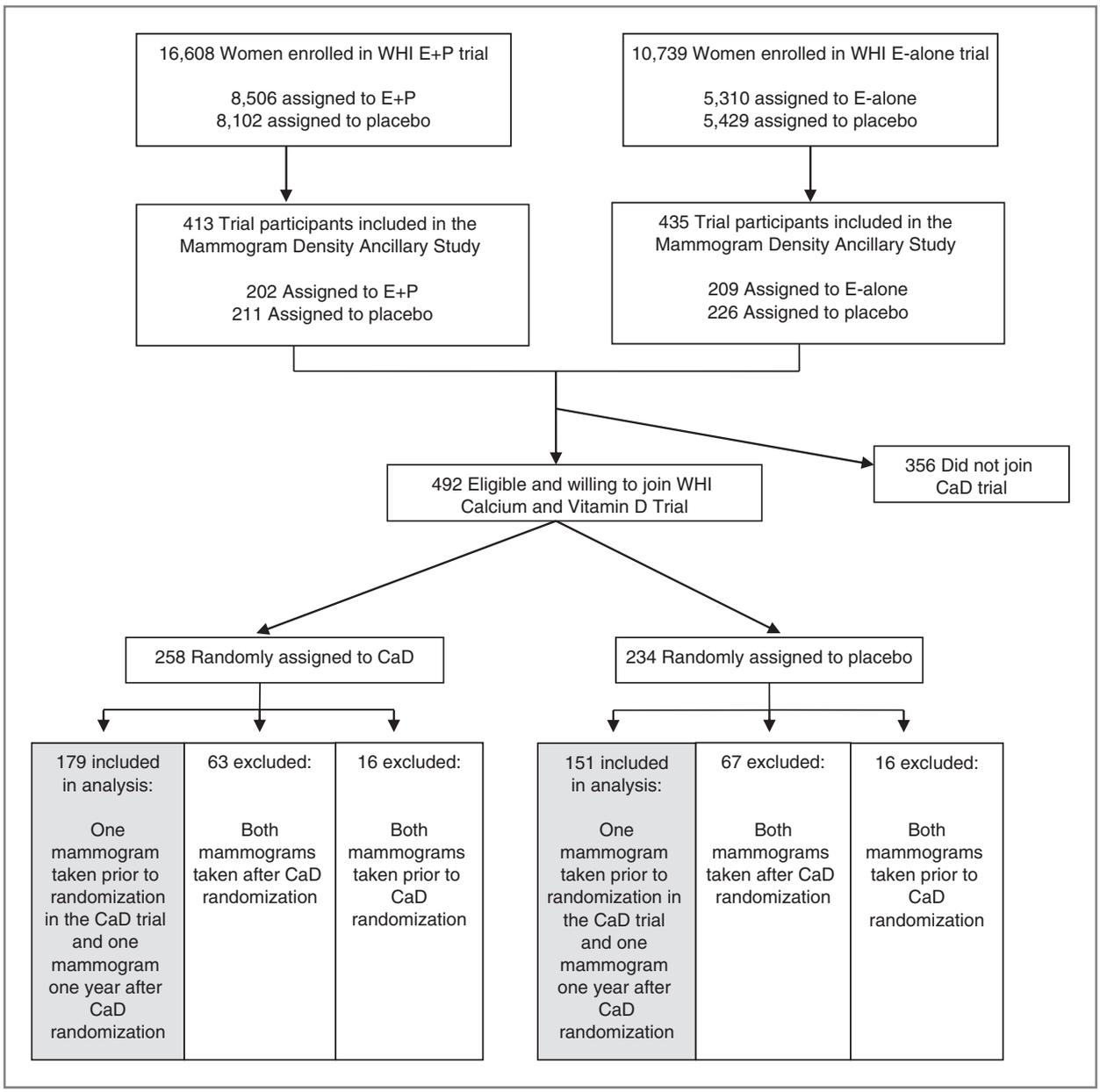

Figure 1. Selection of participants for assessment of calcium and vitamin D assessment and mammographic density in the WHI hormone therapy and calcium and vitamin D Trials. E+P = conjugated equine estrogen plus medroxyprogesterone acetate, E-alone = conjugated equine estrogen, and MPD = mammographic percent density.

Density Ancillary Study. Complete mammogram data showing no evidence of invasive breast cancer were received from 209 women assigned to E-alone and 226 assigned to placebo. Thus, the parent Mammogram Density Ancillary Study included a total of 848 participants (Fig. 1). Of these 848 women, 492 were eligible and enrolled in the CaD trial, 258 of whom were randomized to calcium and vitamin D supplementation and 234 of whom were randomized to placebo.

For this analysis, we limited inclusion to women who had a first mammogram prior to CaD randomization (i.e., "CaD baseline") and a second mammogram approximately one year after randomization (i.e., "CaD follow-up"). This included 179 of the 258 (69.4%) women assigned to CaD supplements and 151 of 234 (64.5%) of women assigned to placebo, for a total of 330 women (Fig. 1).

Assessment of mammographic density

After receiving informed consent, mammograms from each participant were requested from their individual mammography provider and then sent to the University of North Carolina for digitizing. Digitizing of films was

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done on a Lumisys 85 laser digitizer with a maximum resolution of approximately 50 μm and 12-bit depth, with the digitizer recalibrated between sessions. A standard data averaging method was used to convert raw image files to bitmap format for display and measurement of mammographic density. For each film, a unique serial number, the date of exam, laterality, and view were recorded. The technique used to assess mammographic density has been validated previously (38) and used a computer-assisted interactive thresholding technique with software from the Imaging Research Program (Sunnybrook Health Science Center, Toronto, Ontario, Canada).

Mammograms were sorted separately for 2 trained observers (CM, JP), who both reviewed all films. Interobserver reliability for measuring percent density was assessed before the study began and found to be very high (i.e., intraclass correlation coefficients >0.92; ref. 37). Observers independently reviewed mammograms from CaD baseline and CaD follow-up and were blinded to participant identification, randomization status for either trial, the timing of the mammogram (CaD baseline vs. follow-up), result from the other observer, and results of other mammograms from the same woman. The craniocaudal view of the right breast was used if available; otherwise the same view from the left breast was used. Investigators determined the breast edge and noncontinguous areas of mammographic density. The total area of breast and the total combined area of mammographic density were both calculated (pixels) and then the latter was divided by the former to calculate percent density. Density of each participant was then calculated as the mean of the estimates of percent density from the 2 readers.

Assessment of vitamin D intake and other factors

At their baseline clinic visit, participants completed a semiquantitative food frequency questionnaire (FFQ) designed for the WHI and validated in this population (39). Participants reported their usual intake and portion size of 122 foods or food groups in the 3 previous months. Vitamin D intake from food sources was calculated by multiplying the nutrient content of the specified portion size of each food (University of Minnesota Nutrient Coding Center nutrient database) by its frequency of consumption and summing the contributions of all foods. In a validation study in the WHI, vitamin D and calcium intake measured by FFQ correlated well with intake measured with 4 days of diet recalls + 4 days of food records (deattenuated r for intake from foods: vitamin D = 0.70, calcium = 0.73; r for foods + supplements: vitamin D = 0.73; calcium = 0.78; ref. 39).

At clinic visits prior to HT randomization and approximately 1 year later, vitamin D intake from supplemental sources was assessed by trained interviewers using a standard questionnaire that ascertained dose, frequency (pills per week), and duration (months and years) of use of multivitamins, multivitamin mineral, and single supplements. Total vitamin D and calcium intakes were determined by summing intakes from food sources (FFQ) and personal supplements.

Study questionnaires completed during the baseline visits were used to assess breast cancer risk factors, including age, race/ethnicity, previous use of HT, and oral contraceptives, education, alcohol intake, participation in physical activity, history of smoking, age at menarche, and Gail risk score. Weight and height were measured directly and used to calculate body mass index [BMI; weight (kg)/height (m) squared]. For each of the 40 WHI clinical centers, an estimate of annual level of solar irradiance in Langleys (gm-cal) per cm^2 was calculated using measurements from the U.S. Weather Bureau adapted for use in the WHI (40).

Statistical analysis

Comparisons of mean mammographic density by CaD randomization assignment were made by fitting linear models of log-transformed densities on treatment assignment. Means (95% CI) of the logged densities were then exponentiated to yield geometric means (95% CI). Ratios (95% CI) and P values, comparing CaD to placebo, were obtained in a similar fashion by fitting linear models on differences between log-transformed densities (CaD follow-up minus CaD baseline) and exponentiating. The log-transformation and differencing ensured an approximate normal distribution for statistical tests. Subgroup analyses were done to explore whether the effect of CaD was modified by other factors including age, race/ethnicity, total vitamin D intake, HT treatment arm, Gail risk score, BMI, region of residence, and category of mammogram density at CaD baseline. We tested for effect modification by including terms for CaD assignment, the factor (e.g., age), and CaD assignment × the factor in the regression model. Statistical significance was based on the test of interaction in which at most one interaction is expected to be significant at the 0.05 level by chance alone. The influence of nonadherence to protocol-assigned treatment was examined by excluding density measures from participants reported consuming <80% of study medications (CaD or HT) or initiating nonstudy HT. All analyses were conducted using SAS version 9.1.3 (SAS Institute), and all P values were 2-sided.

Results

Characteristics of the 330 women included in this analysis by CaD randomization group are presented in Table 1. Women randomized to CaD supplements did not differ from those randomized to placebo in terms of mean age [61.8 years (SD = 7.5) vs. 62.0 (8.0); $P = 0.85$] or mean BMI [29.9 kg/m^2 (5.7) vs. 29.9 (6.2); $P = 0.94$]. The distribution of other characteristics was similar between randomization groups, including intakes of vitamin and calcium.

We did not find study supplementation with calcium and vitamin D to be associated with a significant change in

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Table 1. Baseline characteristics of mammographic percent density subsample that are participating in the CaD Trial by randomization assignment ($n = 330$),[a] WHI CaD Trial

Characteristic	CaD ($n = 179$) N (%)	Placebo ($n = 151$) N (%)	P^b
Age (y)			0.07
50–59	73 (40.8)	68 (45.0)	
60–69	76 (42.5)	47 (31.1)	
70–79	30 (16.8)	36 (23.8)	
Ethnicity			0.53
White	82 (45.8)	78 (51.7)	
Black	65 (36.3)	54 (35.8)	
Hispanic	25 (14.0)	14 (9.3)	
Asian/Pacific Islander	7 (3.9)	5 (3.3)	
U. S. Region			0.43
Northeast	13 (7.3)	17 (11.3)	
South	73 (40.8)	52 (34.4)	
Midwest	57 (31.8)	54 (35.8)	
West	36 (20.1)	28 (18.5)	
Smoking status			0.40
Never	97 (55.1)	78 (51.7)	
Past	58 (33.0)	47 (31.1)	
Current	21 (11.9)	26 (17.2)	
Body mass index (kg/m^2)			0.53
<25	35 (19.6)	37 (24.7)	
25 to <30	63 (35.2)	50 (33.3)	
≥30	81 (45.3)	63 (42.0)	
Alcohol consumption (drinks/d)			0.21
Nondrinker	106 (59.2)	83 (55.0)	
≤1	65 (36.3)	54 (35.8)	
>1	8 (4.5)	14 (9.3)	
Moderate to strenuous physical activity (# episodes ≥20 min/wk)			0.47
No activity	38 (23.2)	33 (24.1)	
Some activity	71 (43.3)	61 (44.5)	
2 to <4	16 (9.8)	19 (13.9)	
≥4	39 (23.8)	24 (17.5)	
Age at menarche (y)			0.88
≤11	35 (19.7)	33 (21.9)	
12–13	91 (51.1)	76 (50.3)	
≥14	52 (29.2)	42 (27.8)	
Years since menopause			0.59
<5	27 (16.9)	22 (16.5)	
5 to <10	32 (20.0)	24 (18.0)	
10 to <15	28 (17.5)	17 (12.8)	
≥15	73 (45.6)	70 (52.6)	
Parity (number of full term pregnancies)			0.11
Never pregnant/never had term pregnancy	16 (9.0)	14 (9.3)	
1	14 (7.9)	11 (7.3)	
2	25 (14.1)	37 (24.5)	
≥3	122 (68.9)	89 (58.9)	
Age at first birth (y)			0.36
Never pregnant/no full term pregnancies	16 (10.3)	14 (10.1)	
<20	40 (25.8)	36 (26.1)	
20–29	89 (57.4)	85 (61.6)	
≥30	10 (6.5)	3 (2.2)	

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Table 1. Baseline characteristics of mammographic percent density subsample that are participating in the CaD Trial by randomization assignment ($n = 330$),[a] WHI CaD Trial (Cont'd)

Characteristic	CaD ($n = 179$) *N* (%)	Placebo ($n = 151$) *N* (%)	P^b
Benign breast disease and biopsy history			0.68
No	138 (85.7)	121 (89.0)	
Yes, 1 biopsy	16 (9.9)	11 (8.1)	
Yes, 2+ biopsies	7 (4.3)	4 (2.9)	
Family history of female relative w/breast cancer			0.31
No	147 (86.5)	116 (82.3)	
Yes	23 (13.5)	25 (17.7)	
Gail risk score			0.86
<1.25	109 (60.9)	89 (58.9)	
1.25 to <1.75	42 (23.5)	35 (23.2)	
≥1.75	28 (15.6)	27 (17.9)	
Duration of HT use at baseline (y)			0.73
Never-user	35 (19.6)	30 (19.9)	
<5	10 (5.6)	7 (4.6)	
5 to <10	125 (69.8)	102 (67.5)	
≥10	9 (5.0)	12 (7.9)	
Duration of oral contraceptive use (y)			0.75
Nonuser	55 (30.7)	39 (25.8)	
<5	16 (8.9)	14 (9.3)	
5 to <10	92 (51.4)	81 (53.6)	
≥10	16 (8.9)	17 (11.3)	
Total vitamin D intake (IU/d)			0.89
<100	39 (23.1)	39 (26.9)	
100 to <200	48 (28.4)	38 (26.2)	
200 to <400	29 (17.2)	24 (16.6)	
≥400	53 (31.4)	44 (30.3)	
Dietary vitamin D intake (IU/d)			0.78
<100	55 (32.5)	52 (35.9)	
100 to <150	49 (29.0)	37 (25.5)	
150–200	26 (15.4)	26 (17.9)	
≥200	39 (23.1)	30 (20.7)	
Any Supplemental D			0.82
No	114 (63.7)	98 (64.9)	
Yes	65 (36.3)	53 (35.1)	
Total calcium intake (mg/d)			0.67
<500	29 (17.2)	33 (22.8)	
500 to <750	48 (28.4)	38 (26.2)	
750 to <1,000	40 (23.7)	32 (22.1)	
≥1,000	52 (30.8)	42 (29.0)	
Dietary calcium intake (mg/d)			0.43
<500	41 (24.3)	46 (31.7)	
500 to <650	42 (24.9)	29 (20.0)	
650 to <800	25 (14.8)	23 (15.9)	
≥800	61 (36.1)	47 (32.4)	
Supplemental Calcium (mg/d)			0.47
None	42 (23.5)	43 (28.5)	
<500	103 (57.5)	85 (56.3)	
≥500	34 (19.0)	23 (15.2)	

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Table 1. Baseline characteristics of mammographic percent density subsample that are participating in the CaD Trial by randomization assignment ($n = 330$),[a] WHI CaD Trial (Cont'd)

Characteristic	CaD ($n = 179$) *N* (%)	Placebo ($n = 151$) *N* (%)	P^b
HT trial arm			0.49
E-alone	47 (26.3)	33 (21.9)	
E-alone placebo	40 (22.3)	42 (27.8)	
E + P	45 (25.1)	32 (21.2)	
E + P placebo	47 (26.3)	44 (29.1)	
DM trial treatment assignment			0.49
Not in DM trial	125 (69.8)	99 (65.6)	
Placebo	32 (59.3)	35 (67.3)	
Active	22 (40.7)	17 (32.7)	

NOTE: E+P = conjugated equine estrogen plus medroxyprogesterone acetate; E-alone = conjugated equine estrogen.
[a]Includes women in whom mammographic breast density was measured prior to CaD randomization and approximately 1 year after CaD randomization.
[b]χ^2 test of association.

mammographic density between CaD baseline and follow-up, as compared with placebo (Table 2). On average, mammographic density decreased 2% in the CaD supplement group and increased 1% in the CaD placebo group. The ratio of geometric means comparing 1 year change in mammographic density in the CaD supplement group to that of the CaD placebo group was 0.97 (95% CI = 0.81–1.17; $P = 0.77$).

We observed little evidence of effect modification by age, calcium, or vitamin D intake from foods and personal supplements at baseline, Gail risk score, and category of percent density (Table 3). Results stratified by HT treatment arm suggested modest effect modification of supplementation with CaD by HT (P for interaction = 0.08). Among those in the HT trials assigned to placebo, the ratio of geometric mean mammographic density comparing 1 year change in density between CaD and placebo groups was 0.82 (95% CI = 0.61–1.11). In contrast, among HT users, the ratio of geometric mean density comparing CaD and placebo groups was 1.16 (95% CI = 0.92–1.45). Further stratification by E-alone and E+P trial also resulted in a nonsignificant interaction between CaD supplementation and HT ($P = 0.18$ for the CaD by E-alone interaction, and $P = 0.24$ for the CaD by E+P interaction). Among women in the E-alone trial, the ratio of geometric mean mammographic density comparing 1 year change in density between CaD and placebo groups was 1.29 (95% CI = 0.88–1.89) for those randomized to E-alone and 0.86 (95% CI = 0.54–1.36) randomized to the placebo arm. Similarly, among women in the E+P trial, the ratio of geometric mean mammographic density comparing 1 year change in density between CaD and placebo groups was 1.03 (95% CI = 0.80–1.33) for those randomized to E+P and 0.78 (95% CI = 0.53–1.14) randomized to the placebo arm.

In sensitivity analyses limited to women who were adherent (taking at least 80% of CaD supplements and HT medication), differences between groups were slightly greater. Mean mammographic density decreased 0.49% in the CaD group and increased 0.11% in the placebo group (ratio of geometric means comparing 1 year change in density in CaD supplementation and placebo groups = 0.83; 95% CI = 0.86–1.11; $P = 0.21$). Results from analyses

Table 2. Mean[a] mammographic percent density prior to CaD randomization (CaD baseline), approximately 1 year after CaD randomization (CaD Follow-up), and ratio of means by CaD randomization assignment

		Mean% mammographic density (95% CI)				
	n	At CaD baseline	At CaD follow-up	Ratio of CaD follow-up to baseline (95% CI)	Ratio in CaD vs. ratio in placebo (95% CI)	*P*
CaD	179	3.7 (2.9–4.8)	3.6 (2.9–4.6)	0.98 (0.86–1.12)	0.97 (0.81–1.17)	0.77
Placebo	151	2.8 (2.1–3.7)	2.8 (2.2–3.7)	1.01 (0.88–1.15)		

[a]Geometric mean.

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Table 3. Mean[a] mammographic percent density prior to CaD randomization (CaD baseline), approximately one year after CaD randomization (CaD follow-up), and ratio of means by CaD randomization assignment by subgroup

		Mean% mammographic density (SD)				
	n	At CaD baseline	At CaD follow-Up	Ratio of CaD baseline to follow-up (95% CI)	Ratio in CaD vs. ratio in placebo (95% CI)	*P* (int)[b]
Age						0.31
50–57 y						
CaD	59	4.6 (3.0–6.9)	4.2 (2.9–6.1)	0.92 (0.74–1.15)	0.95 (0.71–1.27)	
Placebo	53	3.1 (2.0–4.8)	3.0 (2.0–4.6)	0.97 (0.81–1.17)		
58–65 y						
CaD	64	3.2 (2.1–4.7)	3.2 (2.1–4.9)	1.02 (0.81–1.27)	0.77 (0.56–1.07)	
Placebo	44	1.9 (1.1–3.5)	2.5 (1.5–4.2)	1.32 (1.04–1.66)		
66–79 y						
CaD	56	3.6 (2.2–5.9)	3.6 (2.3–5.5)	1.00 (0.77–1.30)	1.20 (0.83–1.72)	
Placebo	54	3.6 (2.3–5.6)	3.0 (1.8–4.8)	0.83 (0.65–1.08)		
Total vitamin D intake (IU/d)						0.75
<200						
CaD	87	3.6 (2.5–5.2)	3.5 (2.5–4.9)	0.96 (0.79–1.18)	0.97 (0.74–1.28)	
Placebo	77	3.0 (2.1–4.5)	3.0 (2.1–4.3)	0.99 (0.83–1.19)		
200 to <400						
CaD	29	2.4 (1.1–5.3))	2.8 (1.4–5.6)	1.13 (0.73–1.74)	0.89 (0.53–1.47)	
Placebo	24	2.5 (1.3–5.1)	3.2 (1.7–6.1)	1.28 (1.03–1.59)		
≥400						
CaD	53	4.3 (2.9–6.4)	4.0 (2.6–6.0)	0.93 (0.77–1.14)	1.06 (0.75–1.50)	
Placebo	44	2.7 (1.5–4.8)	2.3 (1.3–4.2)	0.88 (0.65–1.19)		
Total calcium Intake (mg/d)						0.51
<750						
CaD	77	3.6 (2.4–5.2)	3.2 (2.2–4.6)	0.90 (0.71–1.14)	0.99 (0.74–1.34)	
Placebo	71	3.1 (2.1–4.6)	2.8 (1.9–4.2)	0.91 (0.75–1.09)		
750 to <1,000						
CaD	40	3.7 (1.9–7.2)	4.4 (2.6–7.5)	1.19 (0.88–1.62)	1.16 (0.75–1.80)	
Placebo	32	2.7 (1.4–5.1)	2.7 (1.6–4.7)	1.03 (0.74–1.43)		
≥1,000						
CaD	52	3.5 (2.3–5.1)	3.3 (2.2–5.1)	0.96 (0.78–1.16)	0.83 (0.61–1.15)	
Placebo	42	2.5 (1.4–4.7)	2.9 (1.7–5.1)	1.14 (0.88–1.49)		
Active HT						0.08
No						
CaD	87	2.7 (1.9–3.8)	2.3 (1.6–3.3)	0.86 (0.70–1.07)	0.82 (0.61–1.11)	
Placebo	86	1.9 (1.3–2.8)	2.0 (1.4–2.9)	1.05 (0.85–1.29)		
Yes						
CaD	92	5.1 (3.6–7.1)	5.6 (4.3–7.3)	1.10 (0.93–1.30)	1.16 (0.92–1.45)	
Placebo	65	4.7 (3.2–6.9)	4.5 (3.0–6.6)	0.95 (0.83–1.09)		
Gail risk score						0.23
<1.25						
CaD	109	3.3 (2.4–4.5)	3.1 (2.3–4.2)	0.95 (0.80–1.14)	0.90 (0.70–1.15)	
Placebo	89	3.0 (2.1–4.2)	3.1 (2.3–4.3)	1.06 (0.90–1.25)		
1.25 to <1.75						
CaD	42	4.2 (2.3–7.6)	4.2 (2.5–7.1)	1.02 (0.75–1.37)	1.01 (0.65–1.57)	
Placebo	35	1.9 (0.9–3.7)	1.9 (1.0–3.6)	1.00 (0.72–1.39)		
≥1.75						
CaD	28	5.2 (3.2–8.5)	5.4 (3.3–8.7)	1.03 (0.81–1.30)	1.22 (0.84–1.78)	
Placebo	27	4.2 (2.5–7.3)	3.6 (1.8–7.0)	0.84 (0.62–1.14)		

(Continued on the following page)

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Table 3. Mean[a] mammographic percent density prior to CaD randomization (CaD baseline), approximately one year after CaD randomization (CaD follow-up), and ratio of means by CaD randomization assignment by subgroup (Cont'd)

		Mean% mammographic density (SD)				
	n	At CaD baseline	At CaD follow-Up	Ratio of CaD baseline to follow-up (95% CI)	Ratio in CaD vs. ratio in placebo (95% CI)	*P* (int)[b]
Race/ethnicity						0.11
White						
CaD	82	2.9 (1.9–4.3)	3.3 (2.4–4.7)	1.17 (0.95–1.42)	1.18 (0.90–1.55)	
Placebo	78	2.9 (1.9–4.3)	2.8 (1.9–4.1)	0.99 (0.82–1.19)		
Black						
CaD	65	4.4 (3.0–6.6)	3.6 (2.4–5.4)	0.81 (0.63–1.03)	0.76 (0.55–1.06)	
Placebo	54	2.6 (1.6–4.1)	2.7 (1.8–4.3)	1.06 (0.86–1.30)		
Hispanic						
CaD	25	4.3 (2.6–7.2)	4.1 (2.5–6.6)	0.95 (0.74–1.23)	0.85 (0.52–1.40)	
Placebo	14	2.3 (0.8–6.4)	2.6 (1.1–5.9)	1.12 (0.66–1.89)		
BMI (kg/m^2)						0.31
Normal (<25)						
CaD	35	6.8 (4.3–10.7)	5.5 (3.3–9.3)	0.81 (0.66–1.01)	0.79 (0.58–1.06)	
Placebo	37	7.5 (4.9–11.5)	7.7 (5.0–12.0)	1.04 (0.83–1.29)		
Overweight (25 to <30)						
CaD	63	3.8 (2.5–5.8)	3.5 (2.3–5.5)	0.94 (0.73–1.20)	1.02 (0.71–1.46)	
Placebo	50	4.2 (2.8–6.4)	3.9 (2.5–6.0)	0.92 (0.71–1.20)		
Obese (≥30)						
CaD	81	2.8 (1.9–4.2)	3.1 (2.3–4.2)	1.09 (0.89–1.34)	1.03 (0.77–1.38)	
Placebo	63	1.2 (0.8–1.8)	1.2 (0.8–1.8)	1.06 (0.86–1.30)		
Solar irradiation (Langleys)						0.13
<350						
CaD	34	2.4 (1.1–5.0)	3.2 (1.8–5.9)	1.36 (0.92–2.00)	1.53 (1.00–2.32)	
Placebo	48	2.7 (1.6–4.5)	2.4 (1.4–4.1)	0.89 (0.71–1.12)		
350 to <400						
CaD	94	3.9 (2.8–5.5)	3.5 (2.5–4.8)	0.88 (0.73–1.06)	0.78 (0.58–1.04)	
Placebo	62	2.5 (1.6–3.9)	2.8 (1.9–4.1)	1.13 (0.91–1.40)		
≥400						
CaD	51	4.5 (3.1–6.6)	4.3 (3.0–6.2)	0.96 (0.80–1.14)	0.98 (0.74–1.31)	
Placebo	41	3.7 (2.2–6.2)	3.6 (2.2–5.9)	0.97 (0.76–1.24)		
Category of mammographic density						0.42
<1%						
CaD	35	0.3 (0.2–0.4)	0.5 (0.3–0.7)	1.78 (1.14–2.78)	1.23 (0.70–2.18)	
Placebo	39	0.3 (0.2–0.4)	0.4 (0.3–0.6)	1.45 (0.99–2.10)		
1%–<10%						
CaD	87	3.6 (3.2–4.2)	3.2 (2.5–4.0)	0.87 (0.73–1.04)	0.97 (0.76–1.24)	
Placebo	71	3.3 (2.8–3.9)	3.0 (2.4–3.8)	0.90 (0.76–1.01)		
≥10%						
CaD	57	19.2 (17.3–21.5)	15.5 (13.1–18.4)	0.81 (0.70–0.92)	0.93 (0.77–1.13)	
Placebo	41	19.3 (16.7–22.4)	16.7 (13.7–20.4)	0.86 (0.76–0.98)		

NOTE: HT = hormone therapy.

[a]Geometric mean.

[b]*P* for interaction.

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stratified by HT treatment arm were also somewhat stronger than in the analysis of all participants. Among HT trial members assigned to placebo, the ratio of geometric mean density comparing CaD and placebo groups was 0.67 (95% CI = 0.41–1.07). In contrast, among women in the HT active arm, the ratio of geometric mean density comparing CaD and placebo groups was 1.07 (95% CI = 0.79–1.47; *P* for interaction = 0.12).

Discussion

In this study of postmenopausal women, daily supplementation of 1,000 mg of elemental calcium and 400 IU of vitamin D_3 for 1 year did not affect mammographic density as compared with placebo.

We observed some evidence that vitamin D and calcium supplementation was associated with slightly lower mammographic density in postmenopausal women not currently using HT and slightly higher density in women using HT, but the test for interaction was not statistically significant (*P* interaction = 0.08). Given our small sample size, especially for subgroup analyses and tests for interactions, this finding may be due to chance. Alternatively, in HT users modest changes related to vitamin D and calcium supplementation may be masked by changes associated with hormone use. Finally, a potential interaction may exist between estrogens and vitamin D as they relate to mammographic density and perhaps to breast cancer risk also. Although interaction with HT has not been observed in previous studies of supplemental calcium and vitamin D and postmenopausal breast cancer, including the WHI CaD trial (6), few studies have evaluated this (16). Potential biologic mechanisms supporting an interaction between estrogen and vitamin D include competition for cell membrane megalin receptors, which play a role in the endocytosis of sex steroid hormones and vitamins, and effects of estrogens on circulating levels of calbindin, a binding protein regulating intracellular free calcium levels and effecting cell proliferation (41). However, it is interesting to note that inverse relationships between vitamin D, calcium, and mammographic density have been more consistently observed in studies of premenopausal women (20, 23, 24, 26), in whom circulating levels of estrogens and mean mammographic density are higher than in postmenopausal women. Additional studies of vitamin D, breast density, and breast cancer should further explore the potential interaction between vitamin D and estrogen-related factors including menopausal status and HT use when possible.

The mean mammographic density among participants in our population (arithmetic mean = 8.4%; SD = 10.2%) was considerably lower than in previous studies of postmenopausal women, perhaps because our participants were somewhat older than women in other studies (16, 20, 23) and because HT trial participants were required to be off hormones for at least 3 months prior to randomization. Studies observing the strongest relationships between density and vitamin D and/or calcium intake generally evaluated women with higher mammographic densities (17, 19). An effect of vitamin D on mammographic density may not be detectable in women who already have very low percent density due to a "floor effect." We did not find an effect of supplementation in subgroup analyses of women with higher mean density including younger women, HT users, or those with baseline density ≥10%, possibly due to the small sample size for these analyses. In addition, it is possible that 1 year of supplementation was insufficient to affect mammographic density, though significant differences in density were observable after 1 year of hormone treatment in both the WHI E+P trial (mean density increased from 3.9% to 9.6% over 1 year in the E+P active group) and E-alone trials (mean density increased from 6.8% to 8.4% over 1 year in the E-alone active group; refs. 36, 37).

The dose of vitamin D tested in our study, 400 IU/d, may have been insufficient to modify breast density within 1 year. It has been proposed that in the absence of sunlight exposure, vitamin D intake of 1,700 to 2,000 IU/d is necessary to achieve 25(OH)D levels of 75 nmol/L (30 ng/dL), which may be needed to lower breast cancer risk (42, 43). The dose used in our study, combined with background personal use of vitamin D supplements and dietary intake, is consistent with current Institute of Medicine guidelines (i.e., 600 IU/d for women ≤70 years, 800 IU/d for women >70 years; ref. 44). Although differences in actual vitamin D intake between intervention groups may have been insufficient to detect an effect on density, overall levels of intake in our study are comparable with those of previous studies that did observe a relation with mammographic density (17, 23, 26). Furthermore, in the CaD trial, vitamin D and calcium supplementation was associated with significantly lower risk of breast cancer in women consuming <200 IU/d of vitamin D at baseline (0.79; 95% CI = 0.65–0.97) but significantly increased in women consuming ≥600 IU/d (1.34; 95% CI = 1.01–1.78; *P* interaction = 0.003; ref. 6). These findings do not support the hypothesis that higher doses of vitamin D than ours are necessary to modify mammographic density and breast cancer risk among older postmenopausal women.

To our knowledge, this is the first observation of the effect of calcium and vitamin D supplementation on mammographic density in the context of a randomized clinical trial. Although we observed no effect of 400 IU/d of vitamin D_3 along with 1,000 mg/d of elemental calcium on mammographic density after approximately 1 year in this small study, questions persist about the potential for interaction between vitamin D and estrogen and warrant further investigation.

Disclosure of Potential Conflicts of Interest

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