No Effect of Aspirin on Mammographic Density in a Randomized Controlled Clinical Trial

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Abstract

Background: Epidemiologic studies suggest a reduced risk of breast cancer among women who regularly use aspirin; a plausible mechanism is through aspirin effect on mammographic breast density, a breast cancer risk factor, possibly mediated through aspirin interference with estrogen synthesis.

Methods: In a 2-arm randomized placebo-controlled clinical trial, we evaluated the effects of 6-months administration of 325 mg/day aspirin on total mammographic breast dense area and percent of the mammographic breast image occupied by dense areas (% density) in 143 postmenopausal women. Eligible women, recruited 2005-7, were healthy, not taking hormone therapy, with elevated mammographic breast density (American College of Radiology Breast Imaging Reporting and Data System (BI-RADS®) density category 2, 3 or 4) within 6 months prior to enrollment.

Results: Women were a mean (s.d.) 59.5 (5.5) years. Geometric mean baseline percent density was 17.6% (95% CI 14.8, 20.9) in women randomized to aspirin and 19.2% (95% CI 16.3, 22.7) in women randomized to placebo. Percent density decreased in women randomized to aspirin by an absolute 0.8% vs. an absolute decrease of 1.2% in controls (p = 0.84). Total breast area and dense area decreased to a similar degree in women assigned to aspirin and in those assigned to placebo, with no differences statistically significantly different between trial arms.

Conclusions: A single daily administration of adult-dose aspirin for 6 months had no effect on mammographic density in postmenopausal women. If aspirin affects breast cancer risk in postmenopausal women, it may do so through alternative pathways than mammographic breast density.

Introduction

Several lines of evidence suggest a role of inflammation in breast cancer etiology, and that blockade of this process has strong potential for cancer chemoprevention. Animal experimental studies have consistently shown that nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit mammary carcinogenesis (1-5). Epidemiologic investigations have provided evidence that NSAIDs may be associated with a reduced risk of breast cancer (6) although a large clinical trial found no effect of alternate day low-dose aspirin on breast cancer risk (7). A recent meta-analysis including 38 epidemiologic studies with 2,788,715 women found that, overall, aspirin use was associated with a 13 percent reduced risk of breast cancer (relative risk 0.87, 95% confidence interval 0.82-0.92) (6).

The biological effects of NSAIDs relevant to breast cancer etiology are not known, and NSAIDs may exert their effects by a number of mechanisms. Aspirin and ibuprofen NSAIDs inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), catalytic enzymes involved in prostaglandin synthesis, by irreversible acetylation and competitive inhibition (8). NSAIDs may lower circulating estradiol levels as COX-2 can potentiate estradiol levels through affecting the aromatase enzyme system (9, 10). NSAIDs may also affect neoplastic growth and development by reducing cell proliferation, increasing epithelial apoptosis, decreasing infiltration by inflammatory cells and subsequent diminished release of destructive enzymes and reactive oxygen species, and modulating tumor immunogenicity (11).

Mammographic breast density (the mammographic representation of the breast composed of epithelial and connective tissue, as opposed to fatty tissue) is positively associated with breast cancer risk (12-19), increases (almost doubling percent density) with administration of estrogen plus progesterone therapy (20) which is known to increase breast cancer risk, and decreases with tamoxifen therapy (21-23) which is known to decrease breast cancer risk (24). In the International Breast Cancer Intervention Study I, a 7.9% vs. 3.5% absolute reduction in percent of the breast occupied by dense tissue was observed with 18 months' tamoxifen vs. control (23). Mammographic density is one of the few modifiable risk factors for breast cancer. Observational studies of the association between NSAIDs and mammographic breast density have shown mixed results (25, 26), and no clinical trials have been reported on aspirin effect on mammographic density.

Given the potential anti-carcinogenic properties of NSAIDs including aspirin, the consistent changes in breast density associated with drugs that increase or decrease risk of breast cancer, and the potential biological effects of NSAIDS on the breast that might be reflected in breast imaging, we tested the effect of 6-months

administration of 325 mg/day aspirin vs. placebo on mammographic density measured with computer-assisted quantification (27) in postmenopausal women with mammographically measurable breast density (BIRAD density classification 2, 3, or 4) (28). We chose aspirin rather than other NSAIDs because of the low risk for cardiotoxic effects of aspirin compared with other NSAIDs, and we chose the particular dose of aspirin because of the many studies suggesting that lower doses commonly used for cardio-protection (i.e., 100 mg/day or less) are not sufficient for reducing breast cancer risk (6) and because higher doses of aspirin are associated with increased risk for adverse events (29, 30). This paper presents the primary results of the trial.

Methods

In a randomized placebo-controlled double-blind clinical trial, we evaluated the effects of aspirin on mammographic density. Women were recruited between 2005 – 2007 in western Washington state through a variety of mechanisms including mass mailings and media placements (Figure 1). Women were screened for eligibility through medical history, review of prior mammography reports for radiologist-determined BI-RADS mammogram density category, and physical exam.

Eligible women were postmenopausal (no menstrual periods for 24 months, follicle-stimulating activity > 50 IU/L for women without a uterus), aged 50 -75 years, not using menopausal hormone therapy, oral contraceptives, or selective estrogen response modulators (SERMS) for the previous six months, with BI-RADS mammogram density category on prior mammograms through their own providers of 2 (scattered fibroglandular), 3 (heterogeneously dense tissue), or 4 (extremely dense tissue present) (28), healthy with no significant co-morbidities including any cancer, not currently using any NSAIDs regularly and willing to avoid NSAID use during the 6-month trial duration, not using any other anticoagulant medication, and with no contra-indications to use of aspirin. Screening blood tests included complete blood count (white blood cells, hematocrit, platelets), prothrombin time (PT), and partial thromboplastin time (PTT). Women with anemia (hematocrit < 35); abnormal bleeding tests; history of bleeding disorders, renal disease, or hemorrhagic stroke; current uncontrolled hypertension, planning extensive weight loss in next 6 months, using > 2 alcohol drinks per day, or with current significant mental illness or alcohol or drug abuse, were also excluded. Potentially eligible women completed a 3-week daily placebo run-in trial, with taking ≥ 80% of placebo run-in capsules (by pill count) required. No woman was excluded solely for noncompliance with placebo run-in.

Baseline measures included anthropometrics (height, weight, body mass index, waist and hip circumferences), resting pulse and blood pressure, and clinical exam including breast exam. Prior to randomization, eligible women underwent screening mammography in our clinical center including cranio-caudal and mediolateral views of both breasts. All mammograms were performed on the same machine by the same technician. Mammograms were interpreted for clinical diagnostic purposes by a radiologist, and any abnormalities were referred to the woman's primary physician.

Informed consent was obtained following the requirements of the Fred Hutchinson Cancer Research Center Institutional Review Board. An independent Data and Safety Monitoring Committee oversaw study protocol and procedures, and reviewed trial data biannually.

A total of 144 women were assigned by simple randomization into one of two arms: 325 mg/day aspirin (N=75), or an identical-appearing placebo capsule/day (N=68). Aspirin and placebo capsules were prepared and blind-packaged by the University of Washington (UW) Pharmacy Services. Participants attended an enrollment visit where they were given their 6-month supply of study medication and instructed to take one capsule daily, instructed regarding potential complications such as bleeding and gastric upset, and provided with a bottle of acetaminophen pills for use for pain/fever during the trial. All study investigators, staff, and participants were blinded to study arm, with the exception of the study statisticians and the UW pharmacy services. Compliance to the aspirin intervention was assessed by capsule count at the 6-month end-of-study clinic visit. Participants were called each month to inquire about safety issues and potential adverse effects (i.e., bleeding episodes, major illnesses, or hospitalizations), and problems with taking pills.

Of the 144 randomized women, one in the aspirin group was found to have a breast cancer on her baseline mammogram after randomization, and was therefore withdrawn from the study as she was determined to be randomized in error.

At 6 months, baseline measures were repeated including screening bilateral 2-view mammogram, breast exam, anthropometrics, and symptoms inventory. The baseline and follow-up films were digitized and the left craniocaudal view was measured for density. All mammograms used for study baseline and follow-up measurements were performed in our clinical center with the same machine by the same technician.

Mammogram Density Determinations

We measured mammographic breast density using the Cumulus program method developed by Boyd *et* al. (28, 29, 31-33). Mammographic films were scanned using the Kodak LS85 laser film digitizer at 88 µm/pixel for small films and 117 µm/pixel for large films. All density measurements were performed by the same reader (E.A.B.) who was blind to participant name, randomization status and to baseline or follow-up status of the films. Films were placed randomly in batches of 52 films. Each woman's baseline and follow-up films were measured in the same batch, and an equal number of films from aspirin and placebo arms were included in each batch. The following items were measured: total breast area, dense area, and percentage breast density, equal to the area of density divided by the total breast area, expressed as a percentage (range 0% - 100%). Percent mammographic density is calculated by dividing the dense area of the breast by the total area. By doing this, % density takes into account the amount of fat in the breast (non-dense tissue) where as dense area does not. The two are often correlated but can demonstrate important differences in density, especially among women with different breast sizes. For example, a woman with very small breasts might have a high % density (because she has very little fat) but a low absolute amount of dense area because her breasts are small. A woman with large breasts with the same dense area would have very low % density because her breasts are much larger. Dense area and total area were measured in pixels and converted to cm2 depending on the size of the film: 7.72 x 10^-5 cm2 per pixel for small films and 1.373x 10^-4 cm2 per pixel for large films. The conversion factors are a product of the scanning resolution and film size. All films were scanned at 2048 pixels per line. For small films (18x24cm), this converts to 88um/pixel (18 / 2048 = 0.008789cm/pixel or 88um/pixel). For large films (24x30cm), this converts to 117um/pixel (24 / 2048 = 0.0117187cm/pixel or 117um/pixel). These numbers are squared to get the conversion factor from pixels to cm^2 per pixel, thus 0.008789^2 = 7.72 x 10^-5 for small films and 0.0117187^2 = 1.373 x 10^-4 for large films.(Chris Peresotti, University of Toronto, personal communication). Mammograms for 5 women were blindly re-measured for QA, with intra-class correlations of 0.999, 0.879, and 0.934 for total breast area, dense area, and percent density, respectively.

Statistical Analysis

Primary analyses were based on assigned treatment at the time of randomization, regardless of adherence or retention status (i.e., intent-to-treat), and all participants' data were included in the primary analyses. The main study endpoints were mean dense breast area and mean percent mammogram density. Geometric means were used for

mammogram density variables since logarithmically transformed data were less skewed. The geometric mean for skewed data is generally close to the median, which is less sensitive to outliers, than the sample mean. The intervention effects were evaluated by the differences in the geometric mean changes at 6 months between the aspirin and placebo groups using the generalized estimating equations (GEE) in order to account for the longitudinal nature of the data. In addition to the GEE approach, we have also applied linear regression in which the outcome and covariates were differences between 6 months and baseline.

We also explored differential intervention effects by baseline age (categorized by median age <59, ≥ 59 years) and BMI (categorized by WHO criteria <25 kg/m^2, 25.0-29.9 kg/m^2, and ≥30.0 kg/m^2). These effect-modification analyses were planned *a priori*.

Secondary analyses included examination of effect modification of the aspirin intervention by adherence with a linear regression model on dense breast area and percent density with the percent of pills as a linear covariate in the model, as well as its interaction terms. In addition, we considered two levels of adherence (≥ 80%, <80% of pills taken). The effect of the intervention was calculated and tested within each adherence level.

All statistical tests were two-sided. Statistical analyses were performed using SAS software (Version 9.1; SAS Institute Inc, Cary, NC).

When designing the trial, we calculated that with N=144, and assuming a mean baseline percent density of 7% (with s.d. 5.8%), allowing for 5% dropout, 1% drop-in and 1% loss to follow-up, we would have 80% power to detect an absolute difference in percent density of 3% between aspirin and placebo arms at the end of the study. The mean (s.d.) information was based on 85 women from a clinical trial testing exercise effect on sex hormones in postmenopausal women (PI: Dr. McTiernan). When the mean percent density is higher, such as 15%, 20%, or others, the power calculation is still applicable to detect an absolute difference in percent density of 3% between the aspirin and placebo arms. The power will reduce if the s.d. is larger than 5.8%. Further notes on power calculation based on baseline mammographic density from our clinical trial data are provided in the discussion section.

Results

All 143 study participants returned for end-of-study measurements including mammogram density and covariates, but the 6-month film for one woman in the aspirin group was not readable, and therefore data for that participant were not included in the analysis.

Women were a mean (s.d.) 59.5(5.5) years of age, had a mean BMI of 26.4(5.4) kg/m^2, most were non-Hispanic white, and more than 70 percent had a college degree or higher (Table 1). Approximately 42, 50, and 8 percent had a category 2, 3, and 4 BI-RADS mammographic density classification, respectively, based on their pre-study outside-provider mammogram reports. Almost one-quarter had a previous breast biopsy. The women's geometric mean percent mammogram density was 18.3 percent (95% CI: 16.3, 20.7): 17.6% (95% CI 14.8, 20.9) in women randomized to aspirin and 19.2% (95% CI 16.3, 22.7) in women randomized to placebo. Baseline characteristics did not differ significantly between aspirin and placebo arms (Table 1), including age, BMI, weight, education, baseline clinical BIRAD mammogram density classification, history of previous breast biopsy, and reproductive history. Women randomized to aspirin and placebo were similarly adherent to study medications (87% pills taken in aspirin; 87% in placebo). A small number of intervention (N=5) and placebo (N=7) reported using NSAID during the 6 months of the trial.

Table 2 shows the total breast area, mammographic dense area, and percent dense area, by pre-study outside-provider BI-RADS density classification. The dense area and percent dense area increased with increasing BI-RADS classifications (P<0.0001, P<0.0001, respectively).

Percent density decreased in women randomized to aspirin by an absolute 0.8 percent vs. an absolute decrease of 1.2% in controls (p = 0.84) (Table 3) Total breast area and dense area also decreased to a similar degree in women assigned to aspirin and in those assigned to placebo, with no differences statistically significantly different between trial arms. When we assessed effect of aspirin on breast density variables by percent of study capsules taken (< 80% vs. ≥ 80%), we also observed no effect of aspirin on density (data not shown). We observed the following changes in BI-RADS density classifications from baseline to 6 months (p=0.54): aspirin 20% increased, 15% decreased, 63% no change, 2% missing classification; placebo 22% increased, 19% decreased, 59% no change.

Aspirin also did not affect density differently than placebo when we looked at subgroups of women characterized by age, BMI, or baseline mammographic density (data not shown). When we reclassified BI-RADS density categories (from pre-study outside provider mammogram reports) using the trial baseline mammograms, 12 women whose mammograms were originally classified as "2" and 2 whose mammograms were originally classified as "3" were re-classified as "1". Eliminating these 14 women from the analysis of aspirin effect on total or percent density did not affect the results (data not shown). In addition to the GEE approach, we have also applied linear

regression in which the outcome and covariates were differences between 6 months and baseline, and there was no effect of aspirin on density.

Adverse events were reported to a similar degree in the two study arms for: chest pain (3 aspirin, 1 placebo), headaches (4 aspirin, 1 placebo), abdominal pain (9 aspirin, 8 placebo), vomiting blood (4 aspirin, 3 placebo), skin rash (4 aspirin, 6 placebo), and vaginal bleeding (2 aspirin, 1 placebo). A larger number of women in the aspirin group than placebo reported increased bruising (18.7% vs. 1.5 %, $p < 0.001$). None of the reports of bleeding events were deemed clinically significant by the women's physicians and none required hospitalization.

Discussion

We found no effect of 325 mg/day aspirin administered over 6 months on mammographic density in a group of postmenopausal women, despite outstanding adherence and retention to the trial. To our knowledge, no previous clinical trials have examined NSAID use in relation to breast density. One recent cross-sectional study of almost 1500 women found no association between years of use of NSAIDS and current mammographic density, and dose was also not reported in that study (25). Another recently published prospective study including pharmacy records from over 29,000 postmenopausal women who had two screening mammograms at a large health maintenance organization found no association with density change (using BI-RADS density classification) from initiation or continuation of NSAIDs (26). However, both initiators and continuers of any NSAIDs were more likely to stay not dense than stay dense [OR, 1.12; 95% confidence interval (95% CI), 1.04-1.20; OR, 1.25; 95% CI, 1.05-1.49, respectively]. NSAID use was measured only through dispensing from the health plan, and did not have the sensitivity to examine dosing (26).

There are several plausible explanations for this lack of effect. First, if aspirin reduces risk for breast cancer, it may do so through a pathway other than reducing mammographic density. Indeed, raloxifene and aromatase inhibitors, which have been shown to reduce risk for breast cancer in high risk women (24, 34) or risk of second primary breast cancers in breast cancer patients (35) have minimal effect on mammographic density, and have not been consistently reported to decrease breast density (36-39). Second, the effect of NSAIDs on breast cancer risk may be dose- or time dependent (40), and it is possible that the single dose of 325 mg/day used in this study was insufficient to produce an effect on mammographic density. Third, 6 months administration may not be sufficient duration to affect change in mammogram density with this particular medication (26). Epidemiologic studies suggest, however, that duration of

NSAID use is not consistently related to risk (6). Fourth, while our measure of mammographic density is a good predictor of breast cancer risk (27), it may not be sensitive enough to capture a small change in mammographic density because of user variability in setting thresholds for total area and dense area (41). Finally, the effect of NSAIDs on mammographic density could be limited to particular sub-populations of women such as premenopausal women, women at the highest level of breast density, or women at high risk for breast cancer. Our trial sample included only 12 women with BI-RADS density category 4, and therefore we could not assess effect on women with the very highest level of density.

In our original design, we assumed that the mean baseline mammographic percent density was 7%, with s.d. 5.8%. The power was not affected by our observed mean baseline percent density, such as the baseline mean of 18.4% from the trial. However, the s.d. from the trial was larger than our original design, which will reduce the power. From our trial, a robust s.d. based on the median absolute deviation was about 9.5%. With the updated mean and s.d., the study would have 80% power to detect an absolute difference of 4.5% between aspirin and placebo arms at the end of the study. This could be an important consideration in designing further studies on intervention effects on mammographic density.

Strengths of this report include the double-blind randomized design, the quantitative, quality controlled mammographic density assessment, and the high degree of participant adherence and retention. Study limitations include the evaluation of a single dose of aspirin, and the relatively short period of follow-up.

In conclusion, use of aspirin for 6 months resulted in no change in mammographic density in postmenopausal women. If aspirin is associated with reduced risk for breast cancer in postmenopausal women, it may do so through pathways other than change in mammographic density.

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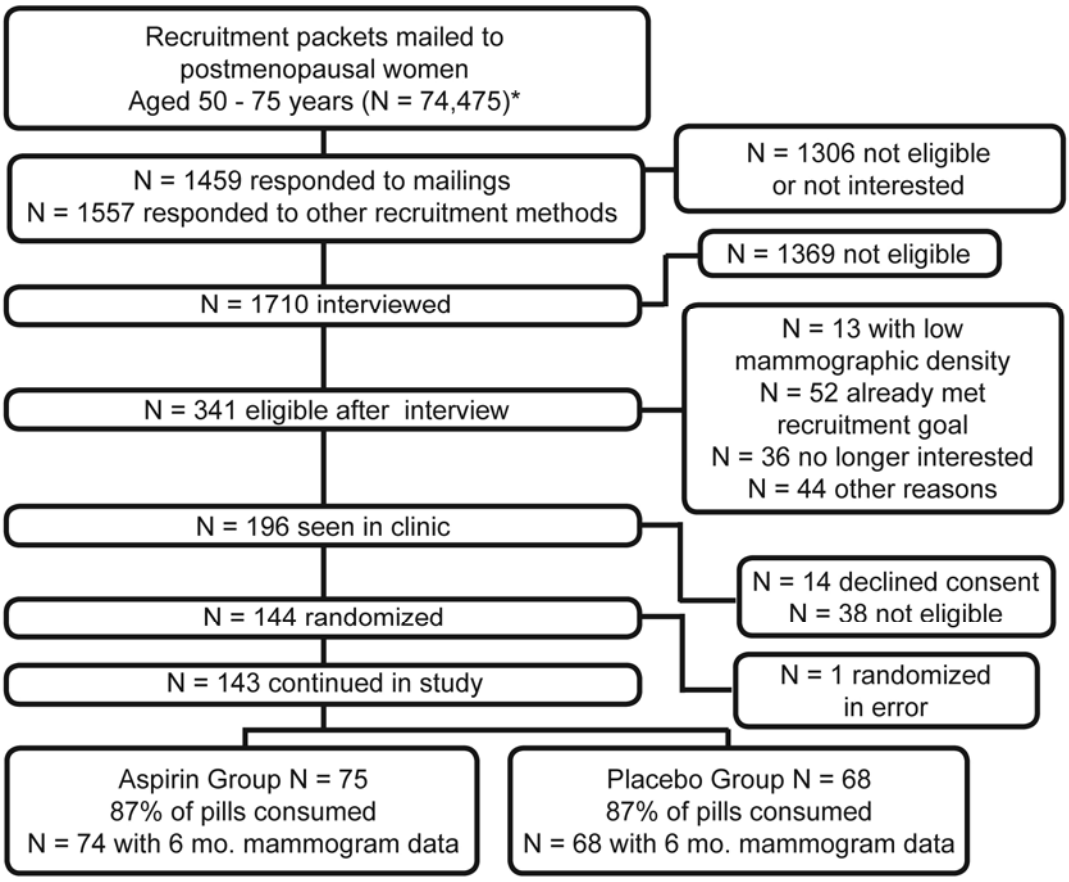
Figure Legend: Participant Screening and Randomization



*Mailings were sent for recruiting for several trials at one time

Table 1. Baseline Characteristics by Study Arm.

	Aspirin N=75	Placebo N=68	P-Value
	Mean (SD)	Mean (SD)	
Age	59.9 (5.5)	59.1 (5.4)	0.41
Median [Range]	58.0 [50.0, 73.0]	59.0 [50.0, 70.0]	
Age at Menopause[1]	49.8 (5.7)	50.1 (5.4)	
Median [Range]	51.0 [30.0,58.0]	51.0 [30.0, 62.0]	0.69
Time Since Menopause[1]	10.0 (7.5)	8.8 (7.7)	
Median [Range]	7.0 [1.0, 30.0]	6.0 [0.0, 39.0]	0.21
BMI	26.9 (6.2)	25.9 (4.1)	0.26
Median [Range]	25.1 [18.2, 47.8] 1 Missing	24.4 [19.1, 40.1]	
Weight (kg)	72.8 (17.3)	70.5 (13.3)	0.37
Median [Range]	67.4 [47.4, 127.0] 1 Missing	67.1 [49.0, 115.2]	
Education	N (%)	N (%)	
High School or Less	2 (2.7)	2 (2.9)	0.99
Some College or Vocational	17 (22.7)	16 (23.5)	
College Degree	27 (36.0)	23 (33.8)	
Post-Graduate Degree	29 (38.7)	27 (39.7)	
Family History of Breast Cancer	N (%) 13 (17.8) 1 Missing	N (%) 18 (26.9) 1 Missing	0.20
BI-RADS mammogram density	N (%)	N (%)	
Class 2	34 (45.3)	26 (38.2)	0.69
Class 3	35 (46.7)	36 (52.9)	
Class 4	6 (8.0)	6 (8.8)	
Previous breast biopsy	N (%)	N (%)	
Yes	15 (20.0) 2 Don't Know	20 (29.9) 0	0.19
Bilateral Oopherectomy	N (%) 7 (9.5) 1 Don't Know	N (%) 7 (10.3)	0.87
Hysterectomy	N (%) 16 (21.3)	N (%) 12 (17.7)	0.58
	Mean (SD)	Mean (SD)	
Number of Pregnancies >6 Months	1.68 (1.84)	1.65 (1.30)	0.90
Median [Range]	2 [0, 11]	2 [0, 5]	
Past Estrogen Use	N (%) 45 (60.0)	N (%) 38 (55.9) 1 Missing	0.53

Mammogram Density Measures	Geometric Mean (95% CI)	Geometric Mean (95% CI)	
Total Breast Area (cm^{-2})	109.5 (98.7,121.5)	106.1 (94.5,119.1)	0.59
Median [Range]	100.2 [46.6 – 395.3]	103.4 [26.5 – 330.2]	
Dense Breast Area (cm^{-2})	19.2 (16.7,22.2)	20.4 (17.4,23.9)	0.60
Median [Range]	19.0 [4.6 – 100.3]	18.9 [4.3 – 100.6]	
Percent Breast Density	17.6 (14.8,20.9)	19.2 (16.3,22.7)	0.46
Median [Range]	19.0 [2.4 -66.7]	20.2 [2.6 – 71.2]	

[1]N=73 for Aspirin group, N=64 for Placebo group.

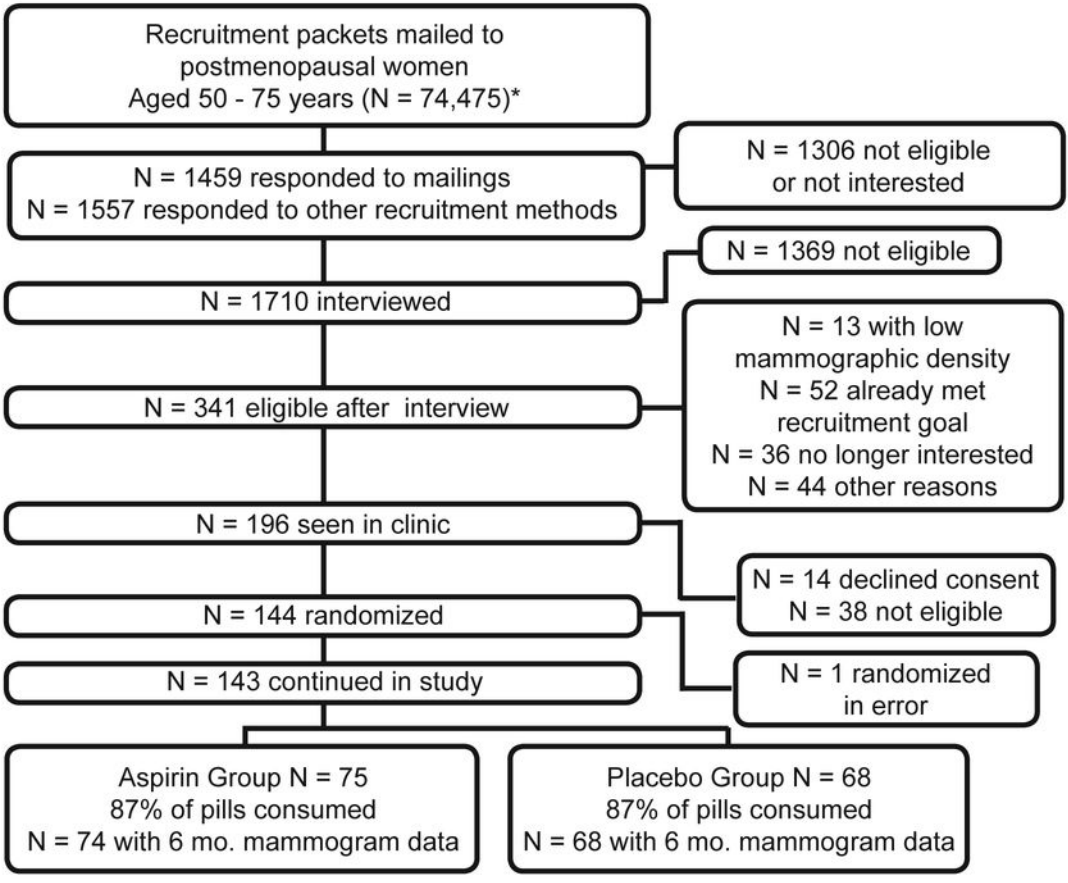
Table 2. Geometric Mean (95% CI) Baseline Mammogram Density in BI-RADS Classification.

	BI-RADS Classification		
	2 **N = 60**	**3** **N = 71**	**4** **N = 12**
Dense Area (cm^{-2})	13.8 (12.0, 15.9)	23.5 (20.6,26.9)	42.5 (34.8,51.9)
% Dense Area	10.8 (9.4, 12.5)	24.2 (21.2, 27.6)	49.1 (43.0, 56.1)
Total Area (cm^{-2})	127.7 (114.0, 143.1)	92.1 (87.3, 108.0)	86.5 (68.7, 109.0)

Table 3. Geometric Means (95% CI) for Baseline and 6 – Months Mammogram Density.

	Aspirin			Placebo		
	Baseline	6 Months	Change	Baseline	6 Months	Change
	N = 75	N = 74	(% Baseline)	N = 68	N = 68	(% Baseline)
Dense Area (cm^{-2})	19.2 (16.7, 22.2)	18.1 (15.5, 21.2) P = 0.64	-1.1 (-5.7%)	20.4 (17.4, 23.9)	18.6 (16.0, 21.7)	-1.8 (-8.8%)
%Dense Area	17.6 (14.8, 20.9)	16.8 (13.9, 20.3) P = 0.84	-0.8 (-4.5%)	19.2 (16.3, 22.7)	18.0 (15.2, 21.4)	-1.2 (-6.3%)
Total Area (cm^2)	109.5 (98.7, 121.5)	107.9 (96.1, 121.1) P = 0.60	-1.6 (-1.5%)	106.1 (94.5,119.1)	103.5 (92.4, 115.9)	-2.6 (-2.4%)

*P-value comparing change from baseline to 6 months in mammogram density measures in Aspirin versus Placebo, using GEE Models, adjusted for change in BMI.



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