Research Article

Cancer Prevention Research

A Randomized Controlled Trial of Green Tea Extract Supplementation and Mammographic Density in Postmenopausal Women at Increased Risk of Breast Cancer

Hamed Samavat[1,2,3], Giske Ursin[4,5,6], Tim H. Emory[7], Eunjung Lee[6], Renwei Wang[2], Carolyn J. Torkelson[8], Allison M. Dostal[9], Karen Swenson[10], Chap T. Le[11,12], Chung S. Yang[13], Mimi C. Yu[14], Douglas Yee[12,15,16], Anna H. Wu[6], Jian-Min Yuan[2,3], and Mindy S. Kurzer[1]

Abstract

Epidemiologic and animal studies suggest a protective role of green tea against breast cancer. However, the underlying mechanism is not understood. We conducted a randomized, double-blinded, placebo-controlled phase II clinical trial to investigate whether supplementation with green tea extract (GTE) modifies mammographic density (MD), as a potential mechanism, involving 1,075 healthy postmenopausal women. Women assigned to the treatment arm consumed daily 4 decaffeinated GTE capsules containing 1,315 mg total catechins, including 843 mg epigallocatechin-3-gallate (EGCG) for 12 months. A computer-assisted method (Madena) was used to assess MD in digital mammograms at baseline and month 12 time points in 932 completers (462 in GTE and 470 in placebo). GTE supplementation for 12 months did not significantly change percent MD (PMD) or absolute MD in all women. In younger women (50–55 years), GTE supplementation significantly reduced PMD by 4.40% as compared with the placebo with a 1.02% PMD increase from pre- to postintervention ($P = 0.05$), but had no effect in older women ($P_{interaction} = 0.07$). GTE supplementation did not induce MD change in other subgroups of women stratified by catechol-*O*-methyltransferase genotype or level of body mass index. In conclusion, 1-year supplementation with a high dose of EGCG did not have a significant effect on MD measures in all women, but reduced PMD in younger women, an age-dependent effect similar to those of tamoxifen. Further investigation of the potential chemopreventive effect of green tea intake on breast cancer risk in younger women is warranted. *Cancer Prev Res; 10(12); 710–8. ©2017 AACR.*

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death among women worldwide (1). Women born in the United States have a 12.4% lifetime risk of developing breast cancer (2). Asian women have historically had lower breast cancer incidence than their counterparts in Western countries (3). The most recent data showed that women in East Asia had only one fourth the incidence rate of women in Western Europe (4). This large disparity in incidence rate has been partially attributed to different lifestyle and environment factors, which are potentially modifiable.

Breast tissue composition is comprised of epithelial and stromal tissues (also known as fibroglandular tissue) that appear white on a mammogram, and fat, which looks translucent or darker on the mammogram. Mammographic density (MD) is the relative amount of fibroglandular tissue to fat. The measure is often reported as percent MD (PMD) that is a proportion of the dense tissue area over the total breast area. MD is a well-established predictor of breast cancer risk (5–7), and previous studies have demonstrated that women with ≥75% MD have more than 4 to 6 times risk of breast cancer than women with less than <10% MD (8–10). The mechanisms linking high MD to high risk of breast cancer are unclear. There are several proposed hypotheses. For example, elevated cellular proliferation through lifetime exposure to sex steroids has been suggested as a potential underlying mechanism (11, 12).

[1]Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota. [2]Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania. [3]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania. [4]Cancer Registry of Norway, Oslo, Norway. [5]Department of Nutrition, University of Oslo, Oslo, Norway. [6]Department of Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, California. [7]Department of Radiology, University of Minnesota Medical School, Minneapolis, Minnesota. [8]Department of Family Medicine and Community Health, University of Minnesota Medical School, Minneapolis, Minnesota. [9]Department of Medicine, Division of Gastroenterology, Hepatology, & Nutrition, University of Minnesota, Minneapolis, Minnesota. [10]Virginia Piper Cancer Institute, Allina Health System, Minneapolis, Minnesota. [11]Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minnesota. [12]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota. [13]Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, New Jersey. [14]Department of Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, California. [15]Department of Medicine, University of Minnesota, Minneapolis, Minnesota. [16]Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota.

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Corresponding Author: Mindy S. Kurzer, Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St Paul, MN 55108. Phone: 612-624-9789; Fax: 612-625-5272; E-mail: mkurzer@umn.edu

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Intervention studies have revealed that postmenopausal hormone use for 12 months increases PMD by 4.7% (13), and in contrast, 18 months intake of tamoxifen, a first-line antiestrogen drug, results in a 4.4% reduction in PMD compared with placebo (14). Given each 1% increase in PMD has been associated with 2% higher risk of breast cancer (5), the above-mentioned changes in PMD caused by the postmenopausal hormone use or tamoxifen therapy can be potentially translated into influencing the risk of breast cancer by nearly 10%.

Green tea, a product of dried tea leaves of *Camellia sinensis*, is one of the dietary agents with potential anticarcinogenic properties. Preclinical studies have shown that green tea or its constituents, particularly, epigallocatechin-3-gallate (EGCG) as the most abundant and biologically active tea catechins (15), suppress mammary tumorigenesis via different molecular pathways (16). Epidemiologic studies have also reported a protective effect of green tea intake against development of breast cancer, although not consistently. In a meta-analysis by Wu and Butler, consumption of green tea was related to 30% [95% confidence interval (CI), 0.61–0.79] lower risk of breast cancer for regular green tea drinkers versus non–green tea drinkers in three case–control studies. However, no association with breast cancer risk was observed for green tea consumption in a meta-analysis of five prospective cohort studies following adjustment for multiple potential confounders (17). The precise mechanisms by which green tea intake may prevent breast cancer are poorly understood, but the primary proposed hypotheses include effects on antioxidant activity (18), sex hormones (19, 20), and MD (21).

Genetic polymorphisms in the catechol-O-methyl transferase (*COMT*) gene have been linked to different responses to green tea intake in relation to breast cancer risk (22). In a case–control study of Asian-American women, green tea drinkers with at least one low-activity *COMT* allele demonstrated significantly lower risk of breast cancer compared with non-tea drinkers, whereas similar results were not observed for those with high-activity *COMT* allele (22). The observed lower risk of breast cancer has been attributed to the lower COMT enzymatic activity due to the transitional mutation of valine108 (cytosolic COMT)/158 (membrane-bound COMT) to methionine (23, 24), which may consequently influence the rate of green tea catechin metabolism. Whether the effect of green tea on breast cancer risk biomarkers including MD is modified by *COMT* genotype has not yet been explored in a human intervention trial.

There is currently very limited evidence for the impact of green tea intake on MD. In a cross-sectional analysis of 3,315 Singapore Chinese women (>90% postmenopausal women; ref. 21), daily green tea drinkers had a statistically significant 2.2% lower PMD than non–green tea drinkers after adjustment for potential confounders ($P = 0.002$). Green tea intake has also been shown to lower estrogens in blood (20), which provides a biological rationale for the study of green tea effect on PMD in this study.

The primary aim of this randomized controlled trial (RCT) was to evaluate the effects of daily consumption of green tea extract (GTE) containing 800 mg EGCG for 12 months on changes in MD measures in healthy postmenopausal women at high risk of breast cancer due to dense breast tissue. We hypothesized that 1-year supplementation with GTE will result in lower PMD and absolute density in a direction accordant with lower risk of breast cancer.

Materials and Methods

Study design

The study design of the Minnesota Green Tea Trial (MGTT) has been described in detail elsewhere (25). Briefly, the MGTT was a phase II, randomized, double-blind, placebo-controlled trial aimed to investigate the efficacy of GTE on biomarkers of breast cancer risk in high-risk (as a result of dense breasts) women with differing *COMT* genotypes. Using a permuted block method, participants were randomly assigned to either GTE or placebo group and were further stratified by *COMT* genotype (low activity: A/A or G/A; high activity: G/G) into four groups: GTE-high *COMT*, placebo-high *COMT*, GTE-low *COMT*, and placebo-low *COMT* genotype activities. Both participants and the study investigators were blinded to the treatment allocation arm. The study conformed to the guidelines explained in the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Boards of the University of Minnesota (Minneapolis, MN), Park Nicollet Institute (Minneapolis, MN), the University of Southern California (Los Angeles, CA), and the University of Pittsburgh (Pittsburgh, PA). Written informed consent was obtained from all participants.

Participant population

The participant population and recruitment has been described in detail previously (26). Participants were recruited from 8 clinical centers in the Minneapolis-St. Paul metropolitan area based on their annual screening mammograms. Eligible participants were required to be healthy postmenopausal women aged 50 to 70 years old and at high risk of breast cancer due to having "heterogeneously dense" or "extremely dense" breast tissues (defined as having >50% fibroglandular tissue) by visual inspection according to the American College of Radiology's Breast Imaging Reporting and Data System (BI-RADS) breast density assessment criteria (26). Women were excluded if they were current smokers, drinking more than one cup of green tea or 7 alcoholic drinks per week, body mass index (BMI, kg/m^2) >40.0 or <18.5, or with compromised liver function (hepatitis B or C viral infection or elevated liver enzyme levels at baseline). Participants were also deemed ineligible if they had ever had breast cancer or any other cancer in the past 5 years, and if they were current or prior (within last six months) users of menopausal hormone therapy, selective estrogen receptor modulators, aromatase inhibitors, methotrexate, or etanercept. Women were additionally excluded if they had experienced a weight change >10 pounds in the last year or if they were planning to participate in any weight loss or gain program during the course of trial. Potential participants were first identified by age and MD criteria from screening mammograms. Next, eligible women received a letter in the mail explaining the study purpose and basics. If interested, participants then attended an orientation session in which the study requirements and specifications were thoroughly described and they had a chance to sign the consent form and schedule their screening clinic visit.

Study procedures and data collection

Full descriptions of all samples and data collection have been described before (25). At the screening visit, nonfasting blood was drawn for *COMT* genotyping, and vital signs and anthropometric measurements were conducted. Final eligibility was determined on the basis of the screening visit results, after which women were randomized into the study.

Enrollment took place between August 2009 and April 2013. All women completed a comprehensive health history questionnaire at the baseline visit by which they provided information on medical and reproductive history, medication and supplement use, demographics, and lifestyle factors. At the beginning and end of intervention, each participant also completed the Dietary History Questionnaire, a validated food frequently questionnaire developed by the NCI (Rockville, MD; ref. 27), to document her daily dietary intake over the past year. This questionnaire contained 124 food items and incorporated the portion size of foods and dietary supplement use into the final analysis using Diet*Calc software. Women were also asked to maintain their routine dietary intake and physical activity level throughout the trial. Hepatic function and potential adverse events (AE) were also

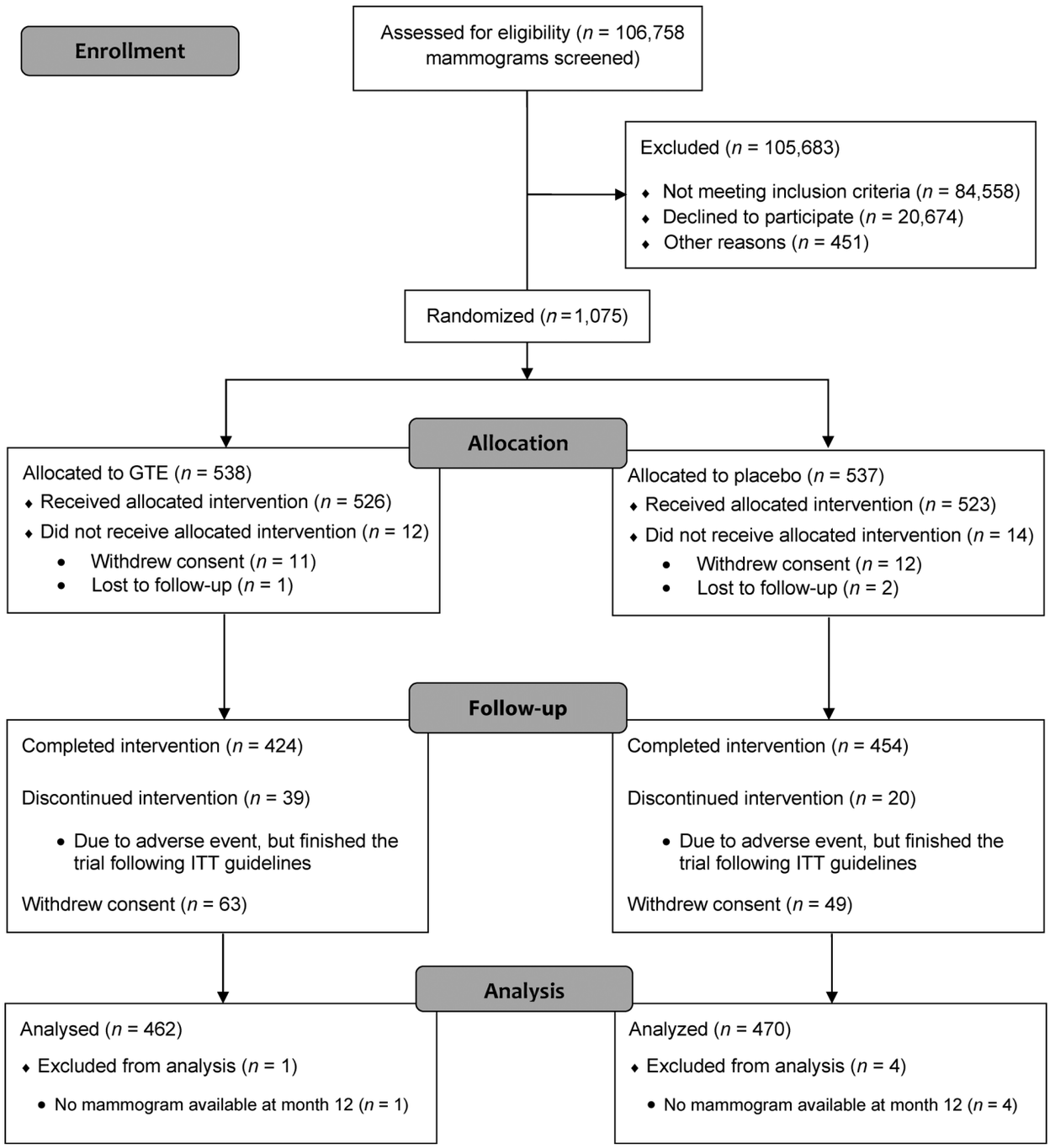

Figure 1.
CONSORT flow diagram of participants through the trial.

closely monitored and identified throughout the trial and described elsewhere (28). Overall, the study intervention was well tolerated and the total frequencies of reported AEs were similar between the two groups.

MD measurement

MD was assessed at month 0 (preintervention) and month 12 (postintervention) by selecting the left cranio-caudal (CC) view of full-field digital mammography (FFDM) screening images. Mammograms were assessed for density by one experienced reader (G. Ursin) using the validated, computer-assisted, area-based quantitative Madena method developed at the University of Southern California as described previously (8, 29–31). In brief, to read the density, the reader draws a region of interest that excludes the pectoralis muscle and other artifacts. The software counts the number of pixels within the region of interest, which represents the area with absolute density. The entire breast area is outlined with an outlining tool on the computer screen

Table 1. Selected baseline characteristics and demographic of study participants ($N = 932$)

	GTE ($n = 462$)		Placebo ($n = 470$)		
Characteristics	**Mean (SD)**	***n* (%)**	**Mean (SD)**	***n* (%)**	***P*[a]**
Age (y)	60.0 (4.9)		59.6 (5.0)		0.22
BMI (kg/m^2)	25.1 (3.7)		25.0 (3.7)		0.52
Race					0.43
White		452 (97.8)		454 (96.6)	
Asian		3 (0.7)		7 (1.5)	
Black		2 (0.4)		5 (1.1)	
Others		4 (0.9)		2 (0.4)	
Unknown		1 (0.2)		2 (0.4)	
Ethnicity					0.94
Hispanic		5 (1.1)		4 (0.9)	
Non-Hispanic		452 (97.8)		463 (98.1)	
Unknown		5 (1.1)		5 (1.1)	
Age at menopause[b] (y)	49.0 (5.7)		49.3 (5.2)		0.42
Time since menopause[c] (y)	11.1 (7.6)		10.3 (7.2)		0.10
Age at menarche[d] (y)	12.9 (1.6)		12.8 (1.9)		0.18
Age at first live birth[e] (y)	27.8 (5.5)		28.2 (6.1)		0.33
Number of full-term births	1.7 (1.2)		1.7 (1.2)		0.90
Past hormone therapy use					0.95
No		269 (58.2)		274 (58.3)	
Yes		189 (40.9)		191 (40.9)	
Unknown		4 (0.9)		5 (1.1)	
Past oral contraceptives use					0.87
No		65 (14.1)		69 (14.7)	
Yes		394 (85.3)		399 (84.9)	
Unknown		3 (0.7)		2 (0.4)	
Parity					0.27
Parous		353 (76.4)		356 (75.7)	
Nulliparous		108 (23.4)		109 (23.2)	
Unknown		1 (0.2)		5 (1.1)	
Breastfeeding[f] (months)	13.7 (17.4)		13.4 (16.1)		0.86
Family history of breast cancer (first-degree relative)					0.54
No		343 (74.2)		357 (76.0)	
Yes		119 (25.8)		113 (24.0)	
Smoking status					0.84
Never		315 (68.2)		320 (68.1)	
Former		145 (31.4)		149 (31.7)	
Unknown		2 (0.4)		1 (0.2)	
Total physical activity[g] (MET-h/week)	45.7 (54.0)		51.2 (107.3)		0.33
Education (degree)					0.16
High school or less		27 (5.8)		31 (6.6)	
Some college		312 (67.5)		285 (60.6)	
Masters/PhD/Professional		121 (26.2)		150 (31.9)	
Unknown		2 (0.4)		4 (0.9)	
COMT genotype					0.33
AA (low activity)		156 (33.8)		143 (30.4)	
GA (intermediate activity)		180 (39.0)		205 (43.6)	
GG (high activity)		126 (27.3)		122 (26.0)	

Abbreviation: MET, metabolic equivalents.

[a]*P* values were calculated from GLM for continuous variables and from the Pearson χ^2 test for categorical variables. Percentages may not add up to 100% due to rounding.

[b]GTE (n) = 441; placebo (n) = 450.

[c]GTE (n) = 439; placebo (n) = 450.

[d]GTE (n) = 458; placebo (n) = 464.

[e]Restricted to parous women; GTE (n) = 354; placebo (n) = 361.

[f]Restricted to parous women; GTE (n) = 334; placebo (n) = 347.

[g]Self-reported; placebo (n) = 469.

(by E. Lee), and the computer counted the total number of pixels, which represents the total breast area. The MD, or the mammographic percent density, is the absolute density (dense area) divided by the total breast area. Mammograms were read in batches of 50, and both baseline and month 12 mammograms for each participant were read in the same batch with the readers blinded to both treatment assignment and time point of each image. In randomly selected 104 mammograms of 52 participants that were read in duplicate, the intraclass Pearson correlation coefficients were 0.94 for absolute density, 0.99 for total breast area, and 0.96 for PMD (all *P*s < 0.0001).

We also assessed the volumetric-based MD from corresponding left CC view of raw (for processing) FFDM images for an available subset of 99 participants using a validated and fully automated method (Volpara version 1.5.0; Volpara Health Technologies; refs. 32–35). This measure was to quantify fibroglandular tissue volume % (FGV%).

Study supplement, intervention, and compliance assessment

The supplement used in this study was a decaffeinated GTE. Each capsule contained 328.8 mg total catechins and 210.7 mg EGCG and less than 4 mg of caffeine. Participants were instructed to consume two capsules in the morning and two in the evening per day for 12 months after a meal to minimize any possible gastrointestinal irritation. Overall, women consumed an average of 1,315 mg catechins, including 843 mg EGCG daily, which is equivalent to five 8-ounce cups (240 mL) of brewed green tea (36). A placebo capsule that was devoid of catechins and caffeine consisted of 204 mg maltodextrin, 202 mg cellulose, and 2 mg magnesium stearate as a flow agent. Further details of the supplement composition have been provided previously (25). Both GTE and placebo capsules were identical in appearance and were supplied in 8 batches by Corban Laboratories (Eniva Nutraceutics).

Compliance was confirmed by pill count, a pill diary, and monitoring concentrations of urinary catechins, including epigallocatechin (EGC) and epicatechin (EC), which has been described elsewhere (25).

COMT genotyping

A whole-blood sample was drawn into a tube containing ethylenediaminetetraacetic acid. Buffy coat was separated from the whole blood following centrifuging and mixing with 0.5 normal saline and was stored in −80°C until DNA extraction. The *COMT* polymorphism genotyping (Val158Met (G/A), rs4680) was conducted at the University of Minnesota Genomics Center as follows. Genomic DNA was extracted from 200 μL of buffy coat samples using the QIAGEN DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen). A TaqMan genotyping assay was defined for the *COMT* genotype, and the assay was performed by an Applied Biosystems TaqMan PCR Core Reagent Kit. Three control samples from Coriell cell lines were used in each run, and genotyping was successful in all DNA samples with the allelic distributions in accordance with Hardy–Weinberg equilibrium for all screened participants ($P = 0.35$).

Statistical analysis

The mean values of PMD and absolute density measures at baseline and 12 months in two treatment groups were calculated, and the change in MD from baseline and 12 months was analyzed as the primary outcome. Relative PMD and absolute density changes were calculated as [(PMD at month 12 – PMD at baseline)/by PMD at baseline] multiplied by 100.

The two-group *t* test (from continuous variables) or χ^2 statistics (for discrete or nominal variables) were used to compare the differences in the distributions of demographic characteristics between two treatment groups. One-way ANCOVA was used to examine the difference in the change in PMD or absolute density between the GTE and placebo groups with adjustment for age and BMI at baseline and BMI at 12-month mammogram, which is equivalent to adjusting for BMI change between baseline and 12-month follow-up. We also conducted stratified analysis by *COMT* genotype, age, BMI, years since menopause, tea drinking, alcohol drinking, and parity. An interaction term between treatment and stratifying variables was included in the model to evaluate the potential modifying role of stratified variables on GTE effect on MD. Statistical analyses were carried out based on the intention-to-treat principle using SAS software version 9.4 (SAS Institute). All *P* values reported are two-sided, and those that were ≤0.05 were considered to be statically significant.

Results

Following assessment of more than 100,000 screening mammograms, 1,075 women were randomized into the MGTT among whom 538 were randomly allocated to GTE and 537 to placebo (Fig. 1). Overall, 937 (87.2%) of the randomized participants (463 in GTE and 474 in placebo) completed the assigned intervention. Four participants in the placebo group and one participant in the GTE group were missing a 12-month screening mammogram; thus, results are presented for a total of 932 participants (462 in the GTE and 470 in the placebo group). The dropout rate was 12.8% ($n = 138$); primary reasons for dropping out were participant request ($n = 93$), adverse effects ($n = 22$), and loss to follow-up ($n = 10$). Baseline characteristics and dietary intake of dropped participants were not significantly different between the two treatment groups except for higher weight and soy intake in the placebo subjects (25).

Table 1 summarizes the baseline demographic characteristics of the study participants by treatment group. All baseline characteristics were equally distributed between the GTE and placebo groups. The mean (SD) baseline age and BMI of all participants ($n = 932$) were 59.8 (5.0) years and 25.1 (3.7) kg/m^2, respectively. More than 97% of the women were non-Hispanic white, and the majority of them were parous, never smokers with some college level education.

Table 2. Mean (95% CI) of PMD at baseline, month 12, and percent change from baseline for PMD and absolute density by treatment group ($N = 932$)

	GTE ($n = 462$)	Placebo ($n = 470$)	P[a]
PMD, %			
Baseline	27.47 (26.18–28.76)	25.91 (24.63–27.19)	0.09
Month 12	26.94 (25.64–28.24)	25.34 (24.06–26.63)	0.09
% Change	−0.58 (−2.52–1.35)	−0.64 (−2.56–1.28)	0.97
P[b]	0.01	0.007	
Absolute density			
% Change	1.06 (−1.07–3.18)	1.70 (−0.40–3.81)	0.67

NOTE: Values are least-square means.

[a]*P* value for difference between the GTE and placebo groups at baseline, month 12, and % changes based on one-way ANCOVA test adjusted for age and BMI at baseline and BMI at 12-month.

[b]*P* value for difference of PMD between baseline and month 12 means within GTE or placebo group based on paired *t* test.

Table 3. Percent changes (95% CI) of PMD and absolute density by treatment group stratified by *COMT* genotype ($N = 932$)

Variable	*n*	GTE ($n = 462$)	*n*	Placebo ($n = 470$)	P^a	P^b
PMD, %						
G/G Genotype	126	0.50 (−3.22–4.22)	122	−1.63 (−5.41–2.15)	0.43	0.54
G/A Genotype	180	−1.55 (−4.66–1.57)	205	−1.24 (−4.17–1.68)	0.89	
A/A Genotype	156	−0.49 (−3.84–2.85)	143	1.23 (−2.26–4.73)	0.48	
P^c		0.68		0.44		
Absolute density						
G/G Genotype	126	2.66 (−1.41–6.72)	122	1.35 (−2.78–5.48)	0.66	0.70
G/A Genotype	180	−1.33 (−4.73–2.08)	205	0.53 (−2.67–3.72)	0.44	
A/A Genotype	156	2.44 (−1.21–6.10)	143	3.77 (−0.05–7.59)	0.62	
P^c		0.23		0.39		

NOTE: Data presented in least-square means (95% CI).
[a]*P* value for comparison between treatment groups based on two-way ANCOVA test adjusted for age and BMI.
[b]*P* value for the interaction between treatment effect and stratifying variable derived from the linear mixed model.
[c]*P* value for change of PMD across stratifying variable within each treatment group based on one-way ANCOVA test with adjustment for age and BMI.

Other than higher intake of vitamin supplements among GTE participants compared with those in the placebo group ($P = 0.038$), baseline energy, food, and nutrient levels were similar between treatment groups (data not shown). Weight, BMI, and energy and dietary intake remained stable over the course of the trial except that those in the placebo group experienced a significant reduction in vitamin C intake compared with the GTE participants ($P = 0.045$; data not shown). Among all participants ($n = 932$), BMI (Spearman correlation coefficient (r) $= -0.32$, $P < 0.0001$), age ($r = -0.10$, $P = 0.001$), and parity ($r = -0.15$, $P < 0.0001$) were inversely correlated with PMD at baseline (Supplementary Table S1).

No differences were observed in baseline PMD between the two groups (Table 2). One-year supplementation with GTE did not result in significantly different changes in either PMD or absolute density compared with the changes in the placebo group after adjustment for age at baseline and BMI at both baseline and month 12.

Changes in MD measures according to *COMT* genotype are presented in Table 3. There was no significant modifying effect of *COMT* genotype with GTE on either PMD ($P = 0.54$) or absolute density ($P = 0.70$).

The GTE supplementation significantly reduced PMD in younger women included in the study. Women aged 50 to 55 years at

Table 4. Percent change (95% CI) of PMD by treatment groups stratified by age, BMI, years since menopause, parity, tea intake status, and alcohol intake at baseline ($N = 932$)

Variable	*n*	GTE	*n*	Placebo	P^a	P^b
Age, y						
50–55	112	−4.40 (−10.11–1.31)	127	1.02 (−4.71–6.75)	0.05	0.07
56–60	156	0.63 (−2.83–4.10)	157	−0.04 (−3.50–3.43)	0.78	
61–70	194	0.53 (−4.05–5.12)	186	−2.16 (−6.80–2.47)	0.22	
P^c		0.13		0.43		
BMI, kg/m^2						
<25	256	−2.45 (−5.87–0.96)	263	−1.12 (−4.57–2.32)	0.48	0.49
25–29.9	146	1.84 (−1.99–5.68)	165	−0.29 (−4.00–3.42)	0.38	
≥30	60	1.28 (−6.72–9.29)	42	1.33 (−7.84–10.51)	0.99	
P^c		0.54		0.57		
Years since menopause, y						
<5	97	−1.60 (−6.20–3.00)	111	0.15 (−4.30–4.60)	0.56	0.55
5–10	121	−2.27 (−6.14–1.59)	138	−0.75 (−4.37–2.88)	0.57	
≥10	221	0.60 (−2.49–3.69)	201	−1.18 (−4.40–2.04)	0.40	
P^c		0.79		0.98		
Tea drinking status						
Past tea drinkers	275	−0.91 (−3.43–1.62)	286	0.67 (−1.80–3.14)	0.38	0.22
Non-tea drinkers	186	−0.24 (−3.30–2.83)	179	−2.33 (−5.45–0.80)	0.35	
P^c		0.69		0.14		
Parity						
Nulliparous	108	0.87 (−3.16–4.90)	109	−1.45 (−5.48–2.57)	0.42	0.59
1–2	249	−1.54 (−4.19–1.12)	258	−1.19 (−3.80–1.42)	0.85	
3+	102	−0.19 (−4.34–3.96)	98	1.84 (−2.39–6.08)	0.50	
P^c		0.61		0.48		
Alcohol, drinks/week						
None	93	−0.37 (−4.71–3.97)	74	−0.55 (−5.42–4.31)	0.96	0.72
0.1–7	308	0.20 (−2.19–2.58)	330	−0.19 (−2.42–2.19)	0.85	
≥7	58	−5.35 (−10.85–0.14)	63	−2.40 (−7.71–2.90)	0.45	
P^c		0.18		0.70		

NOTE: Data presented in least-square means (95% CI).
[a]*P* value for comparison between treatment groups based on two-way ANCOVA test adjusted for age and BMI (where appropriate).
[b]*P* value for the interaction between treatment effect and stratifying variable derived from the linear mixed model.
[c]*P* value for change of PMD across stratifying variable within each treatment group based on one-way ANCOVA test with adjustment for age and BMI (where appropriate).

enrollment in the GTE arm had a 4.40% decrease in PMD compared with those in the placebo with a 1.02% increase in PMD (P for difference = 0.05; Table 4). The interaction between age and GTE supplementation on the change of PMD over 12-month treatment period was statistically borderline significant ($P_{interaction}$ = 0.07). BMI, years since menopause, tea drinking status, parity, and alcohol intake did not significantly modify the effects of GTE intake on PMD (Table 4) or absolute MD (Table 5).

Among a subset (n = 99) of women with available volumetric-based MD (i.e., FGV), we also examined the effect of GTE on the change of this measure. The FGV% was highly correlated with PMD (r = 0.65) and absolute MD was highly correlated with absolute FGV (r = 0.63; P for both <0.0001). We did not observe any effect of GTE supplementation on the percent and absolute FGV (Supplementary Table S2). The *COMT* did not change the null effect of GTE on the FGV measures (Supplementary Table S3).

Discussion

We examined the effects of a GTE supplement with high dose of EGCG on MD measures in an RCT among healthy postmenopausal women at high risk of breast cancer due to high MD. Daily green tea supplementation of 1,315 mg catechins (including 843 mg EGCG) for 1 year had no significant effects on absolute density or PMD. The dose of catechins (particularly, EGCG) used in this trial is at the high end of green tea intake in humans and the highest dose used in a research study of a healthy population.

Our null findings are consistent with the results of the one published intervention study. That small RCT was a study of 40 Caucasian premenopausal and postmenopausal women with a history of hormone receptor–negative breast cancer (37). In that study, daily green tea polyphenols (polyphenon E) supplementation in doses of 800, 1,200, and 1,600 mg of EGCG for 6 months did not result in any significant changes in PMD compared with the placebo counterparts.

Our results differ, however, from the only published epidemiologic study of green tea intake and MD in which regular green tea consumption was linked to 2.2% lower PMD compared with non–green tea drinkers (21). The reasons for the discrepancy in results between the cross-sectional study and our clinical trial as well as the other published clinical trial are not clear, but may be related to the study population (Singaporean Chinese vs. Caucasian women) or difference in the mode of green tea consumption (green tea beverage vs. green tea supplement). In addition, the findings from the observational cross-sectional study reflect the long-term green tea drinking habits, whereas our results are representative of 1-year intervention and the timing of green tea intervention (i.e., postmenopause period) may have been too late. Finally, the age at which the consumption of green tea initiated (e.g., peripuberty or premenopausal) may play a role in response to the change in MD or breast cancer risk, although we are not aware of any study investigating this hypothesis.

The finding of a statistically significant reduction in PMD for the youngest women (50–55 years) enrolled in the study is intriguing.

Table 5. Percent change (95% CI) of absolute mammographic density by treatment groups stratified by age, BMI, years since menopause, parity, tea intake status, and alcohol intake at baseline (N = 932)

Variable	*n*	GTE	*n*	Placebo	P^a	P^b
Age, y						
50–55	112	0.21 (−6.04–6.46)	127	5.12 (−1.15–11.40)	0.10	0.30
56–60	156	3.41 (−0.39–7.21)	157	2.95 (−0.85–6.74)	0.86	
61–70	194	−0.56 (−5.58–4.46)	186	−1.46 (−6.53–3.61)	0.71	
P^c		0.33		0.26		
BMI, kg/m^2						
<25	256	0.61 (−3.13–4.35)	263	0.92 (−2.84–4.69)	0.88	0.96
25–29.9	146	2.61 (−1.58–6.81)	165	3.26 (−0.80–7.32)	0.81	
≥30	60	−0.99 (−9.75–7.75)	42	0.69 (−9.34–10.72)	0.72	
P^c		0.18		0.14		
Years since menopause, y						
<5	97	2.78 (−2.26–7.82)	111	3.48 (−1.39–8.34)	0.83	0.99
5–10	121	1.79 (−2.44–6.02)	138	2.41 (−1.56–6.38)	0.83	
≥10	221	0.02 (−3.37–3.40)	201	0.33 (−3.20–3.86)	0.89	
P^c		0.47		0.92		
Tea drinking status						
Past tea drinkers	275	0.91 (−1.85–3.67)	286	2.81 (0.10–5.51)	0.34	0.39
Non-tea drinkers	186	1.20 (−2.16–4.55)	179	0.32 (−3.10–3.74)	0.72	
P^c		0.87		0.24		
Parity						
Nulliparous	108	1.40 (−3.01–5.80)	109	0.45 (−3.95–4.84)	0.76	0.76
1–2	249	0.28 (−2.62–3.18)	258	0.95 (−1.91–3.80)	0.75	
3+	102	2.33 (−2.20–6.87)	98	5.06 (0.44–9.69)	0.41	
P^c		0.77		0.26		
Alcohol, drinks/week						
None	93	3.75 (−1.00–8.49)	74	0.86 (−4.46–6.18)	0.43	0.51
0.1–7	308	1.06 (−1.55–3.67)	330	2.38 (−0.14–4.90)	0.47	
≥7	58	−3.20 (−9.13–2.89)	63	−0.45 (−6.24–5.35)	0.53	
P^c		0.24		0.60		

NOTE: Data presented in least-square means (95% CI).

[a]P value for comparison between treatment groups based on two-way ANCOVA test adjusted for age and BMI (where appropriate).

[b]P value for the interaction between treatment effect and stratifying variable derived from the linear mixed model.

[c]P value for change of absolute mammographic density across stratifying variable within each treatment group based on one-way ANCOVA test with adjustment for age and BMI (where appropriate).

This result is well in accord with the findings from two intervention studies aimed to investigate the effect of tamoxifen on changes in PMD. Cuzick and colleagues (14) reported a net reduction of 13.4% (95% CI, 8.6–18.1) in MD after 54-month treatment with tamoxifen in women aged 45 years or younger while those older than 55 years only showed 1.1% (95% CI, −3.0–5.1) decrease. Similar findings have also been reported by Meggiorini and colleagues (38). Although the precise mechanisms by which green tea influences MD in younger women of our study are not known, plausible explanations could be due to the hormonal-mediated pathways. In our study population, young women showed higher baseline levels of circulating and urinary estrogens (data not shown) as well as MD (Supplementary Table S1). It would be expected that the impact of any agents targeting the estrogen pathway would be stronger in young women. Our findings support such a notion.

We found no apparent evidence of effect modification by *COMT* polymorphism in this study, possibly due to the small number of participants homozygous for the high activity allele of *COMT*. To the best of our knowledge, no other study has to date examined the interaction between *COMT* genotype and GTE on the changes in PMD.

It is possible that nondifferential misclassification of MD by using the two-dimensional area-based methods may have biased the results toward the null. Limitations of area-based methods for measurement of MD include that they are labor-intensive, subjective, and do not take into account the breast as a three-dimensional object. However, consistent with our main results based on the area-based approach, we observed no significant differences in the volumetric measure of MD based on a subset of the study participants (53 in the GTE and 46 in the placebo group). This finding should be interpreted with caution because of the small sample size for this substudy.

The MGTT had several strengths and limitations. A major strength is its double-blind, randomized placebo-controlled design. This study is the largest intervention trial with adequate statistical power that has evaluated the effects of oral supplementation of GTE on MD with the longest intervention period to date. MD was quantified using a highly reproducible method in which one very experienced reader blinded to the treatment assignment evaluated the density of all mammograms. Furthermore, participation rate was high (dropout rate = 12.8%), and subjects showed excellent compliance with the study protocol and consumed more than 96% of the prescribed treatment capsules. High compliance among the participants consuming the GTE capsules was also confirmed by the results from using a biomarker-based approach in which GTE participants showed significantly increased concentrations of urinary EGC and EC compared with placebo following initiation of study intervention, while as expected, baseline levels of EGC and EC did not differ between the two groups (25). Finally, we showed that participants in both treatment and placebo groups experienced a significant decrease in PMD following 12-month consistent with the reduction in PMD with increasing age (39, 40). Study limitations include a nonethnically diverse study population, which potentially affects generalizability of the findings, and the lack of data available for the duration and amount of green tea consumption in the past. Another limitation of this study was our difficulty with recruiting adequate participants with the high activity *COMT* genotype, which can be explained in part by the fact that the Caucasian populations have a low prevalence of the *COMT Val/Val* genotype (41). As a result, we were statistically underpowered to detect any potential interaction between green tea catechin consumption and *COMT* genotype. Finally, there was a gap of up to 3.5 months between performing mammograms and completing the baseline clinic visit, which may have influenced our results.

In summary, the supplementation with a high dose of EGCG for 12 months had no significant effect on reduction of MD measures in all postmenopausal women in this large clinical trial. However, the statistically significant effect on reduction in PMD for women 50 to 55 years old suggests that green tea supplementation may be effective for women with more dense MD. Future studies on green tea supplementation and prevention of breast cancer are warranted in younger women.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: G. Ursin, C.T. Le, C.S. Yang, M.C. Yu, D. Yee, J.-M. Yuan, M.S. Kurzer

Development of methodology: H. Samavat, M.C. Yu, A.H. Wu, J.-M. Yuan, M.S. Kurzer

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Samavat, T.H. Emory, C.J. Torkelson, A.M. Dostal, K. Swenson, C.S. Yang, D. Yee, M.S. Kurzer

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Samavat, G. Ursin, E. Lee, R. Wang, C.T. Le, C.S. Yang, A.H. Wu, J.-M. Yuan

Writing, review, and/or revision of the manuscript: H. Samavat, G. Ursin, T.H. Emory, E. Lee, A.M. Dostal, K. Swenson, C.T. Le, C.S. Yang, M.C. Yu, D. Yee, A.H. Wu, J.-M. Yuan, M.S. Kurzer

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Samavat, K. Swenson

Study supervision: M.S. Kurzer

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