Effect of Exercise on Markers of Inflammation in Breast Cancer Survivors: The Yale Exercise and Survivorship Study

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Abstract

Physical activity is associated with improved breast cancer survival, but the underlying mechanisms, possibly including modification of the inflammatory state, are not well understood. We analyzed changes in interleukin (IL)-6, C-reactive protein (CRP), and TNF-α in a randomized controlled trial of exercise in postmenopausal breast cancer survivors. Seventy-five women, recruited through the Yale-New Haven Hospital Tumor Registry, were randomized to either a six-month aerobic exercise intervention or usual care. Correlations were calculated between baseline cytokines, adiposity, and physical activity measures. Generalized linear models were used to assess the effect of exercise on IL-6, CRP, and TNF-α. At baseline, IL-6 and CRP were positively correlated with body fat and body mass index (BMI) and were inversely correlated with daily pedometer steps ($P < 0.001$). We found no significant effect of exercise on changes in inflammatory marker concentrations between women randomized to exercise versus usual care, though secondary analyses revealed a significant reduction in IL-6 among exercisers who reached 80% of the intervention goal compared with those who did not. Future studies should examine the effect of different types and doses of exercise and weight loss on inflammatory markers in large-scale trials of women diagnosed with breast cancer. *Cancer Prev Res; 6(2); 109–18. ©2012 AACR.*

Introduction

Breast cancer is the most common cancer diagnosis among American women, accounting for 30% of cancer diagnoses and 15% of cancer-related deaths, with an estimated 230,480 women diagnosed in 2011 (1). Increasing incidence from 1980 to 2001 and improved treatment strategies have resulted in large numbers of breast cancer survivors, a group currently estimated at 2.5 million. However, long-term side effects remain, including a risk of breast cancer recurrence and risk of cardiovascular disease (2). Chronic low-grade inflammation is a risk factor for cardiovascular disease (3), metabolic diseases (4, 5), and breast cancer recurrence and mortality (6, 7). Obesity and sedentary behavior are linked to chronic low-grade inflammation, which could increase cardiovascular disease and recurrence risk in breast cancer survivors (8–10). This potential risk is highlighted by prior findings that more than 50% of breast cancer survivors are overweight or obese (11) and the combination of excess body weight and low levels of physical activity have been linked to one third to one fourth of all breast cancer cases (12). These factors make it critical to understand the effects of lifestyle factors on survivorship and to identify modifiable factors such as promoting a healthy weight and increased levels of physical activity that may improve disease-free survival and quality of life for women diagnosed with breast cancer.

Physical activity is a modifiable lifestyle factor, which has been shown to decrease risk for breast cancer and improve quality of life after a breast cancer diagnosis. In recent systematic reviews by Ballard-Barbash and Löf and colleagues, the authors reviewed the relationship between physical activity and cancer-relevant biomarkers including sex hormones, insulin, adipokines, and inflammatory markers (13, 14). The authors concluded that there was a biologic basis for exercise and breast cancer mortality as exercise may benefit changes in circulating insulin, insulin-like growth factors (IGF), IGF-binding proteins (IGFBP), as well as inflammatory biomarkers (13, 14). The mechanisms of change are not fully understood but could include reduction in adipose tissue, chronic inflammation (15, 16), and through the promotion of an anti-inflammatory environment. Studies have shown that breast cancer survivors have higher levels of circulating cytokines than women without breast cancers; a dysregulation that may persist up to 5 years after diagnosis (17, 18). Identifying factors that reduce chronic inflammation and interventions, which effectively promote an anti-inflammatory environment are important avenues of research.

Several pleiotropic cytokines associated with cancer and chronic low-grade inflammation correlate with sedentary lifestyle, adiposity, and low aerobic fitness (19). Interleukin

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(IL)-6 and TNF-α are proinflammatory cytokines secreted by a variety of cell types and tissues, including tumor cells, infiltrating macrophages, and adipocytes, the latter of which may produce as much as 25% of circulating IL-6 (18). Both cytokines can stimulate the hepatocyte-derived acute phase protein C-reactive protein (CRP), another marker of inflammation. IL-6, CRP, and TNF-α are elevated in breast and several other types of cancer (7, 20, 21). IL-6 has been found to correlate with both disease stage and extent of metastasis as well as breast cancer recurrence (7). In addition, TNF-α is a risk factor in cardiovascular disease and metabolic syndrome, as well as increased in obesity and aging (22, 23). Elevated CRP concentrations are associated with mortality in women diagnosed with breast cancer as well as increased risk for cardiovascular disease (7, 24).

Exercise may provide beneficial changes in circulating levels of nonspecific markers of chronic low-grade inflammation. Preliminary evidence in populations with moderate to high levels of inflammatory markers, such as patients with cardiovascular disease, has found that regular aerobic exercise is associated with reductions in circulating proinflammatory cytokines (25–28). In a yearlong intervention of moderate- to vigorous-intensity aerobic exercise (5 times a week for 45 minutes) in healthy postmenopausal women, higher doses of exercise were associated with lower CRP levels (29). However, participants in the study had lower levels of CRP at baseline, making it unclear whether this approach is beneficial in populations with breast cancer and elevated CRP levels. Results of these studies suggest that these markers are associated with both higher adiposity and lower levels of physical activity (30–32). To date, very few randomized controlled trials of exercise alone (or without dietary weight-loss) in postmenopausal breast cancer survivors have examined the effects of exercise on inflammatory markers (33–35, 37). Given the observed benefits of physical activity interventions on these inflammatory markers in other clinical populations, it is important to understand whether these effects generalize to breast cancer survivors.

The purpose of this study was to examine changes in plasma concentrations of the proinflammatory markers IL-6, CRP, and TNF-α, after 6 months of aerobic exercise versus usual care in breast cancer survivors enrolled in the Yale Exercise and Survivorship Study. Understanding the effects of moderate-intensity aerobic exercise protocol on chronic low-grade inflammation could provide treatment options to decrease risk of not only breast cancer recurrence and mortality, but also cardiovascular risk and mortality in breast cancer survivors.

Materials and Methods

Participants

Participants were recruited into the Yale Exercise and Survivorship Study, described in detail elsewhere (38), by study staff using the Yale-New Haven Hospital Tumor Registry to obtain the names of Connecticut women diagnosed with breast cancer by any Yale-affiliated physician from March 1994 to January 2006 (Fig. 1). Participants were physically inactive (<60 min/wk of recreational physical activity reported in the past 6 months), postmenopausal women diagnosed with stage 0 to IIIA breast cancer and who had completed adjuvant treatment (except endocrine therapy) at least 6 months before enrollment. Women taking aromatase inhibitors or tamoxifen were eligible for participation. Postmenopausal status was defined as women who had not menstruated in the last 12 months before the baseline visit. Women could have gone through natural menopause before diagnosis or before enrollment in our study, but women were also eligible if they went through chemotherapy-induced menopause. Women with type II diabetes, previous cancer, and smokers were excluded because of the potential effect of these factors on outcomes of interest. Seventy-five (9.5%) of 788 patients screened were deemed eligible, consented, and were randomized. Randomization to the exercise or usual care group occurred after completion of all baseline measures using a random number generation. All study procedures were reviewed and approved by the Yale University School of Medicine (New Haven, CT) Human Investigation Committee.

Anthropometric, dual energy X-ray absorptiometry, dietary and medical history measurements

Demographic characteristics and medical history were collected via an interviewer-administered questionnaire at the baseline visit, and clinical data were later confirmed by physician and medical record review. Height, weight, waist, and hip circumference were measured at baseline and 6 months using a digital scale and stadiometer. Circumference measurements were taken at the waist (minimum circumference) and hips (greatest circumference). All measurements were taken twice in succession, by the same technician, and averaged for data entry. A dual energy X-ray absorptiometry (DEXA) scan was completed for each participant at both visits using a Hologic scanner (Hologic 4500, Hologic Inc.) to assess body fat and lean mass. All DEXA scans were evaluated by 1 radiologist blinded to the intervention group of the participant. Dietary intakes were measured with a 120-item validated food frequency questionnaire at baseline and 6 months to control for any changes in diet, though participants were advised to maintain their current dietary habits (39).

Physical activity measures

At baseline and 6 months, participants completed 3 physical activity questionnaires: the physical activity questionnaire (PAQ; ref. 38), the 7-day physical activity log (7-Day PAL; ref. 40), and a 7-day pedometer log (41). The PAQ was used to determine eligibility by verifying participants past 6 months of recreational activity. The 7-Day PAL was completed by all participants before randomization and at the 6-month follow-up visit and was also used to measure adherence in the exercise group recording type and duration of any recreational activity conducted on each day, along with their corresponding heart rate, as measured by a heart rate monitor (Polar). Hours per week spent in moderate- to vigorous-intensity aerobic activity were determined using
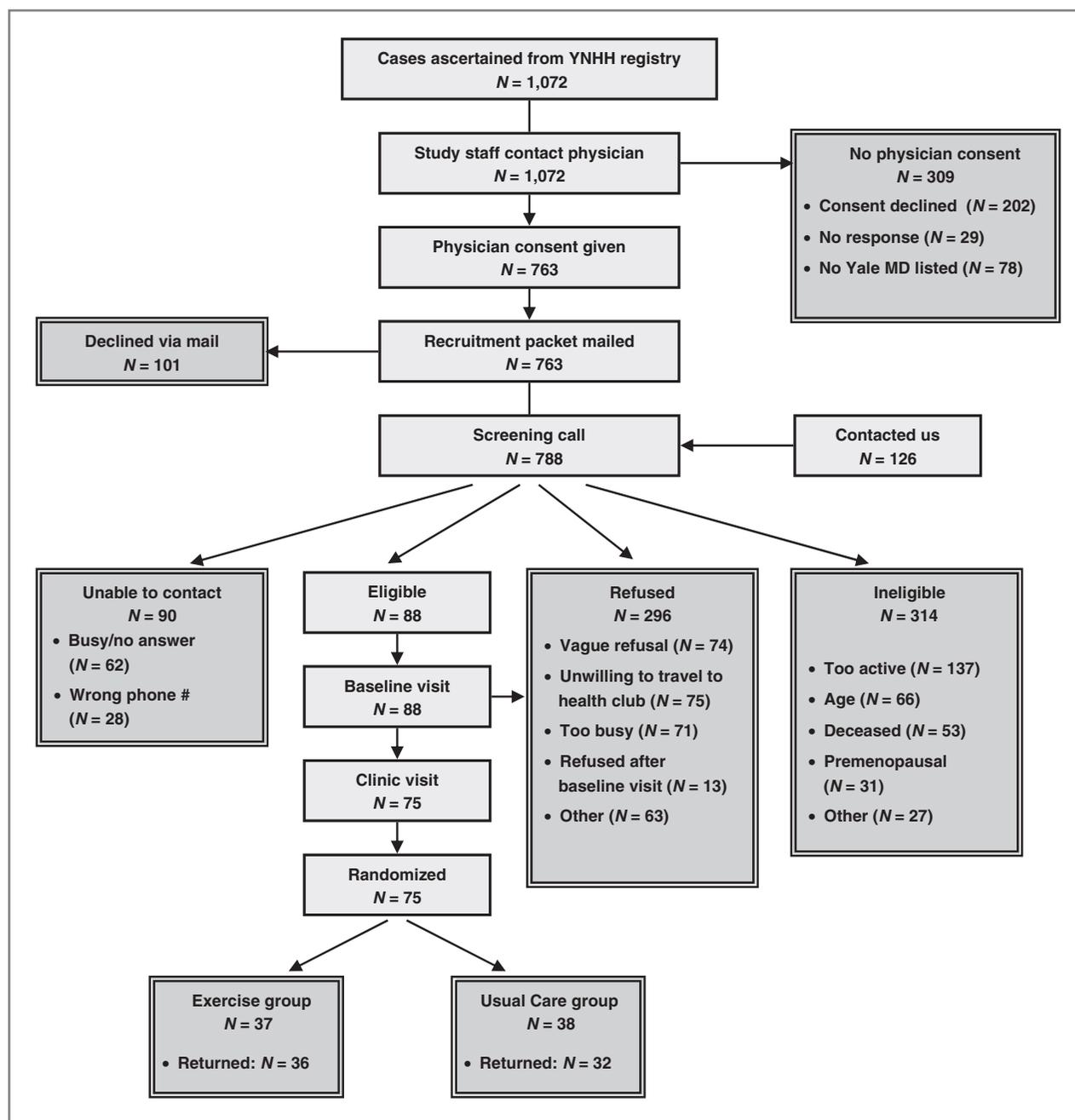
Figure 1. Flow of participants through the YES study.

Ainsworth's Compendium of Physical Activities (42). Participants measured their daily walking steps using the 7-day pedometer log before randomization and at the 6-month follow-up visit.

Exercise intervention

The participants in the exercise intervention were instructed to complete 150 minutes of moderate intensity aerobic exercise, which consisted of 3-weekly certified exercise trainer–supervised exercise sessions at a local health club and twice-weekly unsupervised exercise sessions. Exercise sessions consisted primarily of brisk walking, though participants could meet the exercise goal through other forms of aerobic exercise, such as stationary biking and elliptical training. Activities that did not involve sustained aerobic effort, such as resistance training and yoga, could be conducted but did not count toward the exercise goal for each week. Participants gradually increased minutes of exercise per week by completing 3 15-minute sessions during week 1, building to 5 30-minute moderate-intensity

sessions by week 5. Exercise started at 50% of predicted maximal heart rate (220-age) and was gradually increased in accordance with American College of Sports Medicine (ACSM; Indianapolis, IN) guidelines to approximately 60% to 80% of predicted maximal heart rate. Participants wore heart rate monitors for each exercise session to enable self-monitoring of exercise intensity (Polar Electro). Following each exercise session, participants recorded the type, duration, perceived intensity of activity, and average heart rate during exercise in physical activity logs. Physical activity logs were collected weekly to ensure weekly compliance.

Women in the usual care group were instructed to continue with their usual activities. If a participant wanted to exercise, she was told she could, but that the exercise program and training materials would not be offered to her until the end of the study.

Inflammatory marker assays

Fasting blood draws were collected at the baseline and 6-month clinic visits and plasma samples were stored at $-80°C$ until assayed. Plasma concentrations of IL-6, CRP, and TNF-α were measured using ELISA kits from R&D Systems, Inc.; high-sensitivity kits were used for IL-6 and TNF-α. The assay sensitivities for IL-6, CRP, and TNF-α were 0.039 pg/mL, 0.010 ng/mL, and 0.106 pg/mL, respectively. Samples were assayed in batches from the same lot such that the baseline and 6-month sample from each participant were assayed together and the number of samples from each intervention group was balanced within each batch. Laboratory personnel were blinded to intervention group. Samples were run in duplicate with coefficients of variation for all samples less than 10% and averaging 3.0% for IL-6, 3.1% for CRP, and 3.2% for TNF-α.

Statistical analyses

Baseline and 6-month blood samples were available for 68 of 75 participants (32 usual care and 36 exercisers) because of missing blood draws for 7 women. Participants with CRP concentrations indicative of acute infection, that is, 15 mg/L or higher (43), were excluded from analyses. One woman randomized to the usual care group met these criteria with a CRP concentration of 124 mg/L, resulting in a final sample size of 67. Baseline differences between intervention groups were assessed using χ^2 statistics for categorical variables and t tests for continuous variables. Spearman correlation coefficients were calculated between baseline cytokine and CRP concentrations, adiposity, and physical activity measures. Percentage changes in biomarker concentrations from baseline to 6 months were calculated as follows: [mean baseline to 6-month difference]/[mean baseline value] $\times$ 100. The t tests and generalized linear models (GLM) were used to assess intervention effects according to the intent-to-treat principle. Multivariate models controlling for baseline characteristics including marker concentration, age, race, education, time since diagnosis, tumor stage, radio- and/or chemotherapy treatment, hormone therapy, weight, body mass index (BMI), percentage body fat, and physical activity were similar to univariate

models, and therefore only unadjusted results are presented. All analyses were repeated after logarithmically transforming cytokine values to account for their skewed distributions but are not shown as the results were unchanged. We used GLM to assess *a priori* effect modification of baseline variables (tumor stage, hormone therapy use, radio- and/or chemotherapy treatment, time since diagnosis, BMI, percentage body fat, and body weight) and change in percentage body fat. Finally, the effect of adherence to the intervention within the exercise group was determined using GLM controlling for baseline biomarker concentration. Adherence was defined as meeting 80% of the exercise prescription, that is, 120 min/wk of activity, or 1,590 steps/d based on 1 mile composed of 1,987 steps (44). All analyses were conducted using SAS version 9.1 software (SAS Institute Inc.).

Results

Study subjects

There were no significant differences between the exercise and usual care groups at baseline with regard to demographics, clinical characteristics, body composition, pedometer steps per day, or inflammatory marker concentrations (Table 1). Exercisers had lower stage tumors than usual care women ($P = 0.04$) and borderline significantly higher minutes per week of physical activity; though, activity levels for both groups were quite low. Overall, participants ranged in age from 34 to 79 years with a mean of 56 years and were predominately non-Hispanic White. A majority of women were overweight or obese (mean BMI $= 30.0 \pm 6.6$ kg/m^2) and had low physical activity levels (mean duration physical activity $= 21.8 \pm 38.0$ min/wk). The mean baseline cytokine levels for women were 2.79 ± 4.70 pg/mL for IL-6, 2.45 ± 2.43 mg/L for CRP, and 1.21 ± 0.56 pg/mL for TNF-α.

Baseline correlations

At baseline there was a moderate correlation between IL-6 and CRP ($r = 0.46$; $P < 0.0001$) and modest, nonsignificant correlations between IL-6 and TNF-α ($r = 0.21$; $P = 0.09$) and CRP and TNF-α ($r = 0.22$; $P = 0.08$; Table 2). IL-6 was positively correlated with percentage body fat, body weight, and BMI ($r = 0.49$; $r = 0.63$; $r = 0.65$; $P < 0.0001$, respectively) as was CRP ($r = 0.43$, $P < 0.001$; $r = 0.57$, $P < 0.0001$; $r = 0.60$, $P < 0.0001$). There was a modest correlation between TNF-α and weight ($r = 0.25$; $P = 0.04$) but not with either percentage body fat or BMI. IL-6 and CRP were inversely correlated with pedometer steps per day ($r = -0.42$, $r = -0.44$; $P < 0.001$), but not with minutes per week of physical activity. TNF-α was not associated with either measure of baseline physical activity.

Physical activity levels and intervention adherence

At 6 months, the exercise group had a significant increase in moderate-to vigorous-intensity recreational activity compared with the usual care group (129 min/wk vs. 45 min/wk; $P < 0.001$) as well as a significant increase in daily pedometer steps (1,621 steps or 0.8 miles vs. 38 steps

Table 1. Baseline characteristics of randomized participants ($N = 67$)[a]

	Exercise group (N = 36)	Usual care group (N = 31)	P value
Age, y	56.4 (9.6)	55.4 (7.6)	0.64
Ethnicity			
Non-Hispanic White	30 (83%)	27 (87%)	0.16
African-American	6 (17%)	2 (6%)	
Asian/Pacific Islander	0 (0%)	1 (3%)	
Unknown	0 (0%)	1 (3%)	
Education			
High school graduate	6 (17%)	6 (19%)	0.86
Some school after high school	9 (25%)	9 (29%)	
College graduate and beyond	21 (58%)	16 (52%)	
Time since diagnosis, y	3.5 (2.1%)	3.1 (2.4%)	0.51
Stage			
In situ	4 (11%)	4 (13%)	0.04
Stage I	20 (56%)	7 (23%)	
Stage II	9 (25%)	14 (45%)	
Stage III	3 (8%)	6 (19%)	
Treatment			
None	2 (6%)	4 (13%)	0.36
Radiation only	15 (42%)	7 (23%)	
Chemotherapy only	7 (19%)	7 (23%)	
Radio- and chemotherapy	12 (33%)	13 (42%)	
Hormone therapy			
None	15 (42%)	9 (29%)	0.22
Tamoxifen	11 (31%)	7 (23%)	
Aromatase Inhibitors	10 (28%)	15 (48%)	
Weight, kg	81.3 (17.0)	77.3 (20.0)	0.38
BMI, kg/m^2	30.6 (6.0)	29.4 (7.3)	0.46
% Total body fat (DEXA)	41.3 (6.5)	38.6 (5.7)	0.08
Physical activity[b], min/wk moderate-to-vigorous intensity recreational exercise	30.3 (41.4)	11.9 (31.3)	0.05
Pedometer average, steps/d	5083 (2,313)	5661 (2,740)	0.35
IL-6, pg/mL	3.55 (6.29)	1.91 (1.01)	0.13
CRP, (mg/L	2.47 (2.35)	2.43 (2.55)	0.95
TNF-α, pg/mL	1.15 (0.52)	1.28 (0.60)	0.36

[a]Data are presented as mean (SD) for continuous variables and frequency (percentage) for categorical variables. Percentages may not sum to 100 due to rounding.
[b]Assessed from the 7-day physical activity log administered at baseline.

or 0.02 miles, $P < 0.001$; data not shown). The exercise goal was 150 min/wk of moderate-intensity aerobic exercise; 33% of women achieved this amount. 56% of women achieved 80% of the exercise goal or 120 min/wk, and 75% of women achieved 90 min/wk. Comparison of food frequency questionnaires revealed no significant dietary changes in either exercisers or usual care (data not shown).

Main effects

After 6 months, plasma concentrations of IL-6, CRP, and TNF-α did not differ between randomization groups (Table 3). In the exercise group, IL-6 increased 0.04 pg/mL (1.13%), CRP decreased 0.08 mg/L (3.24%), and TNF-α

increased 0.02 pg/mL (1.74%). In the usual care group, there was no change in IL-6, whereas CRP decreased 0.21 mg/L (8.64%) and TNF-α increased 0.08 pg/mL (5.74%). Adjustments in GLMs for baseline characteristics, including tumor stage, which was slightly unbalanced at baseline, did not significantly affect the results. Results were also unchanged when inflammatory marker concentrations were logarithmically transformed to achieve normality.

Stratified analyses

Analyses were conducted stratified by baseline BMI, percentage body fat, weight, percentage body fat change, tumor stage, hormone therapy, radio- and/or chemotherapy

Table 2. Correlations with IL-6, CRP, and TNF-α (N = 67)

	IL-6 (pg/mL)	CRP (mg/L)	TNF-α (pg/mL)
CRP, mg/L	0.46 $P < 0.0001$		
TNF-α, pg/mL	0.21 $P = 0.09$	0.22 $P = 0.08$	
Percentage total body fat (DEXA)	0.49 $P < 0.0001$	0.43 $P < 0.001$	0.03 $P = 0.79$
Weight, kg	0.63 $P < 0.0001$	0.57 $P < 0.0001$	0.25 $P = 0.04$
BMI, kg/m^2	0.65 $P < 0.0001$	0.60 $P < 0.0001$	0.19 $P = 0.13$
Physical activity, min/wk recreational activity[a]	−0.004 $P = 0.97$	−0.13 $P = 0.28$	−0.07 $P = 0.53$
Pedometer average, steps/d	−0.42 $P < 0.001$	−0.43 $P < 0.001$	−0.11 $P = 0.38$

[a]Assessed from the 7-day physical activity log administered at baseline.

treatment, and time since diagnosis, all of which were selected *a priori*. No statistically significant within-group effects or interactions were found, though statistical power was limited. However, we did observe a borderline statistically significant correlation between change in percentage body fat and change in CRP among women randomized to exercise ($r = 0.27$; $P = 0.069$). The relationship though between change in body fat and change in CRP was not observed in women randomized to usual care.

Adherence effects

Exercisers were stratified by adherence based on achieving 80% of the exercise goal, that is, an increase over baseline of 120 min/wk of physical activity or an increase of 1,590 steps/d. Among women who increased physical activity by 120 min/wk, IL-6 concentrations decreased 0.56 pg/mL (14.29%), whereas among women who did not increase physical activity by 120 min/wk, IL-6 concentrations increased by 0.66 pg/mL (18.54%; $P < 0.01$; data not shown). Results using pedometer logs to measure adherence were similar, though did not reach statistical significance. Changes in CRP and TNF-α did not differ by either measure of adherence.

Discussion

In a 6-month moderate-intensity aerobic exercise intervention, we observed no significant effect of exercise on CRP, IL-6, or TNF-α concentrations between women randomized to exercise versus usual care, though secondary analyses revealed a significant reduction in IL-6 among

exercisers who exercised at least 120 min/wk compared with those who exercised less than 120 min/wk. Consistent with prior studies (45), we found that IL-6 and CRP were associated with higher adiposity as well as with lower levels of physical activity at baseline. Our study also observed changes in body fat associated with changes in CRP among women randomized to exercise.

Fairey and colleagues also conducted a randomized exercise trial in postmenopausal breast cancer survivors and found a nonsignificant 1.39 mg/L decrease in CRP in women randomized to a 15-week, 3 times weekly cycling intervention compared with a 0.10 mg/L increase in the control group (37). Although the authors did not examine circulating IL-6 or TNF-α, they reported no change in the production of either cytokine by cultured blood mononuclear cells (46). This assessment of cytokine production is not reflective of *in vivo* concentration nor does it measure production from other sources including adipocytes.

Compared with a prior study of a yearlong aerobic exercise intervention in healthy women, we did not find differences in CRP. This may be due to design differences. For example, the prior study exceeded ACSM recommendations for weekly exercise over a 12-month time period (26). The present study's exercise prescription met current ACSM recommendations over a shorter duration of 6 months, although on average participants only completed 81% of the recommended weekly exercise, and only 56% of our women randomized to exercise conducted at least 120 min/wk of exercise. Thus, our null findings may be because of too low of a dose of exercise conducted. Changes in IL-6 were

Table 3. Concentrations of IL-6, CRP, and TNF-α at baseline and 6 months[a]

Marker	Exercise group (N = 36)			Usual care group (N = 31)			Difference between groups[b]	P value
	Baseline	6 mo	Mean change	Baseline	6 m	Mean change		
IL-6, pg/mL	3.55 (6.29)	3.59 (6.03)	0.04 (1.32)	1.91 (1.01)	1.91 (1.19)	0.00 (1.20)	0.04	0.91
CRP, mg/L	2.47 (2.35)	2.39 (2.26)	−0.08 (0.74)	2.43 (2.55)	2.23 (2.60)	−0.20 (1.80)	0.12	0.73
TNF-α, pg/mL	1.15 (0.52)	1.17 (0.40)	0.02 (0.22)	1.28 (0.60)	1.35 (0.63)	0.08 (0.47)	−0.06	0.54

[a]Data are presented as mean (SD).
[b]Mean change in exercise group minus mean change in usual care group.

observed among women exercising at least 120 min/wk, further strengthening our hypothesis that exercising at recommended doses of exercise (i.e., 150 min/wk) is necessary. Interestingly, as previously reported, we did observe an effect of exercise on insulin and IGF-1 in this sample of women (47); thus, the dose necessary to elicit favorable changes in breast cancer biomarkers may differ for each biomarker.

There have been several other randomized exercise trials and interventions that, while not conducted in breast cancer populations, have examined circulating cytokines and CRP with inconsistent results (48–56). The interventions have ranged in duration from 4 weeks to 2 years and have included resistance training, aerobic exercise of varying amounts and intensities, and lifestyle changes combining exercise with diet. Consistent with our findings, several reported no change in IL-6 (49, 51, 53, 55, 56), CRP (48, 52, 53, 55), or TNF-α after exercise (49, 53, 55), whereas others have reported decreases in IL-6 (48, 50, 54), CRP (49–51, 54, 56), and TNF-α (54). Some of these studies did not include a control group and most combined exercise with dietary changes (48–50, 54, 56).

One trial with positive results (CRP reduction of 3 mg/L) was conducted in premenopausal, overweight women and used a 1-year, twice-weekly resistance training intervention (51). A second intervention study among nondiabetic lean and obese men and obese male type II diabetics examined the effect of a 12-week, 60-minute, 5-session/wk aerobic exercise intervention on inflammation (48). The authors found a 0.9 pg/mL decrease in IL-6 concentration in the lean and obese nondiabetics and a 3.2 pg/mL decrease in IL-6 in the diabetic group, but no changes in CRP. In addition, there were significant reductions in visceral fat and waist circumference. One of the trials that found no exercise effect randomized 189 overweight/obese men and postmenopausal women to 6 months of inactivity or 1 of 3 exercise groups: low-amount-moderate-intensity, low-amount-vigorous-intensity, or high-amount-high-intensity (53). Fat mass decreased significantly across the exercise groups (6%–13%) relative to the inactive group and adherence rates were high (84%–93%), but no changes occurred for IL-6, CRP, or TNF-α in any of the exercise groups. Finally, in another trial, 316 overweight/obese older adults with osteoarthritis were randomized to 1 of 4, 18-month interventions: control, diet-induced weight loss, exercise (60 minutes of weight training and walking 3 times/wk), or combined diet and exercise (55). Exercise training had no effect on IL-6, CRP, or TNF-α compared with the control group, though these markers were all significantly reduced in the diet alone group. These exercise trials examining changes in inflammation have variable results perhaps owing to the different study populations, the variety in intervention type, duration, and intensity, and the different changes in adiposity occurring over the course of intervention.

Mechanisms through which physical activity may reduce inflammation are not entirely understood but may include release of anti-inflammatory cytokines during exercise, inhi-bition of TNF-α production by epinephrine, effects of muscle-derived IL-6, and reduction in adipose tissue (23, 28, 57). In our study, we found significant decreases in percentage body fat among the exercise group compared with the usual care group (-0.8% vs. 0.4%; $P < 0.01$), but not for change in BMI or body weight. Several of the exercise trials discussed earlier, which found reductions in proinflammatory markers also found decreases in adiposity (48, 50, 56). For example, in Dekker and colleagues, decreases in IL-6 and CRP occurred concurrently with decreases in total fat mass and waist circumference (48). In addition, a study among obese women showed that an approximate 3 kg loss of adipose tissue after a very low-calorie diet was associated with a 0.46 pg/mL, or 17%, reduction in levels of IL-6, but no significant changes in CRP or TNF-α (58). In contrast, some trials reported reductions in inflammation after exercise without any concurrent change in adiposity (49, 51, 54). Further still, some exercise trials have achieved significant fat losses without simultaneous decreases in proinflammatory markers (48, 53, 56). Nicklas and colleagues noted that the decreases in cytokines and CRP seen in the diet-only group were unrelated to changes in BMI. Assessment of body composition in these trials varied, from direct measurement of body fat to indirect measurements such as BMI, and could explain some of the discrepancies across studies. Still, the mediating effect of fat loss remains unclear and may differ for different markers. IL-6 and CRP seem to be more amenable to change through fat loss as compared with TNF-α perhaps because adipose tissue is a significant producer of IL-6, which in turn is a regulator of hepatic CRP synthesis (59), whereas the majority of adipocyte-produced TNF-α is sequestered and contributes a relatively small amount to circulation (60). Indeed, we observed measures of adiposity to be more strongly correlated with IL-6 and CRP than with TNF-α.

Another factor that may modify or mediate the effect of exercise on inflammatory markers, as well as other cancer biomarkers, is endocrine therapy. Evidence suggests that tamoxifen and aromatase inhibitors affect inflammatory and metabolic biomarkers, likely through cross-talk with sex steroid pathways (61–63) In a study of breast cancer survivors initiating endocrine therapy with an aromatase inhibitor, specifically letrozole, increases in CRP were observed within the first 6 months after aromatase inhibitor treatment started (64) Another endocrine therapy trial showed that c-peptide levels increased significantly in breast cancer survivors in the 4 months after initiating tamoxifen ($P < 0.001$), whereas IGF-1 levels decreased ($P < 0.001$; ref. 61) However, other studies consistently show an increase in IGF-1 levels upon initiation of an aromatase inhibitor (62, 63). These findings provide evidence of an interaction between endocrine therapy and biomarkers linked to breast cancer outcomes. Future, appropriately powered, studies should examine the effect of exercise on cancer biomarkers stratified by endocrine therapy. Several factors and study limitations could have influenced our trial results. First, if physical activity's effects on cytokine concentrations are mediated predominantly through fat loss,

our prescribed intervention may not have achieved the necessary reduction in body fat. Although significantly different from the usual care group, changes in percentage body fat among the exercise group were modest (-0.8%), as were changes in BMI ($-0.12\ \text{kg/m}^2$) and weight ($-0.55\ \text{kg}$). Second, imperfect adherence to the intervention may have impacted results; this was explored in secondary analyses. Among women who met 80% of the exercise goal, IL-6 levels decreased 14.4%, whereas those not meeting 80% of the goal had an 18.5% increase; a mean between group difference of -0.69 pg/mL. No significant differences were detected for either CRP or TNF-α. It is also noteworthy that adherers had a mean decrease in body fat of 1.6%, whereas nonadherers had a decrease of only 0.3% ($P = 0.04$). These findings from subanalyses, while suggestive of a possible effect of physical activity on IL-6, must be interpreted with caution as they are not based on the intent-to-treat principle and women who are more adherent may differ from less adherent women. Other limitations include the small sample size for stratified analyses and potential nondifferential measurement error of cytokine concentrations, which were based on single blood draws.

Advantages of this study include randomization to treatment group, inclusion of women with low baseline physical activity, good adherence, and retention rates assessed by thorough exercise monitoring, and prescription of a lengthy, supervised exercise intervention. In addition, valid, objective measures were used for assessment of percentage body fat, physical activity, and biomarker concentrations.

In our trial, we found that baseline inflammatory markers were associated with higher adiposity and lower levels of exercise, but we did not find that the moderate-intensity aerobic exercise intervention significantly altered concentrations of IL-6, CRP, or TNF-α. Future studies should examine the effects of different doses and types of physical activity on cytokines in large-scale trials of breast cancer survivors, as well as determine whether certain factors, including body fat loss and endocrine therapy, modify the potential effect of exercise on inflammatory markers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.B. Jones, M.L. Irwin
Development of methodology: S.B. Jones, H. Yu, M.L. Irwin
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