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Research Article

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A Randomized Double-Blind Placebo-Controlled Trial of the Effect of Vitamin D_3 Supplementation on Breast Density in Premenopausal Women

Jacques Brisson[1,2,3], Sylvie Bérubé[1], Caroline Diorio[1,2,3], Benoît Mâsse[4,5], Julie Lemieux[1,3], Thierry Duchesne[6], Edgar Delvin[5], Reinhold Vieth[7], Martin J. Yaffe[8], and Jocelyne Chiquette[1,3]

Abstract

Background: This double-blind, placebo-controlled parallel group trial assessed whether oral supplementation with 1,000, 2,000, or 3,000 IU/day vitamin D_3 over one year reduces percent mammographic breast density in premenopausal women.

Methods: The trial was conducted between October 2012 and June 2015, among premenopausal female volunteers from Quebec City (Quebec, Canada). Women were randomized with ratio 1:1:1:1 to one of four study arms (1,000, 2,000, or 3,000 IU/day vitamin D_3 or placebo). The primary outcome was mean change in percent mammographic breast density. Participants and research team were blinded to study arm assignment.

Results: Participants ($n = 405$) were randomized to receive 1,000 ($n = 101$), 2,000 ($n = 104$), or 3,000 IU/day ($n = 101$) vitamin D_3, or a placebo ($n = 99$). The primary analysis included 391 participants (96, 99, 100, and 96, respectively). After the one-year intervention, mean ± SE change in percent breast density in the arms 1,000 IU/day (−5.5% ± 0.5%) and 2,000 IU/day (−5.9% ± 0.5%) vitamin D_3 was similar to that in the placebo arm (−5.7% ± 0.5%) (P values = 1.0). In the 3,000 IU/day vitamin D_3 arm, percent breast density also declined but slightly less (−3.8% ± 0.5%) compared with placebo arm ($P = 0.03$). Adherence to intervention was excellent (92.8%), and reporting of health problems was comparable among study arms ($P \geq 0.95$). All participants had normal serum calcium.

Conclusions: In premenopausal women, one-year supplementation with 1,000, 2,000, or 3,000 IU/day vitamin D_3 resulted in a reduction of percent breast density no greater than that seen with the placebo.

Impact: At doses of 1,000–3,000 IU/day, vitamin D supplementation will not reduce breast cancer risk through changes in breast density. *Cancer Epidemiol Biomarkers Prev; 26(8); 1233–41.* ©2017 AACR.

Introduction

Mammographic breast density reflects the amount of epithelial and stromal tissue in the breast and is one of the strongest breast cancer risk indicators (1–3). The risk of breast cancer has been estimated as 4 to 6 times higher among women with >75% breast density as compared with women with few or no density (1, 4).

Vitamin D intake has been shown to be associated with a reduction in breast density in premenopausal women (5–11) although this association was not observed in other studies (12–14). All available studies in postmenopausal women showed no association between vitamin D intake and breast density (5–18).

The purpose of this trial was to determine whether adding oral supplementation with vitamin D_3 (cholecalciferol) at doses of 1,000, 2,000, and 3,000 IU/day to baseline total vitamin D intake over a period of 12 months reduces mammographic breast density among premenopausal women who reside in the Quebec City area, Canada. Adherence to, and safety of supplementation with tested doses of vitamin D_3 over one year were also assessed.

Methods

Trial design

This is a double-blind, placebo-controlled parallel group trial. Participants were randomized to one of four arms in 1:1:1:1 ratio, to receive either one of three vitamin D_3 doses (1,000, 2,000, or 3,000 IU/day) or a placebo.

Participants

The study took place in Quebec City (Canada), which is located at 46° 48′ N latitude. To be eligible, women were required to have no personal history of breast cancer, no personal history of other cancer within last 5 years (except nonmelanoma skin

[1]Centre de Recherche du CHU de Québec-Université Laval, Québec, Canada. [2]Département de Médecine Sociale et Préventive, Centre de Recherche sur le Cancer, Université Laval, Québec, Canada. [3]Centre des Maladies du sein Deschênes-Fabia, Hôpital du St-Sacrement, Québec, Canada. [4]Département de Médecine Sociale et Préventive, Université de Montréal, Montréal, Québec, Canada. [5]Centre de Recherche du CHU Sainte-Justine, Montréal, Québec, Canada. [6]Département de Mathématiques et de Statistique, Université Laval, Québec, Canada. [7]Departments of Laboratory Medicine and Pathobiology and Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada. [8]Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Clinical trial registration ID: The trial is registered at ClinicalTrials.gov with number NCT01747720.

Corresponding Author: Jacques Brisson, Centre de Recherche du CHU de Québec-Université Laval, Hôpital du Saint-Sacrement, 1050, chemin Sainte-Foy, local J0-01, Québec G1S 4L8, Canada. Phone: 418-682-7390; Fax: 418-682-7949; E-mail: jacques.brisson@fmed.ulaval.ca

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cancer), no prior (or planned in the next year) breast reduction or augmentation, nor be pregnant (or planning a pregnancy in the next year) or lactating. In addition, at the time of the study, women should be premenopausal: they should not have undergone bilateral oophorectomy, should have had at least one natural period in the last three months or be aged < 46 years if non smoker or < 44 years if smoker. Women should have normal serum calcium (2.12–2.60 mmol/L) and creatinine (45–85 μmol/L). They should have percent breast density ≥20% based on a recent bilateral mammography performed in one of the participating radiology clinics. They should agree to limit their mean daily supplemental intakes of vitamin D and calcium to 400 IU and 600 mg, respectively, have no contraindications for use of vitamin D supplementation, and not make regular use of drugs suspected of potential drug–vitamin D interactions: drugs interfering with vitamin D absorption or metabolism (e.g., phenytoin or phenobarbital anticonvulsants, cholestyramine, colestipol, mineral oil, orlistat), and drugs suspected to increase blood levels of calcium (digoxin, thiazide diuretic), or of aluminum or magnesium (antacids containing magnesium or aluminum; ref. 19). The study was approved by the Institutional Research Ethics Committee, and was performed in accordance with the ethical standards laid down in the International Conference on Harmonization (ICH) good clinical practice guideline. All study participants provided written informed consent.

Baseline and follow-up information

Data at baseline included breast cancer risk factors (using self-administered questionnaires), past year diet (using the Canadian Diet History Questionnaire I, C-DHQ1; ref. 20), anthropometric measurements, medication use, and vitamin D and calcium supplement use in the past year during in-person interview.

Follow-up phone calls were made 1, 3, 6, and 9 months after randomization, and a second study center visit was made at the end of the one-year intervention. At each follow-up contact, women were questioned on supplemental vitamin D (outside the intervention) and calcium intakes, sun exposure, health issues, medication use, and adherence. Participants with mean daily supplemental intakes >400 IU vitamin D or >600 mg calcium were reminded to decrease their intakes, or otherwise were asked to discontinue temporarily the intervention. Women reporting use of antacids containing magnesium or aluminum were invited to limit their use and provided with advice on alternatives to these products. If women had to start taking thiazide diuretics or digoxin, they were asked to discontinue temporarily the intervention. At the 12-month follow-up visit, women were asked to return any left-over tablets to the research assistant, where number of missed doses at each week of the one-year intervention was recorded. In addition, weight was measured, and blood specimens were collected. Diet in past year was assessed using C-DHQ1.

Intervention

Placebo and vitamin D_3 (1,000 IU) tablets were provided by the same manufacturer in three batches. At reception, study tablets were packaged in "Dispill blister packs." Each blister pack comprises 28 detachable blisters (corresponding to each day for 4 weeks), and each blister contained 3 tablets to be taken each day. Depending on study arm, the three tablets were: (i) placebo (3 tablets placebo/day), (ii) 1,000 IU vitamin D_3/day (1 tablet vitamin D_3 + 2 tablets placebo), (iii) 2,000 IU vitamin D_3/day (2 tablets vitamin D_3 + 1 tablet placebo), (iv) 3,000 IU vitamin D_3/day (3 tablets vitamin D_3). Blister packs of each study arm were then provided to pharmacists in boxes identified solely with a treatment code (A, B, C, D) by an independent statistician (Thierry Duchesne) who had no clinical involvement in the trial and was the only person to know the correspondence between study arms and treatment codes. Mean content of vitamin D_3 tablets reached 1,000 IU in pretrial and intratrial testing performed by the manufacturer and by an independent laboratory.

Outcomes

Change in percent mammographic breast density. For each woman, primary outcome measure is the change in percent breast density during the one-year intervention (breast density at end of intervention minus that at baseline). Participants were asked to have the two mammograms at the same participating radiology clinic. These clinics used digital computed radiography (CR) and digital direct radiography (DR); they were asked to use the same technology for the two mammograms.

Breast density was measured by the percent of the breast showing fibroglandular density on the mammogram, a quantitative measure ranging from 0% to 100% (21), and was assessed by one trained author without any information on women using a computer-assisted method (22). Breast projection is divided in dense fibroglandular and nondense fatty areas. Percent breast density was calculated as the proportion of the dense area within the total area (dense and nondense area) of the breast projection from one randomly selected craniocaudal view for each woman. Mammograms were read at end of study in batches of 80 images. Each batch included the two craniocaudal images of the same breast (at baseline and at 12 months) of 35 women chosen at random among all participants (70 images) and images of 5 women (10 images) chosen at random within the same batch to assess intrabatch variability. The two images of each woman were paired. Duplicates of images ($n = 10$) chosen at random within each batch were also grouped in batches of 80 images and read to evaluate interbatch variability. The within-batch and between-batch intraclass correlation coefficients were 0.96 and 0.94, respectively.

Intervention adherence. Adherence to intervention was defined as use of 80% or more of study tablets during both the first 6 months and the last 6 months of the intervention based on count of remaining tablets in each blister pack at end of intervention. For instance, a woman who took all tablets during the first 6 months but only 70% of tablets in the remaining months was classified as nonadherent. Furthermore, serum 25(OH)D_3 was measured at baseline and at the end of intervention by an isotope dilution LC/MS-QTOF method after derivatization with DMED-TAD. Intra-assay and interassay coefficients of variation were 4.2% and 7.0%, respectively. The mean bias for 25(OH)D_3 ranged between 2.7% and –3.1% at 55 and 80 nmol/L, respectively, within the limits set by the Vitamin D Standardization Program (23).

Adverse events. Possible adverse events were monitored using a structured questionnaire administered by the research assistant at each follow-up contact. A predefined list of problems or symptoms was drawn: nausea, fatigue, headache, weakness, vomiting, hypertension, decreasing appetite, confusion, arrhythmia, hypercalcemia, and kidney disease. At the 12-month follow-up visit, serum calcium and creatinine were assessed.

Blinded harms-related data collected during the trial were submitted periodically to the Safety Monitoring Committee. There were no *a priori* guidelines for early stopping of study recruitment or intervention, but the Committee was responsible to provide the principal investigator with such recommendation should the health of participants be considered compromised.

Sample size

Primary outcome measure is the mean change in percent breast density during the one-year intervention. For comparison of each of the three supplemental arms to the placebo arm, a false positive error rate of 0.83% (2.5% divided by 3) was used. A sample size of 84 women per arm (total of 336) was needed to detect differences in mean change in breast density of at least 5% with a 1:1:1:1 randomization ratio, with 80% power, assuming a SD of 10%. To compensate for potential withdrawals or loss to follow-up, total target sample size was inflated to no less than 376 women. There was no planned formal interim efficacy analysis.

Randomization

The randomization sequence was created by the primary trial statistician (B. Mâsse) who had no clinical involvement in the trial, using a computerized random number generator. The randomization sequence included a randomization number with a treatment code. Randomization was stratified on the six participating radiology clinics (sites) with random permuted blocks of different sizes.

Allocation of intervention was concealed from the research assistants and from the pharmacists, using two sets of opaque, sealed envelopes. Envelopes for research assistants were numbered sequentially and contained randomization numbers, while envelopes for pharmacists were identified with randomization numbers and contained treatment codes.

Once eligibility of a woman was established, the research assistant opened the envelope identified with the next sequential number and provided the pharmacist with the randomization number of the envelope containing the treatment code. The pharmacist then delivered the appropriate blister packs to the research assistant, who sent them to the participant. The research assistants and the pharmacists collected data on each randomization in their respective envelope tracking record [participant identifiers, randomization number, hour and date of randomization, and the treatment code (pharmacy record only)]. Under the supervision of the independent statistician, the two envelope tracking records were periodically compared to ensure that randomization plan was rigorously followed.

Blinding

During the trial, the participants, the investigator staff involved in the data collection or analysis, the pharmacists, and the outcome adjudicator were all unaware of study arm assignments. Vitamin D_3 tablets and placebo tablets were not distinguishable in size, shape, color, and taste. The blister packs bore no information on study arm (treatment code or dose).

Statistical analysis

The statistical analysis plan was written prior to unblinding the trial.

Intention-to-treat analysis. Primary outcome measure is the change in breast density during the one-year intervention. Change in breast density in women assigned to each of the three study arms with vitamin D_3 was compared with that in women in the placebo arm using ANOVA, taking study sites into account. Confidence intervals and *P* values for the three primary comparisons against the placebo were adjusted using the Bonferroni method. A nominal 5% familywise error rate (FWER) level was used. Dose–response analyses were performed using data from the four study arms; a continuous predictor of dose (0, 1,000, 2,000, or 3,000 IU) was included in a regression model that was adjusted for sites. The adequacy and fit of linear, quadratic, and cubic models was compared to identify the best model for the dose–response relationship. Prespecified subgroups allowed dose–response analyses to be conducted according to baseline characteristics: $25(OH)D_3$ status, body mass index, calcium intake, mammographic density, period of the year at time of mammography and technology of mammography. These were performed using regression models above with interaction terms.

Per-protocol analysis. For this analysis, a randomized woman was excluded if one of the following 3 conditions was met: (i) she did not take $\geq$80% of doses during both first and last 6 months of intervention; (ii) the interval between her 2 mammograms was not within 365 $\pm$ 30 days; or (iii) the technology for the 2 mammograms differed.

Adverse events. Adverse events in the three study arms receiving vitamin D were compared with those in the placebo arm using the Fisher exact test. *P* values were adjusted for multiple comparisons using Benjamini–Hochberg method (1995; refs. 24, 25) to control the false discovery rate (FDR). A nominal 5% FDR level was used.

Results

Recruitment

Participants were recruited from October 31, 2012, to July 8, 2014, and the one-year intervention was completed for all participants by June 2015. Among 942 women who were assessed for eligibility, 405 (43.0%) were randomized (Fig. 1). Women who declined participation and those who were randomized were of similar age (mean age: 42.0 vs. 42.7 years, respectively). Only 14 participants were excluded after randomization. Reasons for withdrawals were not related to allocated treatment or harms. A total of 391 participants are thus included in intention-to-treat analysis.

Baseline characteristics and exposures during follow-up

Characteristics of participants at baseline (Table 1) and during follow-up (Supplementary Table S1) showed no substantial imbalance between study arms. During follow-up, changes in hormonal exposures (e.g., menopause, initiation or discontinuation of sex steroid hormone use) were infrequent. One woman had to discontinue the intervention from the moment she decided to use a large dose of vitamin D daily, and another had to discontinue temporarily the intervention for the time she had to use thiazide diuretics.

Description of intervention and breast imaging

Mean interval between the mammogram at entry and the one at the end of intervention was similar among study arm (mean: 374 days; Table 2). The target interval was set in the protocol at 365 $\pm$ 30 days; all but 22 women were within this interval. The two

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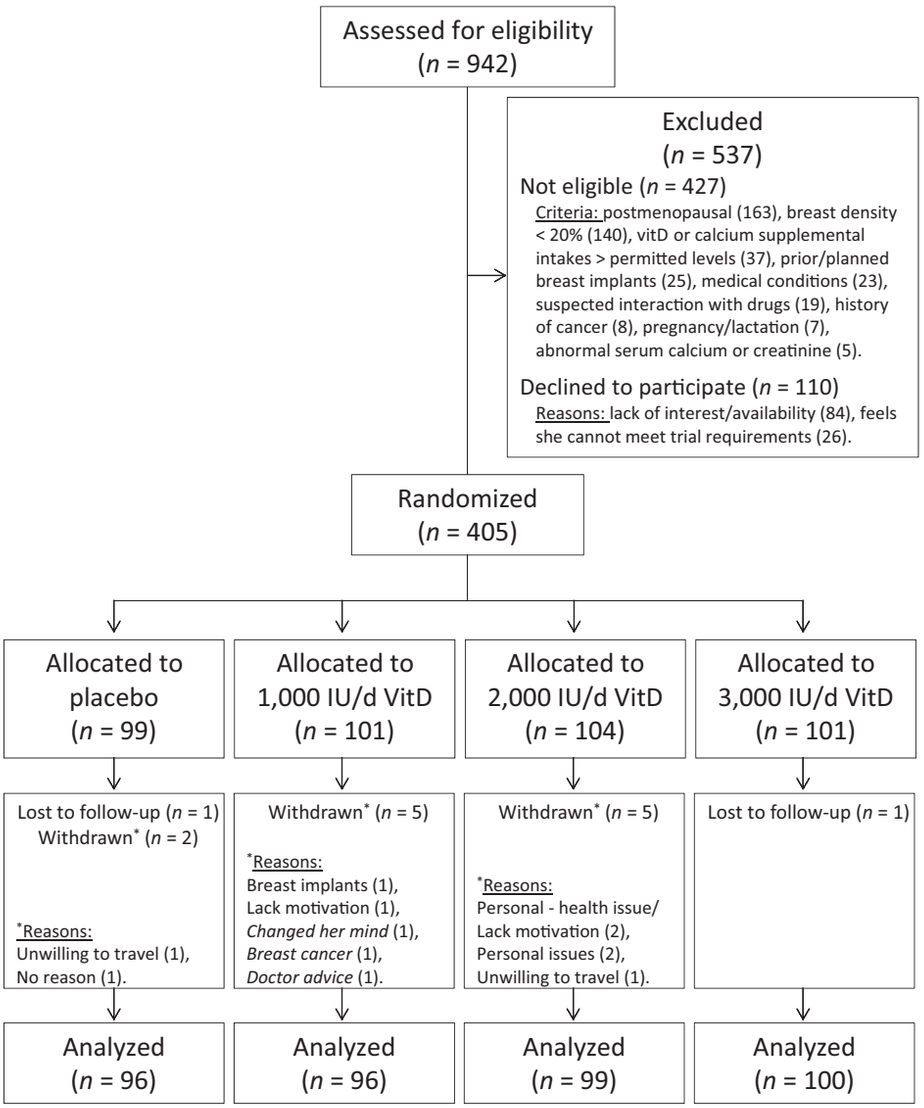

Figure 1.
Flow diagram.

All participants received the intervention as allocated.
Reasons for withdrawals in italics retale to early withdrawals (≤1 month); others relate to late withdrawals (≥6 months).

mammograms were performed with the same technology in all except 7 women. Adherence was 92.8% overall, and was quite similar among study arms, ranging between 90.6% and 96.0%. At the end of the trial, serum 25(OH)D_3 increased linearly with dose of vitamin D_3 with a mean increase of 22.8 nmol/L for increments of 1,000 IU/day vitamin D_3 (Supplementary Table S2); this dose–response relationship was of the same magnitude among subgroups based on baseline serum 25(OH)D_3, body mass index or month of blood sampling (Supplementary Table S3).

Intention-to-treat analysis

Mean changes in breast density after the one-year intervention ranged between –3.8% and –5.5% among study arms (Table 3). Decline in breast density observed in the arms receiving 1,000 or 2,000 IU/day Vitamin D_3 was similar to that in the placebo arm ($P = 1.0$), but the decline in breast density was less marked in the arm receiving 3,000 IU/day vitamin D_3 than that in the placebo arm ($P = 0.03$). In the dose–response analysis, the quadratic term was low but did not quite reach statistical significance ($P = 0.098$). The linear dose–response showed a significant trend ($P_{trend} = 0.02$) of decreasing change in breast density with increasing dose of vitamin D_3. In subgroup analyses, none of studied baseline characteristics (serum 25(OH)D_3, body mass index, calcium intake, percent breast density, timing or technology of mammography) modified the linear dose–response association between vitamin D_3 supplementation and change in percent breast density; all $P_{interaction} \geq 0.10$ (Table 4).

Per-protocol analysis

Among the 391 participants, 53 (between 10 and 16 participants in each study arm) did not meet inclusion criteria for per protocol analysis ($n = 338$). This analysis showed results similar

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Table 1. Characteristics of the 391 participants at baseline by study arm

		Vitamin D_3		
Characteristics of participants	**Placebo ($n = 96$)**	**1,000 IU/day ($n = 96$)**	**2,000 IU/day ($n = 99$)**	**3,000 IU/day ($n = 100$)**
Age (y) (mean ± SD)	42.9 ± 6.2	42.8 ± 5.2	42.7 ± 5.2	42.7 ± 5.5
White race (%)	95.8	99.0	100.0	97.0
Body mass index (kg/m^2) (mean ± SD)	24.4 ± 4.1	24.7 ± 5.1	24.3 ± 4.1	24.0 ± 3.6
Number of full-term pregnancies (mean ± SD)	1.4 ± 1.1	1.7 ± 1.0	1.7 ± 1.2	1.7 ± 1.0
Hormonal contraceptive ever use (%)	91.7	91.7	95.0	93.0
Natural menstrual cycles (%)	64.6	74.0	68.7	75.0
Personal history of breast biopsy (%)	9.4	5.2	9.1	8.0
Dietary vitamin D intake[a] (IU/d) (mean ± SD)	200 ± 169	188 ± 113	177 ± 98	179 ± 113
Use of supplements with vitamin D[b] (%)	33.3	40.6	38.4	43.0
Vitamin D from supplements (IU/d) (mean ± SD)	406 ± 587	302 ± 318	444 ± 433	425 ± 424
Total vitamin D intake[a] (IU/d) (mean ± SD)	337 ± 417	306 ± 260	354 ± 349	366 ± 373
Dietary calcium intake[a] (mg/d) (mean ± SD)	1,024 ± 603	1,028 ± 479	969 ± 389	997 ± 507
Use of supplements with calcium[b] (%)	34.4	39.6	41.4	38.0
Calcium from supplements (mg/d) (mean ± SD)	86 ± 141	116 ± 211	137 ± 200	163 ± 256
Total calcium intake[a] (mg/d) (mean ± SD)	1,054 ± 603	1,075 ± 484	1,027 ± 397	1,061 ± 515
Food energy intake[a] (kcal/d) (mean ± SD)	1,746 ± 664	1,693 ± 506	1,714 ± 584	1,764 ± 639
Minutes/week outdoors[c] (mean ± SD)	470 ± 362	366 ± 237	403 ± 287	417 ± 270
Season at time of baseline mammography (%)				
Winter	31.3	25.0	28.3	27.0
Spring	31.3	37.5	35.4	38.0
Summer	13.5	11.5	12.1	13.0
Fall	24.0	26.0	24.2	22.0
Serum 25(OH)D_3 (nmol/L) (mean ± SD)	64.4 ± 23.1	66.3 ± 26.8	65.5 ± 24.9	60.4 ± 20.6
Screening mammography[d] (%)	90.6	89.6	85.9	87.0
Digital direct radiography[e] (%)	44.8	47.9	50.5	48.0
Radiology clinics[f] (%)				
Site 1	56.3	54.2	52.5	56.0
Site 2	12.5	12.5	12.1	12.0
Site 3	10.4	9.4	10.1	10.0
Site 4	6.3	7.3	6.1	6.0
Site 5	3.1	3.1	4.0	2.0
Site 6	11.5	13.5	15.2	14.0

[a]In the last year, among the 380 women who completed the food frequency questionnaire at baseline (placebo, $n = 95$; 1,000 IU/day, $n = 93$; 2,000 IU/day, $n = 95$; 3,000 IU/day, $n = 97$).
[b]In the last year, based on face-to-face interview at recruitment. Mean supplemental intake is provided among those having supplemental intake.
[c]Between 10 am and 4 pm, in the last year.
[d]Versus mammography for symptoms.
[e]Versus digital computed radiography.
[f]Radiology clinics (sites) where mammograms were done correspond to randomization strata.

to the main analysis (Supplementary Tables S4–S6). Trend of change in density with vitamin D_3 dose was more pronounced when based on mammographic images from CR as compared with DR technology ($P_{\text{interaction}} = 0.02$; Supplementary Table S6). Results of per protocol analysis were unchanged after adjusting for age, body mass index, parity, history of breast biopsy, hormonal contraceptive use, and natural menstrual cycles, the *a priori* list of potential confounders.

Analyses added after unblinding the trial

Changes in dense (fibroglandular) and nondense (fatty) areas of breast tissue on mammogram were used as alternative outcome measures of change in breast density. There was a statistically significant trend of decreasing change in dense area of breast tissue with increasing dose of vitamin D_3 ($P_{\text{trend}} = 0.03$), while vitamin D_3 had no effect on change in nondense area of breast tissue ($P_{\text{trend}} = 0.70$; Supplementary Table S7).

Table 2. Description of the intervention among the 391 participants by study arm

		Vitamin D_3		
Description of intervention	**Placebo ($n = 96$)**	**1,000 IU/day ($n = 96$)**	**2,000 IU/day ($n = 99$)**	**3,000 IU/day ($n = 100$)**
Interval between mammograms[a] (days) (mean ± SD)	376 ± 12	374 ± 12	374 ± 10	374 ± 11
Concordant types[b] of mammograms[a] (%)	99.0	100.0	97.0	97.0
Actual duration of intervention[c] (days) (mean ± SD)	351 ± 19	346 ± 20	348 ± 17	350 ± 17
Adherence to intervention[d] (%)	90.6	92.7	96.0	92.0
Serum 25(OH)D_3[e] (nmol/L) (mean ± SD)	62.9 ± 20.5	92.2 ± 28.1	109.7 ± 26.6	128.9 ± 37.4

[a]The first mammogram at baseline and the second mammogram at the end of the one-year intervention.
[b]Digital direct radiography or digital computed radiography.
[c]From the first Monday after randomization until the day of the mammography at the end of intervention. The actual duration of the intervention ranged between 301 and 406 days.
[d]Use of ≥80% of doses during both first and last 6 months of intervention (%).
[e]Serum 25(OH)D_3 assessed at the end of the one-year intervention.

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Table 3. Comparisons of change in percent mammographic breast density after the one-year intervention between arms receiving vitamin D_3 with placebo

		Vitamin D_3			Linear trend in change in percent density[d]	
Comparisons of percent density	**Placebo ($n = 96$)**	**1,000 IU/day ($n = 96$)**	**2,000 IU/day ($n = 99$)**	**3,000 IU/day ($n = 100$)**	**ß (95% CI)**	***P***
Percent density at baseline (mean ± SD)	40.0 ± 16.5	38.2 ± 14.9	37.3 ± 15.1	38.5 ± 16.6		
Percent density at end of intervention (mean ± SD)[a]	34.2 ± 14.9	32.8 ± 14.2	31.5 ± 14.2	34.7 ± 15.7		
Change in percent density[b] (mean ± SE)	−5.7 ± 0.5	−5.5 ± 0.5	−5.9 ± 0.5	−3.8 ± 0.5		
Difference from placebo[c]	0.0	0.3	−0.1	1.9	0.53 (0.07–0.99)	0.02
(95% CI)[c]		(−1.5–2.1)	(−1.9–1.7)	(0.1–3.7)		
P[c]		1.0	1.0	0.03		

[a]The Pearson correlation coefficient between percent mammographic density at baseline and at the end of the one-year intervention in each of the four study arms ranges between 0.94 and 0.95.
[b]Mean ± SE changes between percent mammographic breast density at the end of the one-year intervention and that at baseline are estimated from ANOVA and adjusted for sites.
[c]Differences in the mean of change in percent mammographic breast density comparing study arms receiving vitamin D_3 with placebo, 95% confidence intervals and *P* values, are estimated from ANOVA and adjusted for sites; 95% confidence intervals and *P* values are adjusted for multiple comparisons (Bonferroni correction).
[d]ß (95% confidence interval) and *P* value for linear trend are estimated from regression models and adjusted for sites. Study arm is treated as a continuous variable, and ß represents mean difference in change in percent mammographic breast density for increments of 1,000 IU/day of vitamin D_3.

Adverse events

Health problems or symptoms were reported by similar proportions of participants in the four study arms (all *P* values ≥0.95; Table 5). At the end of intervention, all participants had normal serum calcium (<2.60 mmol/L).

On two occasions (February and November 2014), the Safety Monitoring Committee had access to blinded data on adverse events based on pooled data. The committee expressed no need to have data by study arm or unblinded, to stop recruitment or intervention, or to modify the study protocol.

Discussion

In the present randomized placebo-controlled trial among premenopausal women living at northern latitudes (46° 48′ N), one-year supplementation with vitamin D_3 at doses of 1,000, 2,000, and 3,000 IU/day resulted in no greater reductions of percent breast density than that seen with the placebo. Actually, supplementation with 3,000 IU/day vitamin D_3 was associated with a slightly smaller decline in breast density compared with placebo. At doses of vitamin D_3 supplementation tested, no harms were detected and serum calcium remained normal.

A U-shaped dose response has been proposed for the relation of nutrients to risk (26) and this type of dose response may apply to the relation of vitamin D_3 to breast density at least among premenopausal women. First, in the majority of observational studies (5–11), increases in vitamin D intakes were associated with lower breast density among premenopausal women (5–14). This trial does not contradict those results because, in observational studies, the range of vitamin D intakes observed was much

Table 4. Comparison of trends in mean change in percent mammographic breast density after the one-year intervention among arms receiving vitamin D_3 or placebo, according to baseline characteristics

		Placebo	Vitamin D_3 1,000 IU/day		Vitamin D_3 2,000 IU/day		Vitamin D_3 3,000 IU/day		Linear trend in change in percent density[b]		Interaction[b]
Baseline characteristics	***N***	**Change in density[a]**	***N***	**Change in density[a]**	***N***	**Change in density[a]**	***N***	**Change in density[a]**	**ß (95% CI)**	***P***	***P***
Serum 25(OH)D_3[c]											
<50 nmol/L	19	−5.4 ± 1.2	24	−5.6 ± 1.1	27	−4.9 ± 1.0	32	−4.0 ± 0.9	0.53 (−0.40–1.46)	0.27	0.99
≥50 nmol/L	77	−5.8 ± 0.6	72	−5.4 ± 0.6	72	−6.2 ± 0.6	68	−3.8 ± 0.6	0.52 (−0.02–1.06)	0.06	
Body mass index											
<25 kg/m^2	62	−6.5 ± 0.7	59	−6.0 ± 0.7	63	−6.0 ± 0.6	73	−4.6 ± 0.6	0.59 (0.03–1.14)	0.04	0.95
≥25 kg/m^2	34	−4.3 ± 0.9	37	−4.5 ± 0.8	36	−5.6 ± 0.9	27	−1.7 ± 1.0	0.55 (−0.26–1.37)	0.18	
Total calcium intake[d]											
<1,000 mg/day	57	−5.8 ± 0.7	44	−5.4 ± 0.8	48	−6.0 ± 0.8	50	−3.8 ± 0.7	0.55 (−0.08–1.19)	0.09	0.92
≥1,000 mg/day	38	−5.8 ± 0.8	49	−5.6 ± 0.7	47	−5.9 ± 0.8	47	−3.8 ± 0.8	0.60 (−0.10–1.30)	0.09	
Percent breast density											
<40%	55	−3.9 ± 0.7	52	−4.0 ± 0.7	64	−5.2 ± 0.6	61	−2.6 ± 0.6	0.29 (−0.29–0.87)	0.32	0.36
≥40%	41	−8.3 ± 0.8	44	−7.1 ± 0.7	35	−7.0 ± 0.8	39	−5.9 ± 0.8	0.72 (0.02–1.42)	0.04	
Month of mammography											
October–February	40	−4.7 ± 0.8	40	−4.8 ± 0.8	37	−5.6 ± 0.9	39	−3.2 ± 0.8	0.34 (−0.38–1.07)	0.35	0.48
March–September	56	−6.5 ± 0.7	56	−5.9 ± 0.7	62	−6.0 ± 0.7	61	−4.2 ± 0.7	0.68 (0.08–1.28)	0.03	
Type of mammography											
CR	53	−6.4 ± 0.7	50	−5.3 ± 0.8	49	−5.4 ± 0.8	52	−3.4 ± 0.8	0.90 (0.27–1.53)	0.006	0.10
DR	43	−4.9 ± 0.8	46	−5.6 ± 0.8	50	−6.3 ± 0.8	48	−4.3 ± 0.8	0.11 (−0.56–0.79)	0.74	

[a]Mean ± SE of the difference between percent mammographic breast density at the end of the one-year intervention and that at baseline.
[b]ß (95% confidence intervals) and *P* values for trend in mean change in mammographic breast density, and $P_{interaction}$, are estimated from regression models. Study arm is treated as a continuous variable, and ß represents mean difference in change in percent mammographic breast density for increments of 1,000 IU/day vitamin D_3. Models are adjusted for sites.
[c]Median serum 25(OH)D_3 was 39 nmol/L in the subgroup with baseline 25(OH)D_3 <50 nmol/L, and 69 nmol/L in that with baseline 25(OH)D_3 ≥ 50 nmol/L.
[d]Among women who completed the C-DHQ1 at baseline ($n = 380$).

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Table 5. Adverse events reported during the one-year intervention by study arm

		Vitamin D_3					
Adverse events	**Placebo ($n = 96$)**	**1,000 IU/day ($n = 96$)**	**P^a**	**2,000 IU/day ($n = 99$)**	**P^a**	**3,000 IU/day ($n = 100$)**	**P^a**
Symptoms[b] (%)							
Nausea	38.5	44.8	1.00	37.4	1.00	31.0	1.00
Fatigue	75.0	79.2	1.00	74.8	1.00	79.0	1.00
Headache	62.5	65.6	1.00	65.7	1.00	69.0	1.00
Weakness	15.6	13.5	1.00	10.1	1.00	22.0	1.00
Vomiting	13.5	10.4	1.00	16.2	1.00	13.0	1.00
Hypertension	1.0	5.2	1.00	4.0	1.00	7.0	0.95
Decreasing appetite	33.3	21.9	0.95	20.2	0.95	22.0	0.95
Confusion	2.1	3.1	1.00	4.0	1.00	5.0	1.00
Arrhythmia	10.4	16.7	1.00	14.1	1.00	14.0	1.00
Kidney disease	0.0	0.0	1.00	0.0	1.00	0.0	1.00
Abnormal serum calcium[c]	0.0	0.0	1.00	0.0	1.00	0.0	1.00
Abnormal serum creatinine[c]	0.0	2.1	1.00	0.0	1.00	0.0	1.00

[a]Arms receiving vitamin D_3 are compared with the placebo arm; adjusted *P* values for multiple comparisons using the Benjamini–Hochberg method.
[b]The structured questionnaire administered at the five planned follow-up contacts included questions on each of these symptoms or problems, which represent main known or suspected symptoms of hypercalcemia. The table presents the frequency with which these symptoms or problems have been reported at least once during the one-year intervention.
[c]Based on blood specimen collected at the 12-month follow-up visit. Two participants had serum creatinine >85 μmol/L (86 and 94 μmol/L, respectively).

lower than in the current trial. In observational studies, mean or median dietary (5, 6, 8, 9, 11, 12) or total (8, 10, 11, 13, 14) vitamin D intakes were <350 IU/day in all but two studies [826 UI/day (9) and 569 IU/day (11)]. It is possible that, below intakes of 1,000 IU/day, increases in vitamin D intake are associated with less breast density.

Second, this trial suggests that, compared with placebo, further reductions in breast density are not seen with 1,000 to 2,000 IU/day supplementation. However, supplementation with 3,000 IU/day slightly slowed the natural decrease in breast density. Thus, our data suggest that, after one year of supplementation with 3,000 IU/day, women would tend to have slightly more breast density (1.9%) than they would have, had they taken a placebo. Such a difference in breast density is small and below the 5% difference that was determined *a priori* to be of clinical significance. This type of dose response was seen overall and in all subgroups examined although the *P* value for the quadratic term of the dose–response relation did not reach formal statistical significance ($P = 0.098$).

Thus, taken together, observational studies and this trial support the idea that the relation of total vitamin D_3 intake (dietary plus supplemental intakes) to breast density, at least among premenopausal women, may be U-shaped. In the lower range of total vitamin D_3 intakes (less than about 1,000 IU/day), increase in intake may be associated with some reduction of breast density as seen in observational studies. In the intermediate range of total intake (about 1,000–3,000 IU/day), increase in intake may have little or no effect on breast density as seen in this trial. Finally, also shown in this trial, in the upper range of total intake (around 3,000–4,000 IU/day), increase in intake may lead to a small increase in breast density. Currently, there are no data on the relation of vitamin D_3 and breast density at intakes above 4,000 IU/day.

Mammographic breast density is one of the strongest breast cancer risk indicators. As for breast density, observational studies have seen reduced risk of breast cancer with increased vitamin D exposure [increased sun exposure (27–29), vitamin D intake (30, 31), or status (31, 32)]. Several mechanisms have been proposed by which vitamin D could plausibly impair cancer development and growth (33). However, a recent meta-analysis of prospective studies suggested a possible nonlinear relationship between vitamin D and breast cancer risk, at least in postmenopausal women (34). According to this meta-analysis, studies of plasma 25(OH)D_3 and breast cancer risk showed no dose–response relationship in lowest range of 25(OH)D_3 levels (<67 nmol/L), an inverse association for plasma 25(OH)D_3 between 67 nmol/L and 87 nmol/L, but a flattening of the dose–response curve above 87 nmol/L; this meta-analysis did not provide information for doses over 100 nmol/L. In the current trial, attained mean serum 25(OH)D_3 after one-year supplementation with 1,000 or 2,000 IU/day vitamin D_3 were 92 nmol/L and 110 nmol/L, respectively, and these doses had no effect on breast density. However, attained mean serum 25(OH)D_3 after supplementation with 3,000 IU/day was still higher (129 nmol/L), and this dose was associated with slightly slower decline in breast density. Thus, our data raise the possibility that sustained long-term supplementation with doses ≥3,000 IU/day vitamin D_3 eventually could result in some increase in breast cancer risk (35). Such a U-shaped relationship has also been suggested between vitamin D status and several other health outcomes, including all-cause and cancer mortality (36).

During this one-year trial, there was a mean decrease of 5.7% in breast density in the placebo arm (Table 3). Although a decrease in breast density is expected with advancing age due to lobular involution of the breast gland (37), this change appears larger than that seen among controls in some previous trials on breast density in premenopausal women (38). However, most of these trials used film mammograms instead of digital mammography. Expected yearly decline with digital mammography is unclear (35, 39). Decline may have been amplified by factors specific to digital mammography such as evolution in image-processing algorithms (40). In addition, assessment of change in breast density was based on the comparison by one reviewer of paired mammograms knowing the time sequence of the mammograms, but unaware of the group status. Thus, yearly decrease in breast density in each study arm may have been overestimated by the reviewer who, *a priori*, knew that breast density is expected to decrease over time. Finally, given the high correlations (≥0.94) between breast density at baseline and that at the end of intervention in all four study arms, regression to the mean is unlikely to

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explain the observed decrease in breast density during the one-year intervention.

The trend between vitamin D_3 supplementation and breast density was stronger with CR than with DR technology. This interaction was not statistically significant in the intention-to-treat analysis but was statistically significant in the per protocol analysis (Table 4; Supplementary Table S6). The CR technology detected 5.6% more breast density than DR technology at baseline (Supplementary Table S8). The CR technology allowed the detection of well-established associations of age, body mass index, and number of full-term pregnancies with breast density; with DR technology, body mass index was the only factor among these to be related to breast density. This raises the possibility that DR technology may have some limitations to study determinants of breast density compared with CR technology, perhaps due to differences in digital image processing.

This randomized trial has strengths and limitations. Three doses of vitamin D supplementation were tested simultaneously within the same population. Doses tested cover the entire range of possible supplementation doses given the 4,000 IU/day "Tolerable Upper Intake Level" specified by the Institute of Medicine (41) for the general population. The one-year duration of the intervention provided sufficient time for the effect of supplementation on breast density to become apparent and provided the necessary control for seasonal variations in serum 25(OH)D_3 and in breast density (17, 42). Allocation concealment was successfully implemented. Blinding of participants and of research team was strictly maintained. Only 14 participants were excluded after randomization, and reasons for withdrawals were unrelated to the intervention. Baseline characteristics and factors contributing to change in vitamin D status or in breast density during the trial (e.g., sun exposure, vitamin D intake, hormonal exposure, change in weight; ref. 43) were well balanced between study arms. Adherence was excellent. Multiple comparisons were taken into consideration. However, while power was adequate for the main analysis (comparison of change in breast density between study arms), sample size calculations did not focus on distinguishing quadratic from linear dose–response or detection of interaction in subgroup analysis and, for these analyses, power was more limited.

Conclusion

In premenopausal women, the reduction seen in breast density after one-year supplementation with 1,000, 2,000, or 3,000 IU/day vitamin D_3 was not greater than that seen in the placebo arm. Thus, at these doses, vitamin D supplementation could not reduce breast cancer risk through reductions in breast density. The trial also suggests caution with respect to sustained long-term use of vitamin D_3 supplementation at dose of 3,000 IU/day, as such a dose appears to slightly delay the decline in breast density that is expected with advancing age.

Disclosure of Potential Conflicts of Interest

M.J. Yaffe has ownership interest in Volpara Solutions (manufacturer of automated density measurement software). No potential conflicts of interest were disclosed by the other authors.

Disclaimer

Volpara Solutions played no role in this work and its software was not used in this study. The Quebec Breast Cancer Foundation had no role in the design, analysis, or writing of this article.

Authors' Contributions

Conception and design: J. Brisson, S. Bérubé, C. Diorio, B. Mâsse, J. Lemieux, T. Duchesne, R. Vieth, M.J. Yaffe, J. Chiquette
Development of methodology: J. Brisson, S. Bérubé, C. Diorio, B. Mâsse, J. Lemieux, T. Duchesne, E. Delvin, R. Vieth, M.J. Yaffe, J. Chiquette
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Brisson, S. Bérubé, C. Diorio, J. Chiquette
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Brisson, S. Bérubé, C. Diorio, B. Mâsse, J. Lemieux, T. Duchesne, R. Vieth, M.J. Yaffe, J. Chiquette
Writing, review, and/or revision of the manuscript: J. Brisson, S. Bérubé, C. Diorio, B. Mâsse, J. Lemieux, T. Duchesne, E. Delvin, R. Vieth, M.J. Yaffe, J. Chiquette
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Brisson, S. Bérubé, C. Diorio, T. Duchesne
Study supervision: J. Brisson, S. Bérubé, J. Lemieux
Other (biostatistician for this trial): B. Mâsse

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