Research Article

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Mammographic Density Change with 1 Year of Aerobic Exercise among Postmenopausal Women: A Randomized Controlled Trial

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Abstract

Background: The Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial examined the influence of aerobic exercise on biological factors that are associated with breast cancer risk. Mammographic density, a secondary outcome, is reported here.

Methods: The ALPHA Trial was a parallel group randomized controlled trial conducted between May 2003 and July 2007. Postmenopausal, sedentary women ages 50 to 74 years (n = 320) were evenly randomized to aerobic exercise (45 minutes, 5 days per week) or control (usual life-style) for 1 year. Dense fibroglandular tissue and nondense fatty tissue were measured from mammograms at baseline and 1 year using computer-assisted thresholding software for area measurements and a new technique that relies on the calibration of mammography units with a tissue-equivalent phantom for volumetric measurements.

Results: Nondense volume decreased in the exercise group relative to the control group (difference between groups = −38.5 cm^3; 95% confidence interval, −61.6 to 15.4; P = 0.001). Changes in total body fat accounted for this decrease. Changes in dense area and dense volume, measures that have previously been associated with breast cancer risk, were not significantly different between the groups ($P \geq 0.26$).

Conclusions: Achieving changes in mammographic measures may require more exercise or a study population with higher baseline levels of sex hormones or a wider range of mammographic density. The data from this study, however, suggest that the protective effect of exercise on breast cancer risk may operate through a mechanism other than mammographic density. *Cancer Epidemiol Biomarkers Prev; 19(4); 1112–21. ©2010 AACR.*

Introduction

Physical activity is associated with a lower risk of postmenopausal breast cancer; a recent systematic review found that most case-control and cohort studies showed risk reductions between 20% and 80% with recreational activity (1). These observational studies are not able to elucidate the mechanisms whereby physical activity reduces breast cancer risk (2). Randomized controlled trials in which physical activity is prescribed and closely monitored with an adequate comparison group can be used to examine how physical activity influences biological factors that are related to breast cancer risk.

Mammographic density is an established risk factor for breast cancer with risk increasing by an estimated four to six times in women who have mammographic density on >75% of the breast area compared with women who have it on <5% (3). Consequently, mammographic density has the potential to be a surrogate of the effects of some factors on breast cancer risk (4, 5). Mammographic density is conceptualized most frequently as a percentage: the area of radiologically dense fibroglandular tissue projected onto a mammogram relative to the total breast area that also includes adipose tissue (percent dense area). The components of this percentage, the absolute area of dense tissue (dense area), and the area with no dense tissue projected

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on it (nondense area) can also be considered but only dense area and percent dense area have been consistently and strongly associated with breast cancer risk (6).

A few correlational studies have suggested that physical activity may be inversely associated with percent dense area (7-10) but most have found no strong association (11-16). An association is biologically plausible because percent dense area seems to be sensitive to hormone levels given that it increases with the initiation of hormone replacement therapy (17, 18) and decreases with selective estrogen receptor modulators (19), and physical activity may decrease sex hormone levels (2). Because no longitudinal studies have examined if mammographic density changes with physical activity, we took the opportunity to examine this hypothesized relation in the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) Trial. This trial was designed to examine aerobic exercise in postmenopausal women in relation to putative biological intermediates of the inverse association between physical activity and breast cancer risk. We previously reported that the intervention resulted in a significant reduction in one of the primary outcomes of the trial, circulating estradiol levels (20); another primary outcome was adiposity. Here, we report the results for mammographic density, a secondary outcome of the trial, as measured with both the standard computer-aided assessment of area and a novel technique for the assessment of volume (21). We hypothesized that the exercise intervention would reduce mammographic measures that are associated with breast cancer risk.

Materials and Methods

The ALPHA Trial was a randomized controlled trial of a 1 y aerobic exercise intervention compared with usual lifestyle conducted in sedentary postmenopausal women. The main methods have been reported elsewhere (20). Here, we provide a summary and a detailed description of our mammographic density measures. The ALPHA Trial was reviewed by the Research Ethics Boards at the University of Calgary, the University of Alberta, and the Alberta Cancer Board. All participants were informed about the nature of the study through contact with study staff and at small group information sessions. They signed a consent form before the collection of study questionnaires and biological measurements for screening and outcome assessment.

Study participants and randomization

Women living in the cities of Calgary and Edmonton in the Canadian province of Alberta were recruited between May 2003 and June 2006 with follow-up through July 2007. They were recruited from the general population largely through two sources: targeted mailings to participants in the Alberta Breast Screening Program that has fixed sites in Calgary and Edmonton and is open to all women in the province ages 50 to 69 y, and media campaigns that included radio, newspaper and television coverage, and pamphlets and posters distributed in public places including physician offices, churches, and food markets. Briefly, eligible women were between 50 and 74 y of age; postmenopausal for at least 24 mo; not using hormone replacement therapy in the last 12 mo; sedentary (<90 min of weekly exercise at moderate intensity or higher or, if between 90 and 120 min, having a maximum oxygen consumption of <34 mL/kg/min); able to do physical activity as assessed by their physician; normal levels of fasting glucose, serum triglycerides, thyroid-stimulating hormone, and alanine aminotransferase; and willing to be randomized to either the exercise or control intervention. Furthermore, women were excluded if the radiologist reading their baseline mammograms rated their breasts to be entirely fatty or if they had breast augmentation or reduction. These eligibility criteria were assessed in several stages: a brief telephone interview, self-administered questionnaires about physical activity level and hormone use, review of radiology reports from the baseline mammograms, family physician assessment of ability to do unrestricted physical activity, blood screen, and fitness test.

Randomization of participants was done on a 1:1 basis into exercise and control groups and stratified by site (Calgary, Edmonton) and body mass index (BMI; <27.5, $\geq$27.5 kg/m^2). The randomization sequence was computer generated by a statistician in randomized blocks sized four to six within strata and was held in sealed opaque envelopes that were opened by the study coordinator in Calgary once eligibility was established and baseline questionnaires, bloods, mammograms, and other scans were collected. Although the participants and the researchers were not blind to the group assignment, all assessors of outcomes including those collecting and analyzing the fitness tests, biological specimens, and scans were blind to group assignment.

Intervention

Women in the exercise group undertook aerobic exercise for 1 y. Over the first 3 mo, the women worked up to exercising at a frequency of five times per week for 45 min in which at least three of the sessions were facility based (Westside Recreational Centre, Calgary; Behavioural Medicine Fitness Centre, University of Alberta, Edmonton) and the remaining two were home based. The target intensity was to be within 70% to 80% of the heart rate reserve, as recorded by a heart rate monitor; time was given for warm-up and cool-down. Examples of aerobic activities done are walking, cycling, or using an elliptical trainer. On-site exercise trainers worked individually with participants. Adherence was monitored through weekly log sheets recording type of activity, duration, and intensity. Women in the control group were asked not to change their current level of activity. Women in both groups were asked to maintain their usual diet.

Mammographic measures

Measurements were made from screening mammograms done at Alberta Breast Cancer Screening Program sites in Calgary and Edmonton. The two sites used the

same type of mammographic unit (LoRad M-IV, Hologic, Inc.) except when this unit malfunctioned, in which case, a mobile unit was used (n = 14 films). Both sites were accredited by the Mammography Accreditation Program of the Canadian Association of Radiologists. The staff at these sites recorded information from the film label, including the thickness of the scanned breast and the filter used. The time between the baseline mammogram and randomization was 63 d (interquartile range, 42-f106) and the time between the follow-up mammogram and the 12-mo date was −6 d (interquartile range, −18 to 3).

The right craniocaudal view was used for measurements except for the 15 cases in which it had a mark on it or information on the film label was missing; in this case, the left side was used for both time points. Films were digitized with a Lumisys 85 laser film scanner (Lumisys) that has a resolution of at least 512 dots per inch (1 pixel = 6.76×10^{-4} cm^2), covers optical densities ranging from 0.03 to 4.1, and has a 12-bit grayscale resolution. Only mammograms of participants having both baseline and 1-y mammograms were digitized. Personal identifiers and dates of examinations were permanently cropped from the images that were read "blind" to all information about the participants including their study identification number and the temporal sequence of the mammograms.

Area measurements were done using a software developed at the University of Toronto, Canada (22). The digitized mammograms were read in batches of 140 films that were set up to ensure that the 2 films from each participant were read in pairs but in random order, the pairs from all women were in random order, and each batch had equal proportions of women from each site, BMI category, and intervention group. First, an experienced reader (NFB; ref. 23) set a threshold on all digitized mammograms, delineating dense from nondense areas within the breast such that nodular densities and homogeneous densities were included as dense area, but fine, loose linear densities were excluded. Then, the breast edge was manually contoured on all digital mammograms by a second reader (CGW). The software calculated the area of the breast and the area above the density threshold within the breast area. For quality control, 30 pairs of films were reread in another batch and, of these, 20 pairs were reread within the same batch. Average reliability, as assessed with intraclass correlation coefficients from these rereads for measures of dense area, nondense area, and percent dense area was 0.94, 1.00, and 0.95, respectively.

Volumetric measurements were also made on the digitized mammograms (21, 24). The mammography units had been calibrated at baseline and every 6 mo thereafter by imaging a tissue-equivalent phantom consisting of a range of thicknesses representing totally fat to totally fibroglandular tissue. A thin aluminum step wedge nonobtrusively imaged with each mammogram compensated for variations in exposures and film processing, adjusting for brightness values relative to the original calibration. A model developed from this calibration data taking into account the image acquisition parameters and compressed breast thickness was used to relate the relative light exposure on the film to the proportion of fibroglandular tissue to fat at each pixel. Thus, dense volume, nondense volume, and percent dense volume across the entire breast could be calculated. Six batches were set up similarly to the batches for area measurements. Thirty pairs of films were remeasured in another batch and, of these, 24 pairs were remeasured within the same batch. Because the volumetric measurements do not rely on subjective reader input, the average reliability of the volumetric measures was very high; the intraclass correlations were 1.00.

Covariate information

Baseline information about demographic variables, menstrual and reproductive history, past hormone use, and if a physician had ever told participants that they had benign breast disease was derived from a self-administered questionnaire. Time-varying characteristics were measured at baseline and 1 y. Estimates of daily intake of energy, fat, and alcohol over the past year were derived from the 124-item Diet History Questionnaire developed at the NIH and modified for use in Canada (25). The Past Year Total Physical Activity Questionnaire was used to assess the frequency; duration; and intensity of occupational, household, and recreational physical activities (26). Volume of activity was calculated using the cost of each activity (metabolic equivalent value) as determined from the Compendium of Physical Activities (27).

Each participant had a fitness test done by trained exercise physiologists at the Human Performance Laboratory at the University of Calgary and the Behavioural Medicine Fitness Centre at the University of Alberta. Maximum oxygen consumption was estimated from submaximal exercise intensities using a modified Balke treadmill protocol (28). Weight and height measurements were made in duplicate; if the two measurements were discrepant, a third measurement was taken. The average of the two closest measurements was used in the analyses. BMI was calculated as weight (kg) divided by the square of height (m^2). Percent body fat was measured from whole-body dual X-ray absorptiometry scans done at the Foothills Medical Centre in Calgary and the Human Nutrition Research Unit at the University of Alberta in Edmonton.

Statistical analysis

Means with SD or frequency distributions were used to describe the characteristics of the participants. The main outcomes were the changes in mammographic measures between baseline and 1 y. Correlations among these outcomes were investigated with Pearson correlations. To test if the outcomes were different between the intervention groups, general linear models were fit adjusting for the baseline value of the mammographic measure and any of several preselected covariates associated with change in any of the mammographic measures at $P < 0.10$. Change in percent body fat was then introduced into the models to see if a change in overall adiposity accounted for any differences in the change in mammographic density

between the intervention groups. Our initial (intention to treat) analyses included all 320 participants who were randomized, assuming that those with missing outcome data experienced no change in the mammographic variables and those with missing values of mammographic variables and covariates had the mean of all the women without missing values. Analyses were repeated including only participants who had both baseline and 1 y measures and no missing values for covariates (complete case). The statistical significance of the interaction terms between intervention group and both study site and baseline BMI, the stratification variables for randomization, was assessed. The interaction term between group and the tertile of each mammographic measure at baseline was assessed to explore the possibility that the intervention effect may have been modified by the baseline value. For exploratory purposes, subgroups of the exercise group were formed according to the mean weekly duration of exercise based on public health guidelines (<150, 150-225, >225 min/wk; ref. 29); changes in mammographic measures between these subgroups and the control group were tested. All analyses were conducted with SAS 9.1 (SAS). The level of significance was set at 0.05; no correction was made for multiple testing.

The ALPHA Trial was planned with a sample size of 150 participants per group to give 80% power to detect differences between the intervention groups of 10% to 20% in serum estrogens, a primary outcome of the trial. With a SD around the mean change in percent dense area of 10% estimated from previous publications (17, 18), a sample size of 150 per group would give 99% power to detect a difference between the intervention and control groups of 5% in percent dense area, 93% power to detect a difference of 4%, and 74% power to detect a difference of 3%. Note that for each unit increase in percent dense area, breast cancer risk has been estimated to increase 2% (30).

Results

Participant recruitment and retention is shown in Fig. 1. Briefly, of the 3,454 women assessed for eligibility, 1,965 were ineligible, 895 refused participation, and 274 were

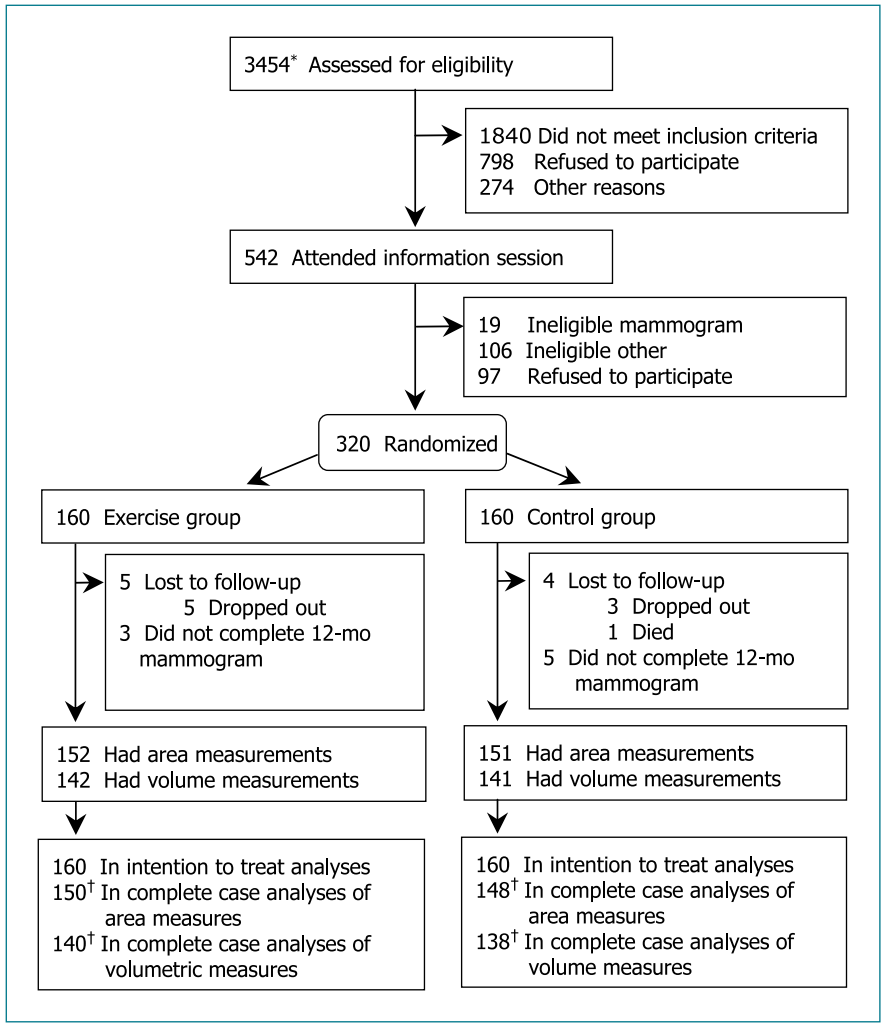

Figure 1. Participant recruitment, randomization, and follow-up. *, from 1,284 responses from mailings to 4,543 screening program participants and 2,170 responses from media campaigns. †, excludes subjects with missing values of covariates (n = 3 controls, n = 2 exercisers).

Table 1. Baseline characteristics of randomized participants

Characteristic	Exercise group (*n* = 160)	Control group (*n* = 160)
Age, mean (SD), y	61.2 (5.4)	60.6 (5.7)
Caucasian, *n* (%)	144 (91)	145 (91)
Full-time employment, *n* (%)	82 (55)	79 (51)
Education beyond high school, *n* (%)	112 (70)	102 (64)
Married or in common-law relationship	113 (71)	125 (78)
First degree relative with breast cancer, *n* (%)	31 (19)	35 (22)
Benign breast disease, *n* (%)	36 (23)	45 (28)
Past hormone replacement therapy use, *n* (%)	75 (47)	71 (44)
Age at menarche, *n* (%), y		
9-11	28 (18)	23 (15)
12	49 (31)	43 (27)
13	40 (25)	51 (32)
14-18	41 (26)	41 (26)
Years since menopause, *n* (%)		
1.1-<5	35 (22)	34 (21)
5-<10	50 (31)	48 (30)
10-<15	41 (26)	42 (26)
15-32.9	33 (21)	36 (23)
Age at first birth, *n* (%), y		
Nulliparous	16 (10)	13 (8)
16-24	81 (51)	87 (54)
25-43	62 (39)	60 (38)
Body composition measurements		
BMI, mean (SD), kg/m^2	29.1 (4.5)	29.2 (4.3)
Waist, mean (SD), cm	88.8 (10.6)	88.8 (10.5)
Percent body fat, mean (SD)	42.2 (4.9)	42.4 (5.7)
Maximal oxygen consumption, mean (SD), mL/kg/min	27.1 (6.2)	26.8 (6.0)
Recreational physical activity, mean (SD), h/wk	2.9 (3.4)	3.3 (3.5)

excluded for other reasons such as loss of contact. A total of 320 women were randomized but 9 did not complete the trial because they were either no longer interested, too busy, or passed away, and 8 did not complete the mammogram at 1 year. Of the 303 women with digitized mammograms, 20 could not have volumetric measures made because 13 had mammograms on the mobile unit without the calibration device, 3 had missing information on the film label, 3 had inadequate scan quality, and 1 had an improbable thickness (0.4 cm).

Baseline characteristics were similar between women in the exercise group and the control group (Table 1). Approximately half of the women were from each site and 91% were Caucasian. The controls were slightly younger than the exercisers [mean (SD), 60.6 (5.7) versus 61.2 (5.4) years]. Participants had a high mean (SD) BMI of 29.1 (4.2) kg/m^2 and reported 3.1 (3.5) hours/week of any intensity of recreational physical activity.

Women in the exercise group were prescribed to exercise over the year of the intervention for a mean of 4.8 sessions per week and a mean duration of 200 minutes per week; they recorded a mean (SD) of 3.6 (1.3) sessions per week for a total duration of 178.5 (76.1) minutes per week. Within categories based on public health guidelines, 39 (25.7%) women averaged <150 minutes/week, 66 (43.4%) women averaged 150 to 225 minutes/week, and 47 (30.9%) women averaged >225 minutes/week). Average heart rate was 62.2 (9.8)% of the estimated heart rate reserve. As assessed with the Past Year Total Physical Activity Questionnaire, recreational activity increased less in controls than in exercisers (3.2 versus 20.2 metabolic equivalent-hours/week; $P < 0.001$). Furthermore, aerobic fitness increased significantly more in exercisers than in controls (3.9 versus 0.7 mL/kg/min; $P < 0.001$). Mean energy intake decreased among controls relative to exercisers (161 versus 45 kcal/day; $P = 0.01$). No adverse effects related to the intervention occurred.

Baseline percent dense area on the mammogram ranged from 0% to 56.1%, with a median of 14.6% (Fig. 2). The percent dense volume ranged from 0% to 32.8%, with a median of 1.2%. At baseline, corresponding area and volume measures were directly correlated (dense, $r = 0.63$; nondense, $r = 0.92$; percent dense, $r = 0.68$). Changes between baseline and 1 year in corresponding area and volume measures were not highly correlated (dense, $r = -0.02$; nondense, $r = 0.52$; percent dense, $r = 0.16$).

Results of analyses that included all women who were randomized, assuming that women with missing data experienced no change in the mammographic variables, were very similar to the results of analyses that were restricted to the women with both baseline and 12-month measurements; thus, results from women with complete measurements are presented. Mean changes in mammographic measures before and after adjustment for change in percent body fat are shown in Fig. 3. Relative to controls, exercisers had a significant decrease in nondense volume ($P = 0.001$) and a nonsignificant decrease in nondense area ($P = 0.08$). Because percent body fat decreased more in the exercise group than in the control group (2.0% versus 0.2%, $P \leq 0.001$) and was correlated with the change in

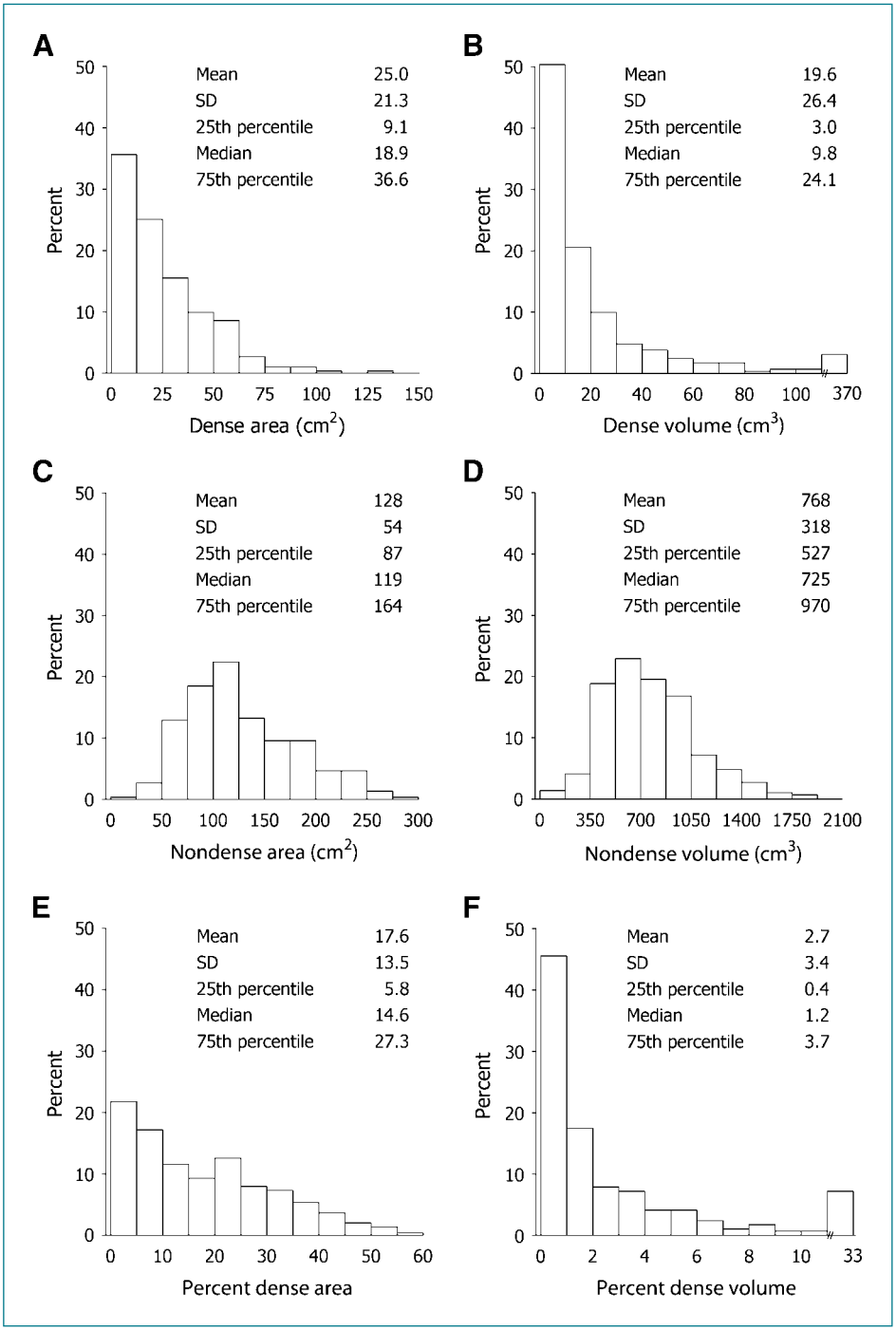

Figure 2. Distribution of mammographic measures at baseline: A, dense area; B, dense volume; C, nondense area; D, nondense volume; E, percent dense area; F, percent dense volume.

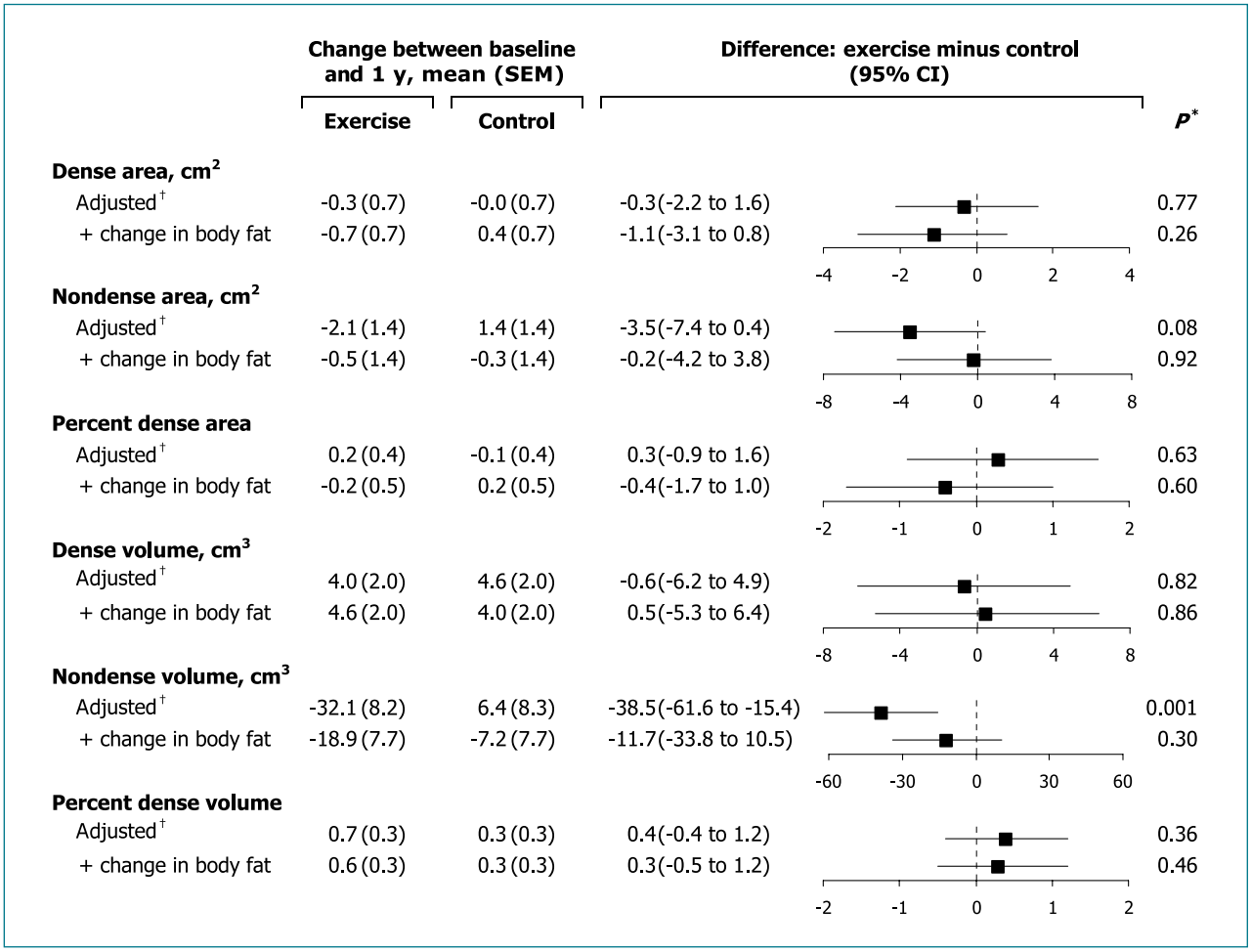

Figure 3. Change in mammographic measures before and after adjustment for change in percent body fat, by intervention group. *, *P* value for difference in mean change between exercisers and controls, from general linear models. †, adjusted for baseline mammographic density, age, age at menarche, age at first birth, and years since menopause. Abbreviation: 95% CI, 95% confidence interval.

nondense volume ($r = 0.45$), after adjusting for change in percent body fat, changes in nondense volume were no longer significantly different between exercisers and controls. No significant differences were observed between the groups in change in dense area, percent dense area, dense volume, and percent dense volume ($P \geq 0.26$). The effect of the intervention was not modified by the stratification variables, site, and BMI ($P > 0.05$). The effect of the intervention on change in each mammographic measure was not modified by its baseline value ($P > 0.54$).

When exercisers were categorized by mean weekly duration of exercise, a significant difference in change in nondense area and volume was seen between the higher duration exercisers and the controls (Fig. 4). Because the change in percent body fat was correlated with mean duration of exercise ($r = -0.37$; $P < 0.001$), no significant differences remained after adjustment for change in percent body fat. The change in the other mammographic measures was not different between any of these exercise duration groups and controls.

Discussion

In this randomized controlled trial, changes in mammographic measures reflecting the amount of fibroglandular tissue in the breast were not affected by a year-long aerobic exercise intervention in postmenopausal women. These measures (dense area and volume) have been associated with breast cancer risk (3, 21). Measures reflecting the amount of fatty tissue in the breast, however, were significantly decreased in the exercise group relative to the control group but the change in overall body adiposity accounted for this decrease. These measures (nondense area, breast area) have not been consistently associated with breast cancer risk (6, 31-33).

Although a few studies suggested that physical activity may be inversely associated with percent dense area (7-10), most have not (11-16) and, in some, weakly positive associations were reported before adjustment for BMI (14-16). The studies that suggested an inverse association have used subjective categorical approaches to

measuring mammographic density (7, 8), or have found the association only in an obese subgroup (9) or a sample with a high proportion of obese subjects (10). Two publications have reported a null association between physical activity and breast area or nondense area after adjustment for BMI (9, 15), although before adjustment, the association was inverse (15). To our knowledge, no other results have been published about the associations between changes in physical activity and changes in mammographic measures, but our null results are consistent with most cross-sectional studies.

Biological mechanisms hypothesized to mediate the inverse association between physical activity and breast cancer risk include a decrease in circulating estrogens, growth factors, adipocytokines, and inflammatory factors (2). These factors are mitogens that could affect the amount of fibroglandular tissue in the breast and consequently, mammographic dense area, and volume (34). Although the sensitivity of percent dense area to sex hormones is shown by its increase with the initiation of exogenous estrogens with progestins (17, 18), variation greater than that influenced by physical activity may be needed to affect it. Consider that, relative to controls, the exercisers in the ALPHA Trial had an average decrease in serum estradiol levels of 1.2 pg/mL (20); 1.2 pg/mL is only about 3% of the increase in serum estradiol seen with the initiation of a 0.05 mg/d estradiol patch that is associated with an average increase in percent dense area of 4% (35). Although the reliability of the mammographic measures was very high, the absolute difference between repeated measures was often higher than the small changes that could be expected to occur after 1 year of aerobic exercise (36). The insensitivity of percent dense area to variation in circulating levels of sex hormones at low levels is further supported by the null or inconsistent associations seen in cross-sectional studies of postmenopausal women not using exogenous hormones (34).

Other plausible mediators of the association between physical activity and breast cancer risk (2) could also be associated with mammographic density. Although insulin-like growth factor-1, markers of oxidative stress, and vitamin D status have been associated with percent dense area in some studies, the association is not consistent (34). Other circulating factors that have been found to be associated with percent dense area, such as prolactin and growth hormone (34), are not strongly related with physical activity or adiposity in postmenopausal women (37).

The exercise intervention in this study resulted in a reduction of the amount of fatty tissue in the breast. In our analysis, no significant effect of the intervention on nondense volume remained after adjustment for change in percent body fat. Thus, in a state of negative energy balance, the fat of the breast seems to be depleted similarly to fat elsewhere in the body, and is not resistant or protected

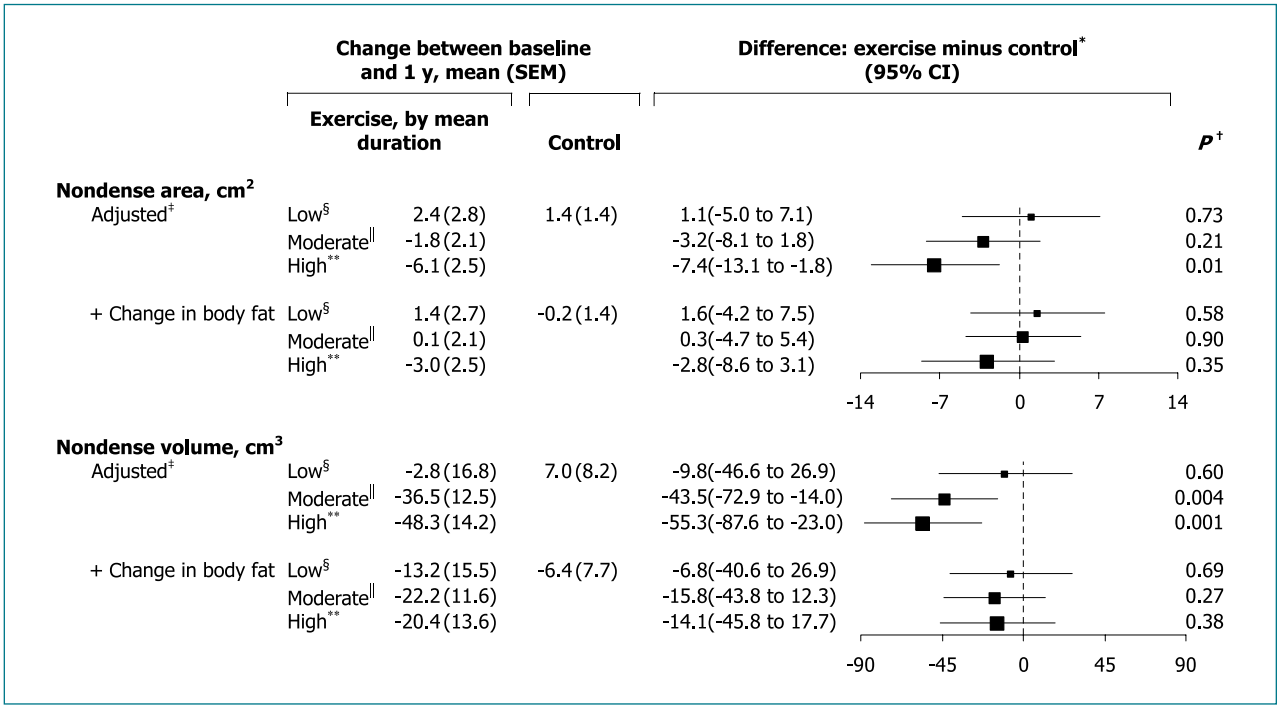

Figure 4. Change in nondense area and nondense volume before and after adjustment for change in percent body fat, by mean duration of exercise. *, difference between mean change in exercisers in that exercise duration group and mean change in controls. †, *P* value for difference in mean change between exercisers in that exercise duration group and controls. ‡, adjusted for baseline mammographic density, age, age at menarche, age at first birth, and years since menopause. §, low duration: <150 min/wk. For analysis of nondense area, *n* = 38, and nondense volume, *n* = 33 exercisers. ‖, moderate duration: 150 to 225 min/wk. For analysis of nondense area, *n* = 65, and nondense volume, *n* = 60 exercisers. b, high duration: >225 min/week. For analysis of nondense area, *n* = 47, and nondense volume, *n* = 47 exercisers.

while other fat depots are depleted. Because adipocytes are a source of sex hormones (38, 39) and inflammatory cytokines (40), a reduction in breast adipose tissue could lower the levels of these factors immediately adjacent to the breast epithelial cells (41, 42). Note that treatment with aromatase inhibitors, which would be expected to reduce the local levels of estrogens in the breast, results in a reduction in proliferation (43) and potentially important changes in gene expression (44) of breast epithelial cells but does not result in a change in mammographic density (43).

Whether mammographic density changes with respect to breast cancer risk factors and chemopreventive agents is being investigated (5, 17-19, 35). The validity of using mammographic density as a surrogate outcome for the effects of many of these factors or agents has not yet been established. It appears from this study, in which an aerobic exercise intervention did not change mammographic measures that are associated with breast cancer risk, that it may not be of value when investigating physical activity interventions. Note that we had a study group that was restricted based on strict eligibility criteria and drawn from a largely Caucasian population, which may limit the generalizability of these findings. It also could be that the protective effect of exercise may operate through a mechanism other than mammographic density; the effect may only occur or be detectable in women with higher baseline levels of sex hormones or mammographic density; a higher amount of exercise is needed to effect changes in the breast large enough to be measurable as mammographic density; or any changes may only be detectable with a measurement of breast composition that is more responsive to change. Other factors, such as sex hormone levels or breast biomarkers measured in sampled breast cells may be alternative surrogate outcomes for the effect of physical activity on breast cancer risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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