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TRANSLATIONAL PHYSIOLOGY

Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors

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Fairey, Adrian S., Kerry S. Courneya, Catherine J. Field, Gordon J. Bell, Lee W. Jones, and John R. Mackey. Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors. *J Appl Physiol* 98: 1534–1540, 2005; doi:10.1152/japplphysiol.00566.2004.—The objective was to determine the effects of exercise training on changes in blood immune function in postmenopausal breast cancer survivors. Fifty-three postmenopausal breast cancer survivors were randomly assigned to an exercise (n = 25) or control group (n = 28). The exercise group trained on cycle ergometers three times per week for 15 wk. The control group did not train. The primary end point was change in natural killer cell cytotoxic activity in isolated peripheral blood mononuclear cells. Secondary end points were changes in standard hematological variables, whole blood neutrophil function, the phenotypes of isolated mononuclear cells, estimations of unstimulated and phytohemaglutinin-stimulated mononuclear cell function (rate of [^{3}H]thymidine uptake), and the production of proinflammatory [interleukin (IL)-1α, tumor necrosis factor-α, IL-6] and anti-inflammatory cytokines (IL-4, IL-10, transforming growth factor-β_1). Statistical tests were two-sided ($\alpha < 0.05$). Fifty-two participants completed the trial. Intention-to-treat analyses, which included the baseline value as a covariate, showed significant differences between groups for change in percent specific lysis of a target natural killer cell at all five effector-to-target ratios (adjusted mean between-group change over all 5 effector-to-target ratios = +6.34%; $P < 0.05$ for all comparisons), the lytic activity per cell (adjusted mean between-group change = −2.72 lytic units; $P = 0.035$), and unstimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes (adjusted mean between-group change = +218 per dpm × 10^6 cells; $P = 0.007$). There were no significant differences between groups for change in any other end point. Exercise training increased natural killer cell cytotoxic activity and unstimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes in postmenopausal breast cancer survivors.

clinical exercise physiology; natural killer cell cytotoxic activity; unstimulated [^{3}H]thymidine uptake

RECENT DATA SUGGEST THAT CANCER and its treatment are associated with pronounced immune deficiency (1, 3, 14, 16, 31) and that blood immune function is positively associated with progression-free and overall survival (5, 17, 19, 20, 30, 37). A recent systematic review found preliminary evidence that exercise can improve blood immune function in cancer survivors (8). The improvements that have been shown include increased natural killer (NK) cell cytotoxic activity, monocyte function, and the proportion of circulating granulocytes (8). However, several methodological limitations of this research were identified, including nonrandomized controlled designs, small heterogeneous samples, and inappropriate statistical analyses (8). Therefore, the effect of exercise training on blood immune function in cancer survivors is not clear.

The Rehabilitation Exercise for Health After Breast Cancer trial was a randomized controlled trial of exercise training in postmenopausal breast cancer survivors who had completed surgery, radiotherapy, and/or chemotherapy with or without current tamoxifen or anastrozole therapy use. We previously reported significant changes in cardiopulmonary function and quality of life (4) and insulin-like growth factors and insulin-like growth factor binding proteins (7). In the present study, we report the effects of exercise training on changes in cell-mediated immune function. We hypothesized that exercise training would increase NK cell cytotoxic activity.

MATERIALS AND METHODS

The trial design and conduct have been previously described (4). In brief, the study was a randomized controlled trial conducted at the University of Alberta and Cross Cancer Institute in Edmonton, Canada. The University of Alberta and Alberta Cancer Board approved the study. Written, informed consent was obtained for all procedures.

Participants. Eligibility criteria included *1*) histologically confirmed stage I to IIIB breast cancer; *2*) diagnosed between January 1999 and June 2000; *3*) completed surgery, radiotherapy, and/or chemotherapy with or without current tamoxifen or anastrozole therapy use; *4*) postmenopausal; *5*) nonsmokers; and *6*) between 50 and 69 yr of age. Eligible participants were not admitted if they had *1*) known cardiac disease, *2*) uncontrolled hypertension, *3*) uncontrolled thyroid disease, *4*) diabetes mellitus, *5*) mental illness, *6*) infection, *7*) immune or endocrine abnormality, *8*) body weight reduction of ≥10% in past 6 mo, and *9*) positive exercise stress test.

Design and randomization. The study was a randomized controlled trial. Participants were stratified by type of adjuvant therapy (previous chemotherapy vs. no previous chemotherapy, and current hormone therapy vs. no current hormone therapy) and randomized to an exercise or control group using a random-numbers table. A block permutation procedure was used to generate the allocation sequence within each strata. The allocation sequence and group assignments were generated by a research assistant and then enclosed in sequentially numbered and sealed envelopes. The contents of the envelopes were concealed from the project director, who assigned participants to

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groups. Investigators and participants had no knowledge of group assignment until the completion of baseline assessments.

Exercise training intervention. The exercise group trained three times per week for 15 wk on recumbent or upright cycle ergometers (Lifestyle Fitness 9500HR, Lifecycle). Exercise intensity was set at the power output that elicited the ventilatory equivalent for carbon dioxide (~70–75% of peak oxygen consumption). Exercise duration began at 15 min for *weeks 1–3* and then systematically increased by 5 min every 3 wk thereafter to 35 min for *weeks 13–15*. Warm-up and cool-down periods consisted of 5 min of cycling at the power output that elicited the ventilatory equivalent for oxygen (~50% of peak oxygen consumption). Exercise physiologists supervised the exercise sessions and monitored heart rate and blood pressure. The control group did not train and were asked not to begin a structured exercise program.

End points. The primary end point was change in NK cell cytotoxic activity in isolated peripheral blood mononuclear cells. Secondary end points were changes in standard hematological variables, whole blood neutrophil function, the phenotypes of isolated mononuclear cells, estimations of unstimulated and phytohemagglutinin (PHA)-stimulated mononuclear cell function {rate of [^{3}H]thymidine uptake, and the production of proinflammatory [interleukin (IL)-1α, TNF-α, IL-6] and anti-inflammatory cytokines (IL-4, IL-10, transforming growth factor-β1)}.

Blinding. Laboratory staff and those who assessed the trial end points were blinded to treatment assignment.

Blood collection. Participants were instructed not to exercise for at least 48 h before blood collection to eliminate immune modulation from an acute bout of exercise (32). Blood was collected between 0700 and 1000 after a 12-h water-only fast with participants in the seated position. Blood was drawn into tubes chilled on ice treated with sodium heparin. Blood samples were collected from 53 participants at baseline and 52 participants at *week 15*.

Purification of blood lymphocytes. Mononuclear cells were isolated on a Ficoll-Hypaque gradient as previously described (11). In brief, cells were counted using a hemocytometer and aliquoted to a cell concentration of 1×10^6 cells/ml for proliferation and cytokine determination. Cell viability was determined using Trypan blue exclusion and was >98% for all groups.

NK cell cytotoxic activity. Two million human K-562 cells (American Type Tissue Culture Collection, Manassas, VA) were labeled with chromium (^{51}Cr; Amersham Pharmacia Biotech) as previously described (9). The cells were seeded in triplicate into a 96-well V-bottom microtiter plate at effector-to-target ratios varying from 3.125:1 to 50:1 for 4-h incubation at 37°C, after which a ^{51}Cr release was determined in a 75-μl aliquot of the supernatant in a TopCount NXT (Canberra Packard). Spontaneous release was determined from the target cells incubated in the absence of effector cells. Maximum release was determined from detergent lysis of labeled target cells. NK cell activity was calculated as follows: percent specific lysis = 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release). Results were also corrected to a per cell basis using the number of CD16$^+$CD56$^+$ NK cells (we did not use CD3 + CD16$^+$CD56$^+$ NK cells because we were forced to delete CD3 based on the fact that our CD3 antibody did not work properly in the laboratory on the day of the test). This was expressed in lytic units (LU/10^3), with LU representing the number of effector cells required to cause 30% lysis of target cells.

Standard hematological variables. White blood cell counts and differential were measured using a Coulter STKS instrument (Coulter Electronics).

Neutrophil function. Size, granularity, and oxidative burst of neutrophils were assessed in a sample of whole blood as previously described (27). In brief, the oxidation of dihydrorhodamine 123 (Cedarlane Laboratories) to rhodamine 123 cells was measured before and at various time points (5, 10, and 15 min) after stimulation with phrobol myristate acetate (ICN). The oxidation was quantified by flow cytometry (FACScan, Becton Dickinson, San Jose, CA) using CellQuest software (Becton Dickinson). The change in granularity and oxidative burst after stimulation was determined using a ratio that compared the value to 0 (unstimulated value).

Blood mononuclear cell phenotypes. Mononuclear lymphocyte phenotypes were identified by an immunofluorescence assay in whole blood as previously described (11). The following anti-human mouse monoclonal antibodies (Sigma, St. Louis, MO, and BD Pharmingen, Mississauga, ON) labeled with FITC, R-phycoerythrin (PE), Biotin (B), or quantum red were used: CD3-B (pan T cells), CD4-FITC (T-helper/inducer cells), CD8-PE or CD8-B (T-cytotoxic/suppressor cells), CD45RO-FITC (antigen-exposed T cells, B cells, granulocytes, and monocytes), CD45RA-quantum red (antigen naive T cells, NK cells, B cells), CD14-PE (monocytes), CD20-FITC (all B cells except plasma cells), CD16-FITC (NK cells and macrophages), CD28-FITC (costimulatory molecule for T-cell activation), CD25-PE (activated T and B cells), and CD56-B (NK cells). Cells (at least 10,000 per monoclonal antibody combination) were analyzed by flow cytometry (FACScan, Becton Dickinson) according to relative fluorescence intensity. Appropriate isotype controls (Sigma) were used for each labeled monoclonal antibody, and resultant percentages were corrected for background fluorescence (<1%).

Estimation of lymphocyte proliferation. Lymphocytes (1×10^6 cells/ml) were cultured for 48 h in 96-well microtiter plates with or without PHA (5 μg/ml, Sigma) or Pokeweed mitogen (55 μg/ml, Sigma) as previously described (10). Each well was pulsed with 1 μCi per well of [^{3}H]thymidine 18 h before the cells were harvested (Amersham Pharmacia Biotech). The cells were harvested onto a Packard UniFilter plate using a Filtermate Harvester, and the plates were counted on a Packard TopCount NXT (Canberra Packard). All assays were performed in triplicate, and stimulation indexes were calculated for each condition as follows: {amount of [^{3}H]thymidine incorporated by stimulated cells [disintegrations per minute (dpm) × 10^6 cells]}/{amount of [^{3}H]-thymidine (dpm) incorporated by unstimulated cells (dpm × 10^6 cells)}.

Cytokine production by blood mononuclear cells. Isolated peripheral blood mononuclear cells were cultured (at 37°C, 5% CO_2, 95% relative humidity for 48 h) in either 2 ml of complete culture medium (0.5×10^6 cells/ml) and PHA (5 μg/ml; PHA-positive culture) or 1 ml of complete culture media (unstimulated tube). Complete culture medium consisted of RPMI 1640 with L-glutamine (300 mg/l) (GIBCO Life Technologies, Burlington, ON) supplemented (40 g/l) with fetal bovine serum (Sigma), 25 mM HEPES buffer (GIBCO Life Technologies), 2.5 μmol/ml 2-mercaptoethanol, and 100 μg/ml antibiotic/antimycotic saline solution containing penicillin G sulfate (10,000 U/ml), streptomycin sulfate (10,000 μg/ml), and amphotericin B (25 μg/ml) (GIBCO Life Technologies). After incubation, tubes were centrifuged at 200 *g* for 10 min at 4°C to pellet cells. Supernatant was collected and stored at −70°C for subsequent cytokine analysis. The ability of peripheral blood mononuclear cells to produce proinflammatory (IL-1α, TNF-α, IL-6) and anti-inflammatory (IL-4, IL-10, TGF-β_1) cytokines was assessed in the unstimulated and mitogen-stimulated supernatant using ELISA kits (BD Bioscience for TNF-α, IFN-γ, IL-6, IL-4, IL-10, and TGF-β_1; R&D Systems, Minneapolis, MN, for IL-1). All of the samples were analyzed using assay kits from a single lot before the expiration date of the assay kit. Each assay was performed in one batch (i.e., baseline and *week 15* assessment of each analyte for exercise and control group participants were performed in one batch). All mean intra-assay coefficients of variation were ≤6.59%.

Adverse events. All adverse events reported by the participant or observed by the investigators were recorded. An adverse event was defined as any adverse change from the participant's baseline condition, regardless of whether it was considered related to exercise training.

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Adherence to the exercise intervention. Exercise trainers monitored adherence to the intervention. Physical activity levels both at baseline and outside of the exercise training intervention during the trial in the exercise and control groups were assessed using the leisure score index of the Godin Leisure Time Exercise Questionnaire (12). Participants completed the leisure score index every 2 wk over the 15-wk intervention.

Statistical analysis. Sample size calculation was based on the primary cardiopulmonary and quality of life end points from the Rehabilitation Exercise for Health After Breast Cancer trial (4). Data were analyzed using the intention-to-treat approach. The last-observation-carried-forward procedure was used for participants who did not complete the trial. Distributions were checked for skewness, kurtosis, and outliers [a priori, we decided that variables with significant skewness or kurtosis ($-t \geq 1.96$) and outliers >3.29 standard deviations from the mean] would be transformed for statistical analysis. No transformations were required for statistical analyses. Baseline comparisons between the two groups were made using independent-sample *t*-tests for continuous data and Pearson's χ^2 tests for categorical data. The primary analyses compared changes from baseline to *week 15* between the two groups using univariate analyses of covariance procedures in which the change from baseline to the *week 15* value was the dependent variable, the baseline value of the same variable was the covariate, and the treatment group was the grouping variable. This adjusted statistical analysis was performed to reduce the impact of baseline differences on study outcomes (given the relatively small sample size of the trial). A two-sided P value of <0.05 indicated statistical significance. No adjustments were made for multiple comparisons. Data are presented as the means ± SD with 95% confidence intervals. In Tables 2 and 3, baseline and *week 15* values are not adjusted, whereas mean change and difference between groups in mean change values are adjusted for the baseline value.

RESULTS

Flow of participants through the trial. Flow of participants through the trial has been described (4). In brief, 370 breast cancer survivors were assessed for eligibility, and 53 (14.3%) were randomly assigned to the exercise ($n = 25$) or control groups ($n = 28$). One participant (4.2%) dropped out in the exercise group compared with 0 participants in the control group ($P = 0.285$). The participant in the exercise group dropped out because of a gastrointestinal complication unrelated to exercise. Overall, 52 of 53 participants completed the trial (98.1%).

Baseline characteristics. Table 1 presents baseline characteristics. No significant differences were observed between groups for age, body mass index, peak oxygen consumption, tumor stage, surgery, radiotherapy, chemotherapy, current tamoxifen or anastrozole therapy use, or past exercise levels.

Self-reported diet intake and medication use. Self-reported diet intake and medication use have been described (7). No significant differences were observed between groups for any variable.

Adherence to the intervention. Adherence to the exercise intervention has been described (4). The exercise group completed 44.3 of 45 (range 38/45 to 45/45) prescribed exercise sessions. Nonprotocol-related exercise was not different between groups ($P = 0.890$). The exercise group reported 15 min of moderate/strenuous nonprotocol-related exercise per week compared with 13 min in the control group.

Change in NK cell cytotoxic activity. Table 2 shows the change in NK cell cytotoxic activity. Intention-to-treat analysis showed significant differences between groups for change in

Table 1. *Baseline characteristics*

Variable	Overall ($n = 52$)	Exercise Group ($n = 24$)	Control Group ($n = 28$)	P Value
Demographic				
Age, yr	59 (6)	59 (5)	58 (6)	0.712
Medical				
Weight, kg	78.7 (18.1)	78.1 (20.4)	79.4 (16.4)	0.801
Body mass index, kg/m^2	29.2 (6.6)	29.4 (7.4)	29.1 (6.1)	0.880
Peak oxygen consumption, ml·kg^{-1}·min^{-1}	18.7 (3.9)	18.6 (3.9)	18.8 (3.8)	0.807
Months postsurgery, RT, and/or CT	14 (6)	14 (6)	14 (7)	0.856
Stage				
I (T1N0)	21 (40%)	10 (42%)	11 (39%)	0.862
IIa (T1N1, T2N0)	17 (33%)	6 (25%)	11 (39%)	0.274
IIb (T2N1, T3N0)	11 (21%)	6 (25%)	5 (18%)	0.530
IIIa (T1N2, T2N2, T3N1-2)	3 (6%)	2 (8%)	1 (4%)	0.463
Surgery				
Mastectomy	28 (54%)	15 (64%)	13 (46%)	0.246
Lumpectomy	24 (46%)	9 (37%)	15 (54%)	0.246
Radiation therapy	37 (71%)	16 (67%)	21 (75%)	0.508
Chemotherapy	21 (40%)	10 (42%)	11 (39%)	0.862
Anthracycline regimen	20 (38%)	10 (42%)	10 (36%)	0.329
Current hormone therapy use	24 (46%)	11 (46%)	13 (46%)	0.966
Past Exercise				
Moderate, min/wk	82 (114)	62 (94)	98 (126)	0.247
Strenuous, min/wk	25 (61)	23 (56)	26 (65)	0.897
Moderate/strenuous, min/wk	106 (129)	85 (102)	124 (146)	0.280
>90 Moderate/strenuous, min/wk	22 (42%)	10 (42%)	12 (42.9%)	0.931

Data are presented as means (SD) for continuous variables and frequency (percentage) for categorical variables. RT, radiation therapy; CT, chemotherapy; HT, hormone therapy.

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Table 2. *Change in natural killer cell cytotoxic activity*

Variable	Baseline[a]	*Week 15*[b]	Mean Change[c]	Difference Between Groups in Mean Change (95% CI)[d]	*P* Value[e]
50:1 E:T ratio, % lysis					
Exercise group	55.5 (12.1)	61.4 (9.8)	+5.5		
Control group	58.0 (12.9)	56.4 (10.5)	−1.4	+6.8 (0.4–13.3)	0.039
25:1 E:T ratio, % lysis					
Exercise group	44.2 (12.8)	49.8 (8.3)	+5.5		
Control group	45.3 (12.1)	44.0 (11.3)	−1.1	+6.8 (0.9–12.7)	0.024
12.5:1 E:T ratio, % lysis					
Exercise group	36.2 (10.6)	41.2 (8.4)	+5.8		
Control group	32.2 (10.1)	33.8 (10.7)	+0.2	+5.6 (0.2–11.1)	0.041
6.25:1 E:T ratio, % lysis					
Exercise group	21.7 (9.0)	27.7 (10.1)	+6.3		
Control group	18.9 (9.4)	19.8 (9.2)	−0.1	+6.5 (1.0–11.9)	0.022
3.125:1 E:T ratio, % lysis					
Exercise group	7.2 (5.1)	12.4 (6.6)	+5.3		
Control group	5.8 (4.5)	5.7 (4.2)	−0.6	+6.0 (2.8–9.1)	<0.001
Total lytic units					
Exercise group	11.98 (6.76)	8.60 (3.40)	−3.42		
Control group	12.72 (8.19)	11.68 (6.00)	−0.70	−2.72 (−5.23 to −0.20)	0.035

Data are means (SD); exercise group ($n = 25$); control group ($n = 28$). E:T ratio, effector-to-target ratio. [a]Baseline value is not adjusted. [b]*Week* 15 value is not adjusted. [c]Mean change value is adjusted for the baseline value. [d]Difference between groups in mean change value is adjusted for the baseline value. [e]*P* value is for the adjusted difference between groups in mean change.

percent specific lysis at the 3.125:1 effector-to-target ratio (adjusted mean between-group change = +6.0%; $P < 0.001$), 6.25:1 effector-to-target ratio (adjusted mean between-group change = +6.5%; $P = 0.022$), 12.5:1 effector-to-target ratio (adjusted mean between-group change = +5.6%; $P = 0.041$), 25:1 effector-to-target ratio (adjusted mean between-group change = +6.8%; $P = 0.024$), and 50:1 effector-to-target ratio (adjusted mean between-group change = +6.8%; $P = 0.039$), and total LU (adjusted mean between-group change = −2.72 LU; $P = 0.035$).

Change in standard hematological variables. Intention-to-treat analysis showed no significant differences between groups for change in any standard hematological variable (data not shown).

Change in neutrophil function. Intention-to-treat analysis showed no significant differences between groups for change in neutrophil function (data not shown).

Change in blood mononuclear cell phenotypes. Intention-to-treat analysis showed no significant differences between groups for change in blood mononuclear cell phenotypes (data not shown).

Change in lymphocyte proliferation. Table 3 shows the change in lymphocyte proliferation. Intention-to-treat analysis showed a significant difference between groups for change in unstimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes (adjusted mean between-group change = +218 per dpm × 10^6 cells; $P = 0.007$) but no significant difference between groups for change in PHA-stimulated [^{3}H]thymidine uptake (adjusted mean between-group change = +9,429 per dpm × 10^6 cells; $P = 0.478$) or PHA stimulation index (adjusted mean between-group change = −7 per dpm × 10^6 cells; $P = 0.478$).

Change in cytokine production by blood mononuclear cells. Intention-to-treat analysis showed no significant differences between groups for change in unstimulated or PHA-stimulated cytokine production by blood mononuclear cells (data not shown).

Adverse events. Adverse events have been described previously (4). In brief, five participants (20.8%) in the exercise

Table 3. *Change in lymphocyte proliferation*

Variable	Baseline[a]	*Week 15*[b]	Mean Change[c]	Difference Between Groups in Mean Change (95% CI)[d]	*P* Value[e]
Spontaneous ([^{3}H]-thymidine incorporated, dpm × 10^6 cells)					
Exercise group	863 (425)	1,042 (290)	+218		
Control group	776 (417)	811 (247)	+1	+218 (63 to 371)	0.007
PHA ([^{3}H]-thymidine incorporated, dpm × 10^6 cells)					
Exercise group	90,098 (49,890)	79,500 (32,218)	−12,243		
Control group	91,279 (54,302)	69,487 (31,540)	−21,671	+9,429 (−5,863–24,720)	0.221
PHA-SI					
Exercise group	112 (54)	80 (38)	−34		
Control group	117 (58)	89 (38)	−27	−7 (−25–12)	0.478

PHA-SI, phytohemagglutinin stimulation index; PHA, phytohemagglutinin; dpm, absolute rate of [^{3}H]thymidine incorporation. Data are means (standard deviation); exercise group ($n = 25$); control group ($n = 28$). [a]Baseline value is not adjusted. [b]*Week* 15 value is not adjusted. [c]Mean change value is adjusted for the baseline value. [d]Difference between groups in mean change value is adjusted for the baseline value. [e]*P* value is for the adjusted difference between groups in mean change.

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group experienced an adverse event compared with two participants (7.1%) in the control group ($P = 0.168$). The adverse events in the exercise group were lymphedema ($n = 3$), gynecologic complication ($n = 1$), and influenza ($n = 1$), whereas the adverse events in the control group were metatarsal fracture ($n = 1$) and bronchitis ($n = 1$).

DISCUSSION

In this randomized controlled trial, we determined the effect of exercise training on blood immune function in postmenopausal breast cancer survivors who had completed surgery, radiotherapy, and/or chemotherapy with or without current tamoxifen or anastrozole therapy use. We found that exercise training had statistically significant effects on change in NK cell cytotoxic activity and unstimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes but no statistically significant effects on change in standard hematological variables, neutrophil function, phenotypes of isolated mononuclear cells, PHA-stimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes, or unstimulated and PHA-stimulated mononuclear cell cytokine production.

Our trial had strengths and limitations. Strengths include the randomized controlled trial design, standardized blood-collection protocols, high exercise adherence rate, and minimal loss to follow-up. Limitations include the 14% recruitment rate, relatively small sample size, short exercise intervention with no long-term follow-up, and use of peripheral blood samples (as opposed to tissue biopsy samples) to determine immune status.

One finding from our trial was the effect of exercise training on NK cell cytotoxic activity. Exercise training increased in vitro NK cell cytotoxic activity at all five effector-to-target cell ratios by 6.45%. In addition, the LU (number of cells required to lyse 30% of the target cells) decreased by 2.72 LU, indicating that the cytotoxic activity increased on a per cell basis. Comparison of our effects with those reported in previous exercise studies is instructive. The effect on percent specific lysis in our trial is similar to that observed in one previous exercise study in cancer survivors. In a nonrandomized trial, Peters et al. (26) showed that 29 wk of cycle ergometer exercise increased percent specific lysis by 9.4% in breast cancer survivors. The effect on percent specific lysis in our trial is also similar to that observed in randomized controlled trials of aerobic exercise training in healthy older adults (6, 34). However, Na et al. (21) showed that 2 wk of combined modality exercise increased percent specific lysis by 18.1% in stomach cancer survivors, whereas Nieman et al. (24) showed that 8 wk of moderate exercise training had no effect on percent specific lysis in breast cancer survivors. Reasons for these differences in magnitude and direction of the effect remain to be determined but may include the exercise parameters, timing of exercise in relation to treatment, experimental design, and/or patient population. Unfortunately, previous exercise trials in cancer survivors and healthy older adults have not reported changes in lytic activity per cell (8), making it difficult to compare our findings. Nonetheless, this finding is consistent with the observation that exercise training can improve NK cell cytotoxic activity beyond that which is associated with normal recovery after cancer therapy (2).

A second finding from our trial was the effect of exercise training on unstimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes. Exercise training increased [^{3}H]thymidine uptake by isolated peripheral blood mononuclear cells when cultured in the absence of mitogen. Interestingly, the increase in the rate of [^{3}H]thymidine uptake was not accompanied by differences in the production of pro- and anti-inflammatory cytokines. The effect on unstimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes observed in our trial is in contrast to two previous observations. In a nonrandomized study, Hayes et al. (15) showed that 3 mo of combined aerobic and resistance exercise training had no effect on unstimulated lymphocyte proliferation in patients receiving high-dose chemotherapy followed by autologous peripheral blood stem cell transplant. Similarly, in a randomized trial, Nehlsen-Cannarella (23) found that 15 wk of moderate aerobic exercise training had no effect on unstimulated lymphocyte proliferation in sedentary, overweight women. However, the effect on unstimulated lymphocyte proliferation in our trial is similar to that observed in one previous study. In a small randomized trial, Rhind et al. (28) showed that 12 wk of cycle ergometer exercise increased unstimulated lymphocyte proliferation in healthy men. Reasons for these discrepant findings are not clear but may include the exercise parameters, experimental design, and/or patient population.

Exercise-induced modulation of blood immune function is biologically plausible. Physiological mechanisms that may explain changes in NK cell cytotoxic activity and [^{3}H]thymidine uptake by peripheral blood lymphocytes have been reviewed and include changes in neuroendocrine status, hematopoiesis, leukocyte apoptosis, muscle damage, protein synthesis, glucose metabolism, and antioxidant defenses (25, 36). Although our trial did not test these mechanisms, these effects may represent clinically significant biological mechanisms of action of exercise training.

The clinical significance of the effects on immune function observed in our trial is not known. However, recent data suggest a positive correlation between good NK cell function and disease-free and overall survival, as well as poor NK cell function and disease relapse (13, 18, 22, 29, 33). For example, Gonzalez et al. (13) showed that NK cell cytotoxic activity was higher in tumor-free survivors compared with those who had tumor-related deaths at the 50:1 effector-to-target ratio (41.7 vs. 28.1%; $P \leq 0.001$), 25:1 effector-to-target ratio (31.9 vs. 20.8%; $P \leq 0.001$), 12:1 effector-to-target ratio (21.4 vs. 13.6%; $P \leq 0.001$), and 6:1 effector-to-target ratio (12.5 vs. 7.3%; $P \leq 0.001$) in patients with laryngeal carcinoma. Sephton et al. (30) found that altered diurnal cortisol rhythms were associated with suppression of NK cell cytotoxic activity and decreased survival in metastatic breast cancer survivors. Liljefors et al. (19) found that pretreatment NK cell cytotoxic activity was positively associated with overall survival (above median percent specific lysis vs. below median percent specific lysis, 71 vs. 30 wk; $P = 0.007$), progression-free survival (above median percent specific lysis vs. below median percent specific lysis, 11 vs. 6 wk; $P = 0.013$), and response rate to monoclonal antibody therapy 17-1A (above median percent specific lysis vs. below median percent specific lysis, 10/26 patients vs. 2/24 patients; $P = 0.019$) in colorectal carcinoma survivors. Unfortunately, to our knowledge, there are no data to suggest that the spontaneous uptake of [^{3}H]thymidine in

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peripheral blood lymphocytes alone is a clinically relevant measure. Therefore, we are unable to comment on this effect.

Rates of adverse events were low and similar between groups. In addition, the exercise training intervention was well tolerated since participants in the exercise group completed 98.4% of prescribed exercise sessions. Although exhaustive exercise training has been shown to suppress immune function (35), there was no evidence of worsening immunologic status. This is probably related to the moderate-intensity exercise that was prescribed in this trial. Overall, therefore, the exercise training intervention was both safe and efficacious.

Future research designed to evaluate the effects of exercise training on blood immune function in cancer survivors is needed (see Ref. 8 for systematic review). In brief, researchers should attempt to confirm our findings in larger samples of cancer survivors and determine whether there are differential physiological effects to be achieved by altering the exercise parameters (frequency, intensity, time, type) or timing of exercise across the postdiagnosis cancer control continuum (e.g., before or after surgery; before, during, or after radiotherapy, chemotherapy, or hormone therapy). Studies should also attempt to obtain tissue biopsy samples, and establish whether exercise-induced alterations in immune system components from peripheral blood correlate to those obtained from tissue. Finally, research is needed to test underlying biological mechanisms of action and to evaluate whether exercise-induced modulation of blood immune function improves clinical outcomes.

In this randomized controlled trial, exercise training increased NK cell cytotoxic activity and unstimulated [^{3}H]thymidine uptake by peripheral blood lymphocytes in postmenopausal breast cancer survivors. Additional randomized controlled trials are needed to determine the clinical implication(s) of these findings.

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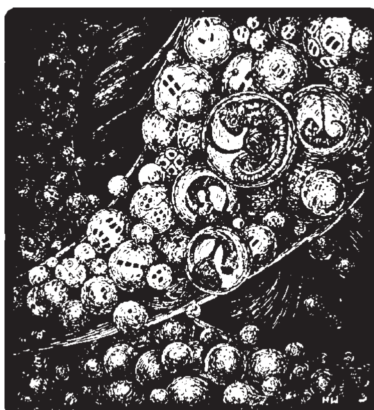

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