Exercise and Lymphocyte Activation following Chemotherapy for Breast Cancer

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[1]The Pennsylvania State University Department of Biochemistry and Molecular Biology, University Park, PA; [2]The Pennsylvania State University Department of Kinesiology and Noll Physiological Research Center, University Park, PA; [3]The University of Connecticut Department of Kinesiology, Storrs, CT; [4]The University of Delaware Department of Health and Exercise Sciences, Newark DE; [5]Centre Medical and Surgical Associates, State College, PA; and [6]Lehigh Valley Hospital, Allentown, PA

ABSTRACT

HUTNICK, N. A., N. I. WILLIAMS, W. J. KRAEMER, E. ORSEGA- SMITH, R. H. DIXON, A. D. BLEZNAK, and A. M. MASTRO. Exercise and Lymphocyte Activation following Chemotherapy for Breast Cancer. *Med. Sci. Sports Exerc.*, Vol. 37, No. 11, pp. 1827–1835, 2005. **Purpose:** To determine whether exercise training would increase lymphocyte activation in patients with breast cancer following chemotherapy. Activation was determined by the presence of $CD4^+CD69^+$ T-helper lymphocytes, mitogen-induced proliferation, and levels of cytokines produced by mitogen-stimulated lymphocytes and in the patients' plasma. **Methods:** Patients with breast cancer ($N = 28$) who participated in a 6-month exercise program were compared with patients ($N = 21$) who did not exercise. Following chemotherapy, and 3 and 6 months later, patients underwent fitness evaluations and had blood drawn. The exercise program consisted of resistance training and aerobic activity at 60–75% functional capacity three times a week with a personal trainer. Immunochemistry and flow cytometry were used to measure the number of $CD4^+CD69^+$ blood lymphocytes. Whole blood was stimulated with concanavalin A (ConA), phytohemagglutin (PHA), or pokeweed mitogen (PWM) to determine proliferation potential. Enzyme-linked immunosorbent assays (ELISA) were used to determine the concentration of interferon-gamma (IFN-γ) and interleukin-6 (IL-6) in the culture medium of mitogen-stimulated lymphocytes as well as the plasma concentrations of IL-6, soluble IL-6 receptor, soluble gp130, and IFN-γ. Analysis of groups across time was done using the Wilcoxon signed rank test, and comparisons of groups were done using the Mann–Whitney U test. **Results:** The exercising patients showed increases in maximal oxygen uptake and upper body strength. This group also showed a greater percentage of $CD4^+CD69^+$ cells and a greater level of tritiated thymidine incorporation (DNA synthesis) when stimulated with ConA, PHA, and PWM at the end of the intervention. Plasma and mitogen-stimulated IL-6 and IFN-γ production were similar in both groups. **Conclusion:** Exercise may improve immune function by increasing lymphocyte activation in patients with breast cancer following treatment. **Key Words:** INTERLEUKIN-6, INTERFERON GAMMA, MITOGEN, PROLIFERATION

The chemotherapeutic drugs that are used to treat breast cancer also target B and T lymphocytes that are critical to the adaptive immune response. All populations of lymphocytes decrease following chemotherapy, but T-helper cells, $CD4^+$, are especially slow to recover (6). For example, in one study, $CD4^+$ T cells decreased by more than 60%, with more than a 90% loss of naïve $CD4^+CD45RA^+$ cells, and levels had not returned to normal even after 18 months (6).

No known therapies exist to increase $CD4^+$ T-cell levels, but an appropriate exercise program holds promise. For example, $CD4^+$ levels increased in patients with early symptomatic acquired immunodeficiency syndrome (AIDS) and human immunodeficiency virus (HIV) following completion of a 3-month aerobic training program (14,18). On the other hand, Hayes et al. (8), found no increase in immune reconstitution following a peripheral blood stem cell transplant in cancer patients who participated in a 3-month, moderate-intensity, mixed exercise program. Nonetheless, exercise training during chemotherapy has been shown to have a positive effect on psychological well-being, physical functioning, neutropenia, thrombopenia, pain, and hospitalization time (3,23).

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In addition to the number of lymphocytes, the functionality of the remaining cells is vital to the patient's immune response. $CD4^+$ cells are necessary for both B- and cytotoxic T-cell responses. Testing the activated $CD4^+$ cells in the blood and in culture in response to mitogens provides a means to assess the potential cell response to antigen. It has been reported that proliferation in response to mitogen is reduced following chemotherapy in cancer patients when the counts are adjusted for the total number of proliferating cells and the number of $CD3^+$ cells (8). Patients with chemotherapy-induced leucopenia have also shown decreased T-cell proliferation in response to anti-$CD3^+$ and exogenous cytokines, further suggesting that the patients' lymphocytes may be functionally different from healthy individuals (25). The response of T cells to exercise varies greatly, even within healthy individuals, depending on the form and intensity of the exercise. For example, the proliferative response of lymphocytes to mitogen was impaired following a 2.5-h marathon but not following a 35-min training run in healthy athletes (4).

On activation, $CD4^+$ T-cells can be characterized by their cytokine profiles. The Th1 response results mainly in the activation of cell-mediated immunity (e.g., activation of T cytotoxic cells, delayed type hypersensitivity) and the promotion and production of opsonizing immunoglobulins (15). Interferon-gamma (IFN-γ) is a defining cytokine of a Th1 response. A Th2 response favors the activation of eosinophils and B cells and the production of large amounts of IgM and IgE. Interleukin-6 (IL-6) is characteristic of a Th2 response. Therefore, these cytokine levels can provide information regarding the status of $CD4^+$ cells (15).

Levels of IL-6 are reported to be less than 10 $pg \cdot mL^{-1}$ in healthy individuals (9). On the other hand, in patients with breast cancer, plasma IL-6 levels were found to be elevated above levels of healthy individuals with a higher plasma level correlating to a poorer prognosis (9,26). The clinical implications for IFN-γ are less well defined. Elevated IFN-γ levels have been observed in patients with advanced cancer; however, this finding is inconsistent with another study correlating an increase in IFN-γ to a positive response to therapy (2). Although investigators have looked at various aspects of immunity related to cancer and exercise, no studies have specifically examined the effect of a mixed exercise program on the Th1 and Th2 response in patients with breast cancer following therapy.

This study was conducted to determine whether exercise would increase lymphocyte activation following chemotherapy. In addition, we wished to identify the subpopulations that were activated by their *in vitro* and *in vivo* cytokine production.

METHODS

Subjects. Recovering patients with breast cancer were assigned to one of two groups, those with a formal exercise intervention, "exercisers" (mean age 48.5 ± 10.6 yr, range 29–69 yr), and those without a formal exercise intervention, "nonexercisers" (mean age 52.3 ± 9.2 yr, range 38–71 yr) (Table 1). In total, 28 exercisers and 21 nonexercisers completed at least the first 3 months of the study. Of these, 7 exercisers and 6 nonexercisers dropped out after the midpoint of the study, resulting in 21 exercisers and 15 nonexercisers completing the 3- to 6-month study period. If a sample was obtained, exercisers were included in the analysis whether or not they complied with the protocol.

Subjects were recruited through physicians and advertisements in central Pennsylvania. Because the protocol would not allow a blind study, recruitment was done in two phases. Patients recruited in the State College area were recruited as exercisers and had the option of enrolling as a nonexerciser if they were not able to make the training sessions, whereas patients from the Hershey area were not informed of the exercise intervention and were entered into the nonexercise group. All exercise sessions were carried out at the University Park Campus General Clinical Research Center (GCRC) in the State College area.

Of the 49 subjects, 44 were diagnosed with stage I–III breast cancer, 26 had had a lumpectomy, 22 had had a mastectomy, and 35 had undergone radiation in addition to chemotherapy (Table 2). The stage of cancer was undetermined in five patients and the type of surgery was not known for two. Chemotherapy lasted 3 to 6 months and radiation lasted 1 to 2 months. At least 2 wk, but occasionally up to 2 months after completion of treatment, patients underwent fitness evaluations and began the intervention. We waited for at least 2 wk after completion of treatment to allow any cells that might have been damaged by the chemotherapy or radiation to clear from the system.

Study design. To qualify for the study, the patients had to be scheduled for, or be in the process of receiving, chemotherapy for breast cancer; be between the age of 25 and 80 yr; not be on medications that alter the immune system; and have their physician's approval. The protocol was reviewed and approved by the institutional review boards of the Hershey Medical Center, Hershey, Pennsylvania; the Centre Community (Mt. Nittany) Hospital, State College, Pennsylvania; and the office for regulatory compliance institutional review board biomedical and biosafety committees of the Pennsylvania State University (PSU), University Park, Pennsylvania. All patients signed an informed consent before beginning the program.

Blood samples were collected before chemotherapy (T1), after treatment but before beginning the exercise program (T2), after 3 months of exercise (T3), and after 6 months of exercise (T4). It was difficult to recruit patients before chemotherapy; therefore, data from this point (T1) were not included in the final analysis because of low participant numbers. Occasionally, it was impossible to obtain a blood sample. Fitness evaluations in both the exercisers and nonexercisers were carried out posttreatment (T2) and at 3 months (T3) and 6 months (T4) following treatment.

Exercise intervention. All patients with breast cancer underwent a physical and fitness evaluations following previously established protocols at the PSU-GCRC (12,13,17). The 28 participants in the exercise intervention group met with a trainer for one-on-one sessions three times a week. Of

TABLE 1. Patient physical measures.

Variable	Time	Exercisers (Mean ± SD)	*N*	Nonexercisers (Mean ± SD)	*N*
Physical measures					
Age (yr)	T2	48.5 ± 10.6	28	52.3 ± 9.2	21
Weight (kg)	T2	73.6 ± 13.7	28	72.4 ± 11.2	21
	T3	73.31 ± 12.39	28	71.61 ± 12.22	19
	T4	69.83 ± 15.43	21	73.11 ± 10.91	13
BMI ($kg \cdot m^{-2}$)	T2	26.67 ± 5.35	28	26.63 ± 4.13	21
	T3	26.75 ± 4.64	28	26.67 ± 4.54	19
	T4	25.88 ± 4.89	21	27.33 ± 4.64	13
Body fat (%)	T2	33.45 ± 8.07	28	33.03 ± 8.00	20
	T3	32.87 ± 6.88	27	31.55 ± 8.21	18
	T4	32.74 ± 6.61	20	32.98 ± 7.20	13
Exercise measures					
Frequency of breaths ($L \cdot min^{-1}$)	T2	63.09 ± 13.41	28	59.54 ± 14.12	19
	T3	69.81 ± 14.20**	27	58.94 ± 14.07	17
	T4	71.87 ± 15.48#	19	65.20 ± 11.47	12
Volume of oxygen intake ($mL \cdot min^{-1}$)	T2	1466.21 ± 252.63	28	1364.53 ± 345.70	19
	T3	1543.52 ± 304.65	27	1356.11 ± 345.35	18
	T4	1623.68 ± 311.56#	19	1427.77 ± 295.37	13
Peak heart rate (bpm)	T2	163.64 ± 18.18	28	162.68 ± 15.95	19
	T3	167.70 ± 12.73**	27	159.65 ± 19.23	17
	T4	172.47 ± 10.81#	19	162.46 ± 17.61	13
Grip test left hand (lb)	T2	23.39 ± 7.50	27	23.00 ± 4.36	19
	T3	25.77 ± 6.13**	28	23.24 ± 5.31	19
	T4	27.00 ± 6.32**#	20	21.92 ± 4.87	13
Grip test right hand (lb)	T2	25.33 ± 6.88	27	25.11 ± 4.21	19
	T3	27.93 ± 6.26**	27	25.37 ± 4.73	19
	T4*	29.03 ± 6.58**#	20	23.69 ± 3.98	13
Bicep curl (lb)	T2	17.02 ± 4.33	28	16.58 ± 3.54	18
	T3*	18.69 ± 4.29**	28	15.83 ± 4.08	18
	T4	19.12 ± 3.48#	19	16.90 ± 3.33	13
Triceps curl (lb)	T2	23.01 ± 5.70	28	22.02 ± 5.80	19
	T3*	24.63 ± 6.18**	28	22.79 ± 4.95	19
	T4*	25.59 ± 7.60#	20	20.90 ± 6.80	13

* Measure significantly different between the exercisers and nonexercisers ($P < 0.05$).
** Measure significantly increased from the previous point within the group ($P < 0.05$).
# Measure significantly increased from the start (T2) to the end (T4) of the intervention ($P < 0.05$).
T2, posttherapy; T3, 3 months posttherapy; T4, 6 months posttherapy.

these subjects, 12 participated in the exercise intervention for only the first 3 months. After 3 months, 10 women continued to exercise with their trainer and 6 exercised at home on their own. Women who exercised at home were periodically contacted by the trainer to review their progress over the phone. They also underwent a fitness evaluation at the end of the second 3 months. All exercisers maintained a fitness log for the length of the intervention whether working with a trainer or at home.

The training consisted of a 5-min warm-up of light aerobic exercise and stretching, resistance training using Flexbands (Jumpstretch Inc, Boardman, OH), and aerobic activity (treadmill for outdoor running and walking). The women completed four upper and lower body exercises beginning with one set (8–12 repetitions) and gradually increased to three sets by week 4. Aerobic training began at 10–20 min and progressed to 20 min at 60–75% functional capacity. The total session time ranged from 40 to 90 min. Based on an expected frequency of three sessions per week for 3 months, of those who were exercising at the time, the exercise compliance was 82.2% for the first 3 months and 75.9% for the second 3 months.

Training frequency, intensity, and mode were chosen based on recommendations from the American College of Sports Medicine (21). Each woman in this group had acceptable cardiovascular health and was treated as unfit with a starting intensity of 60%, which has been used for cardiac populations. An upper limit of 75% provided the ability for each woman to progress in fitness without undue stress. Resistance exercise was included because of reports that resistance training could increase immune cell function and because we had seen an increase in CD4+ T-cell levels in a group of young women following a 6-month resistance exercise program ((24), and Miles and Mastro, unpublished data 1997).

Blood samples. Approximately 10 mL of blood was collected into heparinized tubes at either the PSU or Hershey GCRC. Blood was drawn at least 48 h after the last exercise session. Of this, approximately 7.0 mL was centrifuged at 1200 RPM for 10 min (IEC Centra, Needham Heights, MA), and the plasma removed and stored at −80°C. The numbers of $CD3^+$, $CD4^+$, $CD8^+$, B, and natural killer (NK) cells in fresh blood were measured by immunochemistry and flow cytometry (Beckman Coulter, Fullerton, CA). The activation of T-helper lymphocytes was determined by the percentage of $CD4^+$ cells also expressing $CD69^+$. True count tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) were used to determine the absolute numbers of $CD4^+$ cells present. $CD4^+$ monoclonal antibodies conjugated with fluoroisothyocyonate (FITC), and CD69+ monoclonal antibodies conjugated with phycoerythrin (PE) were used to determine the percentage of

TABLE 2. Patient demographics.

Variable	Exerciser (*N*)	Nonexercisers (*N*)
Marital status		
Married	22 (78.6%)	15 (71.4%)
Widowed	1 (3.6%)	2 (9.5%)
Single	3 (10.7%)	3 (14.3%)
Divorced/separated	2 (7.1%)	1 (4.8%)
Job type		
Business	9 (32.1%)	4 (19.0%)
Nonbusiness professional	11 (39.3%)	7 (33.3%)
Retail/sales	0 (0.0%)	3 (14.3%)
Other	2 (7.1%)	4 (19.0%)
Unemployed/retired	6 (21.4%)	3 (14.3%)
Employment status		
Full-time	19 (67.9%)	12 (57.1%)
Part-time	3 (10.7%)	2 (9.5%)
Unemployment/retired/other	6 (21.4%)	7 (33.3%)
Education		
High school	3 (10.7%)	6 (28.6%)
Some college	4 (14.3%)	7 (33.3%)
College degree	11 (39.3%)	1 (4.8%)
Graduate school	6 (21.4%)	3 (14.3%)
Other/not given	4 (14.3%)	4 (19.0%)
Stage of cancer		
I	6 (21.4%)	5 (23.8%)
II	19 (67.9%)	10 (47.6%)
III	2 (7.1%)	2 (9.5%)
Unable to stage	1 (3.6%)	4 (19.0%)
Type of surgery		
Lumpectomy	15 (53.6%)	11 (52.4%)
Mastectomy	11 (39.3%)	10 (47.6%)
Unknown	2 (7.1%)	0 (0.0%)
Chemotherapy treatment		
Adriamycin/cytoxan	13 (46.4%)	12 (57.1%)
Adriamycin/cytoxan + taxol	6 (21.4%)	5 (23.8%)
Cytoxan, methotrexanate, 5-flouricil	5 (17.9%)	2 (9.5%)
Other	4 (14.3%)	2 (9.5%)
Radiation treatment		
Yes	19 (67.9%)	16 (76.2%)
No	9 (32.1%)	5 (23.8%)

$CD4^+$ that was also $CD69^+$. Lymphocyte common antigen (LCA), CD45 conjugated with phycoerythrin-texas-red (ECD) fluorochrome was added to every sample and used to gate on the lymphocyte subset of leukocytes. A normalized human peripheral blood leukocyte sample, CD Chex Plus (Streck Labs, Omaha, NE) was used as a control.

Lymphocyte proliferation was determined by mitogen assays over a range of concentrations of the T-cell activators phytohemagglutinin (PHA), concanavalin A (ConA), and the T- and B-cell activator pokeweed mitogen (PWM). Whole blood was diluted 10-fold with phosphate-buffered saline (PBS) and incubated with the mitogen in 96-well plates. No mitogen was added for the control, and all assays were done in replicates of six. Mitogens were added over a range of concentrations—5, 10, and 50 $\mu g \cdot mL^{-1}$ PHA; 3, 12, 25 and 50 $\mu g \cdot mL^{-1}$ ConA; and 0.25, 2.5 and 5 $\mu g \cdot mL^{-1}$ PWM—to determine the optimal concentration. For the last 4 h of a 72-h incubation, cells were pulsed with tritiated thymidine (10 μL, 100 $\mu Ci \cdot mL^{-1}$, 6.7 $Ci \cdot mM^{-1}$) and then harvested onto glass fiber filters. The amount of radioactivity incorporated was measured as the counts per minute with a beta-plate counter (PerkinElmer Wallac, Boston, MA). The average of the six replicates was reported.

Apoptosis was measured in isolated white blood cells using APO 2.7, a detection antibody for a 38-kd mitochondrial membrane protein that is exposed on cells undergoing programmed cell death. APO 2.7 was detected using flow cytometry. Because of almost nondetectable levels and no difference seen between the groups, this expensive and laborious assay was discontinued half way through the study.

Cytokine production was determined in the culture medium of the activated lymphocytes as a measure of their functionality. Cell culture was performed as previously described using 10 $mg \cdot mL^{-1}$ PHA (1). IFN-γ and IL-6 were chosen as indicators of the Th1 and Th2 subpopulations, respectively (i.e., IFN-γ being the predominant Th1 cytokine and IL-6 being the predominant Th2 cytokine). Their use is well documented in the literature and the assays were validated in our laboratory. Whole blood was diluted as in the mitogen assay and incubated with 10 $mg \cdot mL^{-1}$ PHA for 48 h. The culture medium was then collected and frozen at −80°C until assay for the cytokines by ELISA at the PSU-GCRC as previously reported (22).

The blood plasma concentration of IL-6, soluble IL-6 receptor (sIL-6R), soluble glycoprotein 130 (sgp130), and IFN-γ as well as the activated lymphocyte supernatant concentration of IFN-γ and IL-6 were determined by ELISA at the PSU-GCRC as previously reported (22). The range of detection for all assays was 10 to 10,000 $pg \cdot mL^{-1}$. The concentration of the cytokines from the mitogen-activated lymphocytes was divided by the number of CD4+ cells present, as determined by flow cytometry, to standardize the values.

To determine if the plasma IL-6 was capable of eliciting a cellular response or if it was being moderated by its soluble receptors, the concentration of sIL-6R and sgp130 were also determined. The amount of biologically active IL-6 (BAIL-6) was calculated as the ratio of agonistic to antagonistic IL-6 binding by the formula: (IL-6] × [sIL-6R])/ [sgp130] (22).

Statistical analysis. All numerical data were nonparametric. Analysis of baseline differences in physical measures between the two breast cancer groups was performed by a Mann–Whitney U test. A chi-square test was used to compare descriptive characteristics (e.g., job and educational status) between the two breast cancer groups. Analyses of groups from T2–T3, T3–T4, and T2–T4 were done using the Wilcoxon signed rank test. Comparisons of the cytokine mean at a given point between the two groups were performed using the Mann–Whitney U test. The change in cytokine concentration between points was calculated and the difference in the change score between two groups analyzed again with the Mann–Whitney U test. All analyses were performed using SPSS 10.0 for Windows and SPSS 11.0.2 for Macintosh OSX (SPSS Inc., Chicago, IL). Values are expressed as the mean ± the SD and a P value less than 0.05 was considered significant unless otherwise noted.

RESULTS

No statistical difference was found between the groups in age, weight, body mass index, or percent fat at the post-therapy point (T2) (Table 1). The exercisers and nonexer-

cisers did not differ at the posttherapy baseline in their marital status, type of job, employment status, education, stage of cancer, type of surgery, or treatment (Table 2). This result suggests that the recruitment at two locations resulted in an appropriate randomization. At the end of intervention, the exercisers showed an increase in their fitness level as indicated by the volume of oxygen intake ($\dot{V}O_{2max}$), grip test, bicep curl, and triceps curl (Table 1). The nonexercise group did not show an increase in fitness, despite any exercise they may have performed on their own.

Activation. The exercisers showed an increase in fitness over the course of the intervention as measured by their $\dot{V}O_{2max}$ and upper body strength. No difference in the mean number of $CD3^+$, $CD4^+$, $CD8^+$, B, or NK cells were seen between the exercisers and nonexercisers. Both groups exhibited mean $CD3^+$, $CD8^+$, NK, and B-cell levels that were below the interquartile range in healthy individuals at all points following therapy (6).

The percentage of $CD4^+$ cells that were also $CD69^+$ as measured by immunochemistry, and flow cytometry was significantly elevated in the exercisers compared with the nonexercisers at the end of the intervention (Table 3). When the total numbers of $CD4^+CD69^+$ cells were compared, however, no significant difference was seen between the two groups at any points. At the start of the intervention, the mean for the percent and total $CD4^+CD69^+$ were higher, with a larger standard deviation, in the exercisers compared with the nonexercisers. This difference was the result of one person with 21% $CD4^+$ cells that were also $CD69^+$ and a total $CD4^+CD69^+$ count of 124 cells per liter of blood. When this outlier was removed, the percentage and total $CD4^+CD69^+$ counts and standard deviation were similar between the two groups. In the nonexercisers, this percentage significantly decreased from the start to the middle and end of the intervention period. In both groups, the mean percentage and number of $CD4^+$ $CD69^+$ cells was lower at the end of the study than the start, although the decrease was not statistically significant.

The average counts per minute caused by incorporation of tritiated thymidine were plotted for each mitogen to determine which concentration gave the maximal proliferative response. The optimal concentration was 25 $mg \cdot mL^{-1}$ for Con A, 50 $mg \cdot mL^{-1}$ for PHA, and 5 $mg \cdot mL^{-1}$ for PWM. The counts per minute were significantly higher in the cells from the exercisers compared with those of the nonexercisers at the end of the study for all mitogens at their optimal concentration (Table 4).

Activated cell supernatant cytokines. Cytokine concentrations from the culture supernatant of activated lymphocytes were measured by ELISA as an indication of the lymphocytes' functionality. IFN-γ represented a Th1 response, and IL-6 corresponded to a Th2 response. The ratio of IFN-γ to IL-6 was also computed. The production of IFN-γ, IL-6, and the ratio of IFN-γ to IL-6 did not change over the course of the study in either the exercisers or nonexercisers (Table 5). Also, no difference was found in the cytokine production or ratio for the exercisers when compared with the nonexercisers at all points.

Plasma cytokines. The change in the mean IFN concentration in the plasma of exercisers decreased from 215.4 $\pm$ 490.5 $pg \cdot mL^{-1}$ (T2) to 202.7 $\pm$ 481.6 $pg \cdot mL^{-1}$ (T3), whereas that in the nonexercisers increased from 133.2 $\pm$ 236.1 $pg \cdot mL^{-1}$ (T2) to 331.1 $\pm$ 561.6 $pg \cdot mL^{-1}$ (T3) when the change in concentration between the postchemotherapy to the 3-month point was calculated for each subject. This change in the mean plasma IFN-γ concentration between the two groups was significantly different. This was the only difference between the two groups, and no significant change was found in the mean in either the exercisers or the nonexercisers over the course of the study.

The plasma IL-6 concentrations following chemotherapy were highly variable within both groups (Table 6). The plasma concentrations of the exercising subjects were not significantly different than the nonexercising subjects at any times, although the concentration was higher in the exercisers. Also, no significant difference was found in the change in IL-6 over time for either group.

The plasma concentration of sIL receptor and sgp130 were measured to calculate the level of biologically active IL-6 (see Methods). Neither sIL6-R nor sgp130 themselves were significantly different between the exercisers and nonexercisers, nor did they change significantly over time (Table 6). Also, neither the exercisers nor the nonexercisers showed a change in BAIL-6 over the study points or when one point was compared between the exercisers and nonexercisers.

When the ratios of IFN-γ to IL-6 were compared at the postchemotherapy point (T2), the mean of the exercisers was significantly higher than that of the nonexercisers ($P <$

TABLE 3. Percentage and absolute numbers of CD4+CD69+ lymphocytes from breast cancer patients following chemotherapy and an exercise intervention.

		Exercisers			Nonexercisers		
		N	No. cells/μL blood Mean	SD	*N*	No. cells/μL blood Mean	SD
% $CD4^+CD69^+$	T2	28	1.40	4.00	21	0.63	0.82
	T3	27	0.37	0.29	20	0.41$	0.61
	T4	21	0.51#	0.46	16	0.34#~	0.61
Total $CD4^+CD69^+$	T2	27	7	24	21	4	8
	T3	27	2	2	20	2	3
	T4	21	3	3	16	2	4

Blood was drawn from patients following chemotherapy as indicated in the Methods section.
CD4+CD69+ lymphocytes were labeled with fluorescently conjugated antibodies and analyzed by flow cytometry.
Both percentages and absolute numbers of lymphocytes were determined.
Measure is significantly different between the exercisers and nonexercisers.
$ Significantly different from measure at previous time.
~ Significantly different from start of intervention.

TABLE 4. DNA synthesis in mitogen-stimulated lymphocytes from exercising and nonexercising breast cancer patients following chemotherapy.

		Exercisers			Nonexercisers		
		Tritiated Thymidine Incorporation			Tritiated Thymidine Incorporation		
	Time	*N*	Mean CPM	SD	*N*	Mean CPM	SD
ConA 3 $\mu g \cdot mL^{-1}$	T2	27	7981	6973	21	7142	8291
	T3	28	8924	6643	19	8282	6723
	T4	20	10,218	7149	15	6224	3150
ConA 12 $\mu g \cdot mL^{-1}$	T2	27	14,077	9456	21	11,645	12,099
	T3	28	16,479	12,000	19	14,512	14,085
	T4	20	16,995#	8750	15	10,264#	5962
∧ConA 25 $\mu g \cdot mL^{-1}$	T2	27	14,128	10,437	21	10,289	8038
	T3	28	16,352	16,079	19	12,771	14,661
	T4	20	17,445#	9587	15	9669#	6274
ConA 50 $\mu g \cdot mL^{-1}$	T2	27	8487	8131	21	7817	9768
	T3	28	10,775#	12,726	19	6800#	8311
	T4	20	10,760	8604	15	5541	4084
PHA 5 $\mu g \cdot mL^{-1}$	T2	27	10,932	16,202	21	12,245	13,290
	T3	28	6985#	6563	19	17,328#	19,892
	T4	20	11,680	12,971	15	7898∼	8565
PHA 10 $\mu g \cdot mL^{-1}$	T2	27	19,603	20,928	21	19,302	12,149
	T3	28	21,027	14,001	19	27,549	27,663
	T4	20	24,919	16,788	15	18,454	12,974
∧PHA 50 $\mu g \cdot mL^{-1}$	T2	27	34,600	27,277	21	23,694	17,630
	T3	28	39,285	30,853	19	28,770	23,537
	T4	20	39,321#	21,207	15	26,444#	18,296
PW 0.25 $\mu g \cdot mL^{-1}$	T2	27	3314	2562	21	5397	11,356
	T3	28	3899	3680	19	4511	5774
	T4	20	5479	5430	15	3788	2961
PW 2.5 $\mu g \cdot mL^{-1}$	T2	27	4455	3193	21	3510	2382
	T3	28	5009	3529	19	5009	4698
	T4	20	6618	5134	15	4348	2844
∧PW 5 $\mu g \cdot mL^{-1}$	T2	27	4345	3077	21	5501	10,184
	T3	28	5213	3872	19	4581	4097
	T4	20	6754#	5426	15	4192#	2741

Whole blood was prepared as described in the methods and incubated with a range of concentrations of mitogens.
Tritiated thymidine was added for the last 4 h of a 72-h culture. Cells were collected on glass fibers and the radioactivity incorporated was determined with a beta plate counter. Data are presented as the mean CPM incorporated ± the standard deviation.
* Measure is significantly different from the start to end of study.
# Measure is significantly different between the exercisers and nonexercisers.
$ Significant difference from measure at previous time.
∼ Significant difference from start of intervention.
∧ Optimal concentration of mitogen.

0.025) (Table 6). The ratio for the exercising group also increased significantly from 3 to 6 months after the start of intervention (1.2 to 1.6, $P < 0.02$). This change in ratio reflected an increase in IFN-γ that exceeded the increase in IL-6. The ratio did not change over time in the nonexercising group.

DISCUSSION

In this study, patients with breast cancer were enrolled in a 6-month moderate exercise program very soon after completing chemotherapy or radiation treatment. These patients showed improvements in fitness as well as immunologic indicators compared with patients who did not exercise. The exercising group showed a greater percentage of proliferating $CD4^+CD69^+$ T-helper cells. This proliferation results from activation of the T cells and suggests that, although the level of $CD4^+$ cells was reduced below normal as a result of the treatment, the cells remaining were functional and capable of responding to antigen. The results were not skewed by a difference in apoptosis between cells from the two groups (data not shown). We felt that it was important to measure apoptosis because there might be an increased turnover of lymphocytes caused by programmed cell death. Very low levels of apoptosis were detected and no difference seen between the two groups. The difficulty in detecting apoptotic lymphocytes is not surprising because apoptotic cells are rapidly removed *in vivo*.

A greater level of activation in the exercisers' $CD4^+$ lymphocytes as measured by expression of $CD69^+$ supported the mitogen assays. With all three mitogens, the lymphocytes of the exercisers showed a greater level of proliferation at the end of intervention compared with those of the nonexercisers. PWM stimulates both B and T cells to proliferate. Increased levels of tritiated thymidine incorporation for the exercisers when lymphocytes were stimulated with PWM may suggest an increase in B-cell activation in addition to $CD4^+$ activation.

These findings may be clinically relevant because cancer patients often experience secondary infections following chemotherapy because of their immunosupressed state. It is known that lymphocyte numbers are reduced after chemotherapy; however, the total number of cells is not necessarily indicative of the ability of an individual to mount an appropriate immune response. These findings suggest that, although the lymphocyte numbers were reduced up to 6 months following treatment, exercise may prove to be a useful form of immunotherapy. By participating in a mod-

TABLE 5. Cytokines secreted by PHA activated lymphocytes.

Group	Time	IFN ($ng \cdot mL^{-1}$)	*N*	IL-6 ($pg \cdot mL^{-1}$)	*N*	IFN/IL-6	*N*
Exercisers	T2	24.5 ± 48.2	20	339.8 ± 1060.9	20	0.2 ± 0.6	17
	T3	6.6 ± 11.0	25	72.5 ± 114.4	22	7.8 ± 35.5	21
	T4	6.0 ± 11.0	18	49.8 ± 71.3	16	0.2 ± 0.7	16
Nonexercisers	T2	15.4 ± 18.6	12	72.8 ± 179.7	12	0.3 ± 0.4	8
	T3	19.3 ± 44.3	14	53.8 ± 87.3	14	1.2 ± 3.6	12
	T4	13.4 ± 16.6	12	886.2 ± 2918.4	12	0.5 ± 0.5	10

Lymphocytes were treated as described in the legend to Table 5 except that only PHA was used (10 $\mu g \cdot mL^{-1}$).
Values are expressed as the mean ± the standard deviation.
* Measure is significantly different between the exercisers and the nonexercisers.
$ Measure is significantly different the measure at precious time.
~ Measure is significantly different from the start of intervention.
T2, posttherapy; T3, 3 months posttherapy; T4, 6 months posttherapy.

erate exercise program, patients with breast cancer exhibited greater levels of $CD4^+$ cell activation. This may be important for cancer patients following therapy to prevent secondary infections, recurrence, and metastasis that can result from a depressed immune system.

Despite a greater level of lymphocyte activation in the exercisers, no difference was seen in the cytokine concentrations in the culture supernatant of activated lymphocytes when the exercisers were compared with the nonexercisers. Also, no significant change was seen in the cytokine production from lymphocytes taken over time in either group. In both groups and at all points, the amount of IFN-γ produced was close to the limit of detection, making any difference between the two groups too small to be accurately measured or statistically significant. When IL-6 was examined, the variation in both groups was so great that no significant difference was detected between the two groups. The low level of IFN-γ production and variability of IL-6 makes this a poor indication as to which subpopulations of lymphocytes were activated.

Several cytokines were examined in the plasma of the patients with breast cancer to see whether the difference in potential lymphocyte activation produced a difference in circulating cytokine concentrations. IFN-γ and IL-6 as well as the ratio of IFN-γ to IL-6 were examined to determine whether the immune response was predominantly Th1 or Th2. No significant difference was found in IL-6 between the two groups or over time in either group. The change in the mean concentration of IFN-γ decreased in the exercisers from the start to the middle of the intervention, whereas it increased in the nonexercisers. The change in the mean IFN-γ concentration was significant when compared between the two groups. No difference was noted, however, in the mean concentration of IFN-γ between the two groups at any one point or over time within either group. Despite no significant differences in the concentration of IFN-γ or IL-6 at the posttherapy point, the ratio of IFN-γ to IL-6 was significantly different between the two groups. This result was random and not a result of exercise, because the intervention had not yet begun. From these data, the increased lymphocyte activation in the exercisers at the end of the intervention did not cause a difference in the mean concentration of IFN-γ, IL-6, or the ratio over the course of the intervention.

This finding suggests that, despite the difference in activation, no apparent long-term significant changes occur in cytokine production or in the relationship between the Th1 and Th2 responses as a result of exercise. One explanation to account for similar cytokine production in the two groups is that a large number of näive or Th_0 cells exist. Additionally, cytokines are meant to act locally, and the circulating level most likely does not represent the true amount produced by activated lymphocytes.

New research suggests that the plasma concentration of IL-6 may not accurately represent the concentration capable of participating in cellular signaling. Binding of IL-6 to its alpha chain receptor induces a conformational change that allows the beta chain glycoprotein 130 (gp130) to also bind (16,20). Both IL-6R and gp130 exist in soluble forms (sIL-6R, and sgp130) (16). When sIL-6R binds IL-6, it reduces IL-6 proteolysis and extends its half-life and increases the biological function of IL-6 by allowing the IL-6–sIL-6R complex to bind gp130 (10,19). This process of transsignaling is important for the biological activity of IL-6, because many cell types do not express IL-6R but do express gp130, a receptor chain common to several cytokines (10,20). sgp130, however, inhibits IL-6 bioactivity by blocking the ability of the IL-6–sIL-6R complex to bind to membrane-

TABLE 6. Plasma cytokine concentrations from breast cancer patients following chemotherapy from breast cancer.

Group	Time (Mean ± SD)	IFN ($ng \cdot mL^{-1}$)	*N*	IL-6 ($pg \cdot mL^{-1}$)	*N*	SIL-6R ($ng \cdot mL^{-1}$)	*N*	sgp130 ($ng \cdot mL^{-1}$)	*N*	BAIL-6 ($pg \cdot mL^{-1}$)	*N*	IFN/IL-6	*N*
Exercisers	T2	215.4 ± 490.5	17	384.4 ± 1103.8	19	22.2 ± 10.7	20	234.5 ± 118.9	19	14.7 ± 34.6	16	1.31 ± 0.85*	18
	T3	202.7 ± 481.6	24	495.2 ± 1948.8	26	26.8 ± 15.5	26	280.4 ± 91.5	26	87.4 ± 361.4	26	1.19 ± 1.09	25
	T4	279.2 ± 546.2	18	690.9 ± 2333.5	18	23.9 ± 20.5	19	309.8 ± 93.5	20	133.4 ± 461.0	17	1.59 ± 1.69	18
Nonexercisers	T2	133.2 ± 236.1	11	204.4 ± 321.6	14	23.5 ± 8.0	18	276.0 ± 74.7	19	15.3 ± 28.2	13	0.70 ± 0.45*	11
	T3	331.1 ± 561.6	12	176.1 ± 312.9	15	21.6 ± 6.2	16	264.9 ± 89.3	16	9.5 ± 14.6	14	1.29 ± 0.90	12
	T4	239.6 ± 596.8	11	227.9 ± 388.5	14	18.3 ± 12.4	12	282.8 ± 93.8	14	18.4 ± 29.3	12	3.74 ± 9.69	11

* Measure is significantly different between the exercisers and the nonexercisers.
T2, posttherapy; T3, 3 months posttherapy; T4, 6 months posttherapy.

bound gp130 (20). As with membrane-bound gp130, sgp130 can only bind IL-6 if the IL-6 is associated with the alpha sIL-6R (10). Therefore, sgp130 and sIL-6R concentrations were also measured to compute the concentration of biologically active IL-6, BAIL-6 (see Methods).

No significant differences were found in sgp130, sIL-6R, or BAIL-6 production between the two groups at any point or over time. This finding would indicate that IL-6 was not being differentially regulated in one group compared with the other. Because IL-6 is indicative of a Th2 response, this supports the IL-6 data, and suggests the Th2 response was not significantly affected by exercise.

It is known that chemotherapy affects the number of lymphocytes, especially $CD4^+$ T-helper cells, and often results in a decrease in their function. This study suggests that moderate, mixed exercise may prove beneficial in increasing the activation of lymphocytes in patients with breast cancer following chemotherapy. Despite the increased numbers of activated cells, no difference was seen in the concentration of cytokines produced in the plasma or in when they were activated with mitogens. This study may prove to have important clinical manifestations. In addition to traditional treatments, many patients and doctors look to augment therapy with lifestyle changes such as diet and exercise. Little data, however, support the benefit or disprove any negative effects that may result. This study suggests that a moderate, mixed exercise program following treatment for breast cancer may heighten the function of the immune system. A more functional immune system may prove valuable in fighting secondary infection, recurrence, or possibly metastasis. A longer, more inclusive study investigating the rates of infection, recurrence, and survival would be valuable to examine the relationship between exercise-induced immune activation and clinical outcomes.

After the submission of this manuscript, a paper reporting the results of ergometer exercise on breast cancer survivors appeared in print (5). Although the experimental design was different, it is interesting to note that the exercise group in this study showed an increased number of lymphocytes undergoing DNA synthesis (^{3}H-thymidine incorporation) without stimulation *ex vivo*, similar to our finding of increased CD69+ lymphocytes in the blood from exercising cancer patients.

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