Impact of Acute Intermittent Exercise on Natural Killer Cells in Breast Cancer Survivors

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Abstract
Background. Current research examining the effect of exercise on immune responses in cancer survivors is limited. Objective. The aim of this pilot study was to examine the effect of 1 bout of intermittent exercise on natural killer (NK) cell numbers in breast cancer survivors. Methods. A total of 9 women with stage I to III invasive breast cancer who were 3 to 6 months posttreatment and 9 sedentary women without a history of cancer completed 10 three-minute intervals of aerobic exercise on the cycle ergometer at 60% of VO₂peak (peak oxygen uptake). Whole blood samples were taken pre-exercise, immediately postexercise, and at 2 hours and 24 hours postexercise. NK cell counts were assessed using flow cytometry. Results. In both groups, NK cell counts significantly increased immediately postexercise compared with pre-exercise (P = .004-.008) and returned to near pre-exercise levels during recovery (P = .129-.547). Absolute NK cell counts were significantly lower in breast cancer survivors immediately postexercise when compared with controls (P = .046). Conclusions. The breast cancer survivor group exhibited NK cell responses to 30 minutes of moderate-intensity intermittent aerobic exercise that were comparable with that in the group of physically similar women without a history of cancer. Immune changes related to cancer treatments may be related to the lower absolute NK cell counts observed in the breast cancer survivor group. Although the results of this study are preliminary in nature, they suggest that this type of exercise does not disrupt this aspect of innate immunity in recent breast cancer survivors, thereby supporting current exercise recommendations for this population.

Keywords
NK cells, innate immunity, breast cancer, aerobic exercise, intermittent exercise, moderate intensity

Introduction
In the United States, breast cancer is the most common cancer among women except for skin cancer.¹ The American Cancer Society estimates that in 2015, approximately 231,840 new cases of invasive breast cancer will be diagnosed and that approximately 40,290 women will die from breast cancer.¹ Major treatments for breast cancer, including surgery, chemotherapy, and radiation therapy, can cause significant physical distress that may persist for months or years after the completion of therapy.¹ To this end, numerous studies over the past 20 years have tested various strategies to alleviate these complications, with many focusing on exercise. Many of these first-generation studies have shown that exercise training can mitigate many treatment-related side effects and improve physical functioning and that regular exercise appears to be safe and well tolerated and is associated with few adverse events.³⁻⁵ Exercise is an attractive adjunct therapy for cancer patients and survivors because of its potentially positive influence on the immune system.⁶ One particular component of the immune system, the natural killer (NK) cell, is especially relevant because of its ability to target and kill virally infected cells as well as cells that have undergone malignant transformation.⁷ In healthy athletic and nonathletic individuals, NK cells are extremely responsive to acute aerobic exercise. They follow a profile that typically shows marked elevations in both cell number and activity immediately

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postexercise while displaying decreases during the recovery period for up to several hours. It is also thought that exercise-related enhancements in NK cell function may confer a protective effect against pathogen invasion, whereas exercise-related decreases in NK cell function may lead to increased incidence of viral infection and potential illness.

Examining and understanding NK cell responses to acute aerobic exercise in breast cancer survivors is particularly important when considering exercise prescription for this population. Currently, aerobic exercise prescriptions for cancer patients and survivors are largely modeled after those for the general population. Whereas much research has been conducted in healthy individuals, immune responses to exercise have not been systematically measured in breast cancer survivors. Because cancer treatments can lead to immune system deficiencies that may continue for extended periods of time posttreatment, one could hypothesize that a cancer patient’s weakened immune system may not respond to exercise in the same way as that of a healthy individual. Typically, aerobic exercise prescriptions are constructed of an accumulation of multiple acute exercise sessions per week. Therefore, exercise specialists and clinicians need to understand how long it takes for a breast cancer survivor’s immune system to recover from 1 bout of aerobic exercise before prescribing the next bout of aerobic exercise. Current studies examining NK cell responses to exercise in breast cancer patients and survivors have shown promising results. However, these studies only examined resting NK cell function at the beginning and end of an exercise training period; none examined the acute exercise response, and none compared these responses with that of individuals who had never experienced the physiological toll of cancer treatments. Studying NK cell changes and function may provide insight into whether the exercise is contributing to enhanced immune function or leading to periods of potential immunosuppression. Therefore, the purpose of this investigation was to compare the effect of a single session of intermittent aerobic exercise on NK cell numbers in breast cancer survivors and healthy controls.

Methods

Participants

Participants were recruited into a breast cancer survivor group and a control group. Participants in the breast cancer survivor group included women who had been diagnosed with stage I, II, or III invasive breast cancer; had received chemotherapy; and had completed all planned surgery, chemotherapy, and radiation therapy 3 to 6 months prior to enrollment. They were recruited from the Medical Oncology and Radiation Oncology clinics at the North Carolina Cancer Hospital and from the waitlist for the Get REAL & HEEL Breast Cancer Rehabilitation Program in the Department of Exercise and Sport Science at the University of North Carolina at Chapel Hill (UNC-Chapel Hill). Participants in the control group included women who did not have a history of cancer diagnosis or treatment, were sedentary (ie, had not participated in regular organized physical activity within the past year), and were free from cardiovascular and musculoskeletal disease that would render aerobic exercise participation unsafe. They were recruited from the faculty, staff, and student populations at UNC-Chapel Hill as well as from across the surrounding geographic communities. All participants, regardless of study group, were between the ages of 40 and 70 years, were not regular users of anti-inflammatory medications, and had not experienced a menstrual period for approximately 1 year prior to enrollment (either as a result of being postmenopausal or as a result of cancer treatment). No participant reported suffering from any other disease states or conditions. Approval from the Protocol Review Committee in the Lineberger Comprehensive Cancer Center, the Institutional Review Board in the Department of Exercise and Sport Science, and the Biomedical Institutional Review Board at UNC-Chapel Hill were obtained before proceeding with participant recruitment. All participants were provided with hard copies of written informed consent documents. All participants, as well as the research team member obtaining consent for participation, signed all written informed consent documents prior to performing any testing procedures.

Overview of Procedures

Each participant visited the laboratory on 3 separate occasions. Prior to each laboratory visit, all participants were asked to refrain from eating for at least 2 hours prior to testing, exercise and caffeine for at least 12 hours prior to testing, alcohol use for at least 48 hours prior to testing and to maintain adequate hydration. Visit 1 included an orientation to the study, medical and physical screening, and the assessment of peak aerobic capacity (peak oxygen uptake [VO2peak]) on the cycle ergometer. During visit 2, the participants performed a moderate bout of intermittent aerobic exercise at 60% of VO2peak for 30 minutes on the cycle ergometer. Blood samples were collected during this laboratory visit, immediately prior to exercise, immediately postexercise, and 2 hours postexercise. Visit 3 occurred 24 hours after the completion of exercise and consisted of only a follow-up resting blood draw. All laboratory visits occurred in the Integrative Exercise Oncology Research Laboratory and/or in the Applied Physiology Laboratory in the Department of Exercise and Sport Science at UNC-Chapel Hill.
Visit 1: Medical/Physical Screening and VO_{2peak} Test

During the first visit to the laboratory, participants received an explanation of the study protocol, information regarding the potential risks of participation in the study and were asked to sign the informed consent documents. All participants completed a comprehensive medical history questionnaire and underwent a 12-lead resting electrocardiogram (ECG) and a physical exam by either a physician or other certified professional. Participants were screened for exclusion based on the criteria put forth by the American College of Sports Medicine as contraindications to exercise testing.\(^1\)

Age, race, height (portable stadiometer, Perspective Enterprises, Portage, MI), and body mass (calibrated balance-beam scale, Detecto, Webb City, MO) were recorded for all participants upon enrollment into the study. Height and body mass were used to calculate body mass index (BMI). Percentage body fat was assessed using a Discovery dual energy X-ray absorption scanner (Hologic, Inc, Bedford, MA). For participants in the breast cancer survivor group, cancer treatment type was also recorded.

The final component of visit 1 was an exercise test to measure VO_{2peak}. The purpose of measuring VO_{2peak} was to assess peak aerobic capacity which would then be used to estimate the submaximal workload that participants would perform during laboratory visit 2. The VO_{2peak} test was performed on an electronically braked cycle ergometer (Lode, Groningen, Netherlands) using the Astrand Cycle Ergometer Maximal Test Protocol.\(^19\) Expired gases were collected and analyzed using a TrueMax 2400 Metabolic System (Parvo Medics, Salt Lake City, UT). Participants began the test sitting quietly on the cycle ergometer for 3 minutes while resting metabolic data were collected. Participants then began the first stage of the test by cycling at 50 W for 3 minutes. At the end of the 3-minute stage, the workload was increased by 25 W every 3 minutes until volitional fatigue. Heart rate (HR), rating of perceived exertion (RPE),\(^20\) expired gases, and 12-lead ECG monitoring were performed throughout the test. HR and RPE were recorded at the end of every minute. The highest VO\(_2\) measured by the metabolic system during the last stage of the test was recorded at the participant’s VO_{2peak}, and the corresponding workload on the cycle ergometer was recorded as the participant’s peak workload. Once the VO_{2peak} test was complete, the participant pedaled at a very low workload (<20 W), with ECG and blood pressure monitoring continuing until they had returned to near-baseline levels.\(^21\)

VO_{2peak} was calculated by the metabolic system, whereas the peak workload (W) was the workload at which the participant was exercising when VO_{2peak} was achieved. From these results, the workload (W) at 60% of VO_{2peak} was estimated following the Karvonen procedures, by regressing VO\(_2\) values for each stage against the corresponding workload for that stage. This intensity was chosen because it is widely used in exercise prescriptions for this population.\(^2,12,17,22-29\)

Visit 2: Acute Aerobic Exercise Session

During the second visit to the laboratory, participants performed an acute bout of moderate aerobic exercise on the cycle ergometer at a workload corresponding to 60% of the participant’s VO_{2peak} for 30 minutes. This combination of intensity and duration is commonly used in many studies that have examined the effects of aerobic exercise on physical functioning in breast cancer patients and is, therefore, representative of exercise prescriptions that are widely used for this population.\(^3,12,17\) However, previous pilot testing in the laboratory with recent breast cancer survivors demonstrated that exercising on a cycle ergometer for 30 continuous minutes at 60% of VO_{2peak} was rather challenging. Therefore, to ensure that all participants would be able to complete the exercise session, an intermittent protocol was used, such that participants alternated 10 three-minute intervals of exercise with 90 s of rest, for a total of 30 minutes of exercise in a 43.5-minute period. All participants started the exercise session between 7:00 and 10:00 AM to control for daily variations in the study variables.

At the beginning of visit 2, participants rested in the supine position for approximately 20 minutes while a catheter was inserted into an antecubital vein in the arm for blood sampling. A 3-syringe technique was used for blood sampling. The first draw was a small amount to discard the contents of the catheter. The second draw contained the sample. The third injected a small amount of sterile saline to keep the catheter patent. A pre-exercise blood sample was drawn into K\(_2\)EDTA Vacutainer tubes, which were used for the measurement of NK cell counts.

After the pre-exercise blood sample was obtained, participants sat quietly on the cycle ergometer for 3 minutes while resting metabolic data were collected. After approximately 5 minutes of warm-up, participants began the 30-minute moderate-intensity exercise bout at the prescribed workload. HR and RPE were recorded at the end of every 3-minute period of exercise. Expired gases were monitored during the 1st, 3rd, 7th, and 10th exercise intervals. If necessary, adjustments in workload were made to ensure that the participants were exercising as close to 60% of VO_{2peak} as possible for the entire 30 minutes. Immediately after completion of the exercise bout, participants dismounted the cycle ergometer and returned to the supine position. Blood sampling immediately postexercise was performed in the same manner as described above.

Participants were then allowed to rest comfortably in the laboratory. They were allowed to drink water ad libitum; however, no food or other beverage could be ingested. A
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final blood sample was obtained at 2 hours postexercise in the same manner as the other 2 samples. The catheter was then removed, and any necessary bandaging was performed.

Visit 3: 24-Hour Follow-up Session

The purpose of the third visit was to obtain a blood sample 24 hours after the completion of the aerobic exercise bout. The blood sample was drawn from an antecubital vein using a standard venipuncture technique.

Determination of Immunological Parameters

Complete blood counts were obtained from all blood samples using a COULTER Ac•T diff Hematology Analyzer (Beckman Coulter, Inc, Brea, CA). Whole blood samples were stained using fluorescently labeled monoclonal antibodies for the cell surface markers CD3, CD16, and CD56. The specific fluorescent labels were fluorescein isothyocyanate for the CD3 marker, phycoerythrin for the CD16 marker, and allophycocyanin for the CD56 marker. Fluorescent staining was done to identify the proportion of lymphocytes that express the CD3-CD16+CD56+ phenotype that is characteristic of NK cells. Stained samples were further prepared for flow cytometric analysis according to procedures adapted from protocols made available by Becton Dickinson.30 Samples were fixed in a 1% paraformaldehyde solution and analyzed within 24 hours of preparation using a CyAn 3 laser/9 color flow cytometer (Beckman Coulter, Inc). Flow cytometry data were viewed and analyzed using Summit 4.3 software (Dako North America, Inc, Carpinteria, CA). Absolute NK cell counts were calculated by multiplying total lymphocyte counts by the NK cell proportions.

Calculation of Plasma Volume Shifts

Plasma volume shifts (changes in plasma volume caused by exercise) were calculated according to the equation given by Dill and Costill.31 The hematocrit and hemoglobin values that were used in these calculations were obtained from the complete blood count data pre-exercise immediately postexercise, 2 hours postexercise, and 24 hours postexercise. Plasma volume shifts were reported to estimate the effect that exercise and posture had on fluid shifts, which may affect concentrations of leukocytes.

Statistical Analysis

Statistical analyses were performed using SAS version 9.3. All study variables were compared between groups using Wilcoxon rank sum tests and within groups using Wilcoxon signed rank tests, with significance set a priori at \( P < .05 \). In Tables 1 to 3 and in the text of the Results section, data are presented as mean ± standard deviation (SD). In Figure 1, data are displayed as boxplots to show the median and interquartile ranges of the NK cell counts. Although nonparametric statistics were used for analysis, data are largely presented as mean ± SD to allow for easier comparison between the results of the current study and results from other published studies.

Results

Participants

This study included a total of 18 participants, with 9 participants in the breast cancer survivor group and 9 participants in the control group. Physical characteristics for the 18 participants are presented in Table 1. Age was the only physical characteristic for which there was a statistically significant difference between groups, where the average age of the control group was approximately 9 years more than that of the breast cancer survivor group (\( P = .013 \)). Although the BMIs do not suggest obesity, the percentage of body fat (−42%) for both groups was very high. In addition, the \( \text{VO}_{2\text{peak}} \) results suggest very low aerobic fitness for both groups.

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**Table 1. Participant Physical Characteristics and \( \text{VO}_{2\text{peak}} \).**

| Characteristic                  | Breast Cancer Survivor Group (n = 9) | Control Group (n = 9) |
|--------------------------------|-------------------------------------|----------------------|
| Age, years                     | 50 ± 6b                             | 59 ± 5b              |
| Race, number of women          | Caucasian, 8; African American, 1   | Caucasian, 9         |
| Height, cm                     | 164.7 ± 5.8                         | 163.8 ± 5.9          |
| Weight, kg                     | 76.9 ± 12.6                         | 77.7 ± 13.3          |
| Body mass index, kg/m²         | 28.4 ± 5.0                          | 28.9 ± 4.6           |
| Percentage body fat (%)        | 41.6 ± 4.5                          | 42.1 ± 4.0           |
| \( \text{VO}_{2\text{peak}}, \text{ mL/kg/min} \) | 18.1 ± 2.7                         | 18.5 ± 5.1           |
| Peak workload, W               | 107 ± 19                            | 106 ± 17             |

Abbreviation: \( \text{VO}_{2\text{peak}} \), peak oxygen uptake.

*Values given are mean ± standard deviation.

\( P < .05 \) for comparing age between groups.
All participants in the breast cancer survivor group received surgery, chemotherapy, and radiation therapy and had completed these treatments within 3 to 6 months prior to enrollment. Four participants underwent mastectomy, and 5 underwent lumpectomy. A total of 6 participants received the chemotherapy combination of doxorubicin, cyclophosphamide, and paclitaxel, and one of these participants also received carboplatin; 1 participant received the chemotherapy combination of cyclophosphamide and docetaxel, and 2 participants received the chemotherapy combination of carboplatin and docetaxel; 6 participants were receiving adjuvant hormonal therapy, with 5 receiving tamoxifen and 1 receiving letrozole; and 2 participants were receiving adjuvant trastuzumab. Finally, 2 participants received additional medications related to their cancer treatments, with one receiving lapatinib (an anti-HER 2 protein

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### Table 2. Participant Heart Rate, Rating of Perceived Exertion, and Metabolic Responses During the Submaximal Aerobic Exercise Session.a

| Parameter          | Breast Cancer Survivor Group | Control Group |
|--------------------|-------------------------------|---------------|
| Submaximal VO₂, mL/kg/min | 12.1 ± 1.3                   | 11.1 ± 2.1    |
| Submaximal workload, W   | 59 ± 9                        | 62 ± 11       |
| Resting HR, beats/min    | 68 ± 6                        | 66 ± 9        |
| Exercise HR, beats/min   | 122 ± 18                      | 119 ± 16      |
| RPE                 | 12 ± 1                         | 12 ± 1        |

Abbreviations: VO₂, oxygen uptake; HR, heart rate; RPE, rating of perceived exertion.

aValues given are mean ± standard deviation.

### Table 3. Blood Cell Counts and Plasma Volume Shifts Across Time.a

| Parameter                  | Breast Cancer Survivor Group | Control Group |
|----------------------------|-------------------------------|---------------|
| Total leukocytes (cells/µL)|                               |               |
| Pre-exercise               | 3666.7 ± 1659.1               | 4377.8 ± 1072.1 |
| 0h Postexercise            | 4964.7 ± 1973.4b              | 5540.1 ± 1089.4b |
| 2h Postexercise            | 5264.7 ± 1645.0c              | 5552.5 ± 1214.8c |
| 24h Postexercise           | 3642.6 ± 1370.8               | 4288.8 ± 1105.6 |
| Total granulocytes (cells/µL)|                               |               |
| Pre-exercise               | 2633.3 ± 1320.0               | 2844.4 ± 610.6 |
| 0h Postexercise            | 3608.3 ± 1445.9b              | 3570.5 ± 475.4b |
| 2h Postexercise            | 3986.3 ± 1285.0c              | 3610.7 ± 714.1c |
| 24h Postexercise           | 2560.1 ± 1068.4               | 2707.5 ± 744.5 |
| Total monocytes (cells/µL) |                               |               |
| Pre-exercise               | 255.6 ± 133.3                 | 400.0 ± 165.8  |
| 0h Postexercise            | 370.0 ± 125.9b                | 449.1 ± 169.7  |
| 2h Postexercise            | 308.6 ± 104.8                 | 399.3 ± 181.3  |
| 24h Postexercise           | 309.2 ± 133.1                 | 364.8 ± 139.0  |
| Total lymphocytes (cells/µL)|                               |               |
| Pre-exercise               | 788.9 ± 355.1                 | 1111.1 ± 401.4 |
| 0h Postexercise            | 987.0 ± 498.9d                | 1529.9 ± 513.8d |
| 2h Postexercise            | 945.8 ± 646.4e                | 1542.5 ± 654.2e |
| 24h Postexercise           | 761.5 ± 309.5f                | 1205.7 ± 358.8f |
| Plasma volume shifts (%)   |                               |               |
| 0h Postexercise            | −11.8 ± 3.8                   | −11.5 ± 4.6    |
| 2h Postexercise            | −2.8 ± 6.3                    | −0.1 ± 9.2     |
| 24h Postexercise           | −7.3 ± 6.7                    | −6.6 ± 7.1     |

Abbreviations: 0h, immediately postexercise; 2h, 2 hours postexercise; 24h, 24 hours postexercise.

aValues given are mean ± standard deviation.

bP < .05 for pre-exercise versus 0h postexercise.

cP < .05 for pre-exercise versus 2h postexercise.

dP < .05 for comparing 0h postexercise between groups.

eP < .05 for comparing 24h postexercise between groups.
kinase inhibitor) and one receiving bevacizumab (an angiogenesis inhibitor).

HR, RPE, and metabolic responses that the participants achieved during the moderate-intensity exercise session are presented in Table 2. Submaximal VO\textsubscript{2} and workload were similar between groups (\(P = .267-.726\)). Additionally, pre-exercise resting HR as well as exercise HR and RPE responses during the exercise session were similar between groups (\(P = .596-.757\)).

**Leukocyte Subset Cell Counts and Plasma Volume Shifts**

Cell counts for total leukocytes, granulocytes, monocytes, and lymphocytes as well as plasma volume shifts at each study time point are presented in Table 3. Total leukocyte, granulocyte, monocyte, and lymphocyte counts were adjusted to reflect the exercise-induced shifts in plasma volume. In the breast cancer survivor group, blood samples were not able to be obtained from 1 participant immediately postexercise (0h postexercise) and from 1 participant at 2 hours postexercise because of difficulty in obtaining a blood sample through the angiocatheter. Exercise and recovery patterns of total leukocyte, leukocyte subsets, and plasma volume shifts were largely similar between breast cancer survivors and controls.

**NK Cell Counts**

Cell counts for NK cells are presented graphically as box-and-whisker plots in Figure 1. In this figure, the line inside each box represents the median value, whereas the “x” inside each box represents the mean value. The upper and lower bounds of the box represent the interquartile range (IQR; ie, the middle 50% of the data). The upper and lower whiskers show the values that are within 1.5 × IQR of the 75th and 25th percentiles, respectively, whereas the “x” above and below the whiskers denote outlier values. NK cell count at 24 hours postexercise was not able to be determined for 1 control participant because of difficulty obtaining enough blood for sample preparation and analysis. When comparing groups, NK cell count was significantly lower immediately postexercise in the breast cancer survivor group (172.8 ± 118.8 vs 286.8 ± 134.2 cells/µL, \(P = .046\)). Although statistically nonsignificant, NK cell counts were also consistently lower in the breast cancer survivor group pre-exercise (70.3 ± 37.9 vs 108.9 ± 51.7 cells/µL, \(P = .130\)), at 2 hours (93.0 ± 82.2 vs 131.3 ± 81.0 cells/µL, \(P = .285\)), and at 24 hours postexercise (90.9 ± 58.0 vs 123.3 ± 62.4 cells/µL, \(P = .285\)).

Percentage changes for NK cell counts between study groups and across the 3 postexercise time points (relative to pre-exercise values) were also examined. Both groups displayed notably large percentage increases in NK cell counts immediately postexercise, relative to pre-exercise values (125.6% ± 75.6% and 184.4% ± 90.9% for the breast cancer survivor group and control group, respectively). At 2 and 24 hours postexercise, the percentage change in NK cell counts indicated only slight differences compared with pre-exercise levels in both groups (26.0% ± 51.3% and 24.2% ± 61.1% at 2 hours postexercise and 32.6% ± 66.8% and 14.8% ± 41.8% at 24 hours postexercise for the breast cancer survivor group and control group, respectively). Magnitudes of change in NK cell counts were similar between groups at each of the 3 postexercise time points (\(P = .182\) to .813).

**Discussion**

This investigation is the first to examine the effect of acute aerobic exercise on any cellular immune parameter in breast cancer survivors. Absolute NK cell counts were consistently lower in the breast cancer survivor group at each study time point compared with the control group, and this difference was most notable immediately postexercise. This study found that the magnitude of change for NK cell counts appears similar across time (0-24 hours postexercise) when comparing the breast cancer survivors and the controls, as evidenced by similar NK cell count percentage changes for both study groups.

**Changes in NK Cell Counts**

Absolute NK cell counts were consistently lower in the breast cancer survivor group across time compared with the control group—a finding that was statistically significant immediately postexercise. This result was not entirely
surprising, particularly after noting that total lymphocyte counts were also consistently lower in the breast cancer survivor group across time compared with the control group, a finding that was also significant immediately postexercise as well as 24 hours postexercise (see Table 3). Immune system deficiencies such as lymphopenia can continue for extended periods of time posttreatment, and so the decreased total lymphocyte and NK cell counts observed in the breast cancer survivor group may have been related to the multitude of treatments that they had recently experienced. However, NK cell counts did significantly increase immediately postexercise compared with pre-exercise levels in both groups, and then decreased toward pre-exercise levels at 2 hours and 24 hours postexercise. This is encouraging, suggesting that the breast cancer survivors seem to experience similar NK cell number recruitment compared with controls during and after 30 minutes of intermittent moderate-intensity aerobic exercise.

Additionally, the majority of the study participants did not experience below-baseline decreases in NK cell counts that are typically seen during the recovery period from exercise.

Previous studies that have examined changes in NK cell counts in response to moderate-intensity and moderate-duration aerobic exercise in healthy men and women of various ages and fitness levels have reported similar results. Additionally, Maisel et al. examined the effect of an acute bout of treadmill exercise to exhaustion on circulating lymphocyte subpopulations in congestive heart failure patients (another clinical population that often presents with low aerobic fitness) and matched healthy controls, finding that NK cell counts increased immediately postexercise in both groups, with magnitudes similar to those observed in the current study.

Several biological mechanisms may be associated with the rise and fall of NK cell counts during and after moderate-intensity acute aerobic exercise. Increased cardiac output during exercise may act synergistically with increased catecholamine levels to affect vascular endothelial lymphocyte adhesion, which would recruit NK cells from their marginal pools and other reservoirs.

During the recovery period, decreases in NK cell counts could be related to exercise-induced changes in catecholamines and cortisol, the latter of which may be mediated by changes in the inflammatory cytokine interleukin-6. The purpose of this current study did not involve direct exploration of potential biological mediators of the NK cell response to the moderate-intensity acute aerobic exercise bout. However, separate analyses have been performed on participants’ stored blood samples, examining catecholamine and cortisol responses in response to the moderate-intensity exercise bout, the results of which are being considered elsewhere. In short, these analyses showed that although significant changes in plasma catecholamine and cortisol levels did occur in both study groups as a result of the moderate-intensity exercise bout, there were no significant correlations between changes in catecholamine or cortisol responses and changes in NK cell counts. This was likely a result of the small study sample size as well as the discontinuous nature of the exercise session, which may not have elicited enough of a catecholamine response to have an impact on β-adrenergic stimulation of NK cells.

In the current investigation, it was interesting to note that even though absolute NK cell counts were lower in the breast cancer survivor group, the magnitude of change in NK cell counts was similar between groups. After observing these results, one question that arises is whether or not there were any differences in other markers of exercise intensity and perceived stress between the groups as reflected in the HR, RPE, and other metabolic responses. As shown in Table 2, no significant differences were observed between the breast cancer survivors and the controls for exercise HR responses, RPE responses, submaximal VO$_2$, or submaximal workload during the moderate-intensity exercise session. Additionally, percentage exercise intensity based on HR reserve (53% ± 15% vs 56% ± 15%; $P = .546$) as well as percentage exercise intensity based on submaximal VO$_2$ and VO$_{2\text{peak}}$ (68% ± 8% vs 64% ± 10%; $P = .408$) were also similar between the breast cancer survivors and controls. These similarities suggest that even though the cancer treatments may have affected the absolute immune cell counts of breast cancer survivors, the exercise bout resulted in an immune response, the magnitude of which paralleled that of controls who had never experienced cancer treatment.

In addition to examining the changes in NK cell counts themselves, another parameter that may be useful to investigate is changes in NK cell activity (NKCA) in response to exercise. The current study was able to explore changes in NKCA for 6 participants in the breast cancer survivor group, using a whole-blood flow cytometry assay adapted from Fondell et al. The NKCA results (measured as percentage target cell cytolysis in 500 mL of blood and expressed as mean ± SD) for the 6 participants in the breast cancer survivor group were 73.6% ± 30.3%, 74.4% ± 21.0%, 67.6% ± 12.5%, and 60.2% ± 37.1% at pre-exercise, immediately postexercise, at 2 hours postexercise, and at 24 hours postexercise, respectively. This exploratory analysis found that NKCA did not change significantly across the study time points for these 6 breast cancer survivors. This exploratory finding is interesting because one would expect that a measure of NK cell function would change in a manner similar to that of NK cell counts. From a biological standpoint, the exact mechanisms are not clear. A similar result was observed by van der Pompe et al. during and after graded exercise up to VO$_{2\text{max}}$ postmenopausal women, in which the authors speculated that a component of the signaling pathways triggered by agonist stimulation of the β-adrenergic receptors on NK cells may be impaired in this population of participants.
observed relatively small changes in NKCA postexercise despite large changes in NK cell counts hypothesized that the NK cells that were recruited into circulation during exercise may have had decreased cytotoxic function compared with the NK cells circulating pre-exercise. In the current study, the sharp increases in NK cell counts postexercise accompanied by the relatively constant NKCA across time may likely be more related to the effects of increased cardiac output on NK cell cytosis rather than β-adrenergic stimulation, especially considering that the exercise bout was discontinuous and of moderate intensity and duration. However, no definite conclusions can be drawn from this exploratory analysis without data from more breast cancer survivors as well as from healthy controls for comparison. Furthermore, additional investigation of NKCA in breast cancer survivors using other assay techniques may be warranted to allow a more comprehensive picture of NK cell function in this population.

Strengths, Limitations, and Recommendations for Future Research

As with any study, there are inherent strengths and limitations. The study sample was very homogeneous and included a comparative control group of women. With the exception of age, the 2 study groups were matched very closely with regard to the other physical characteristics and aerobic fitness. Additionally, this study is one of the few to describe immune responses to acute aerobic exercise in the oncology patient population and is the only study to investigate the effect of acute aerobic exercise on NK cell counts in breast cancer survivors. Furthermore, it is one of the few studies to also include NK cell responses to acute aerobic exercise in healthy postmenopausal women as well as in a study sample with a low VO2peak (<20 mL/kg/min). However, this study was limited by its small sample size. Additionally, NK cell count data were not available for 2 cancer survivors (one immediately postexercise and one 2 hours postexercise) and 1 control at 24 hours postexercise because of difficulties obtaining blood samples from those participants at those times. Finally, a comprehensive picture of the effect of the exercise bout on NK cell function (NKCA) as well as all potential biological mediators of the NK cell response was not explored in this study. Therefore, biological mechanisms driving these results are largely speculative, especially when concerning results pertaining to the breast cancer survivor group.

As mentioned previously, immune responses to exercise have not been systematically measured in breast cancer survivors. In addition to further examining NK cell responses to acute exercise in cancer patients and survivors, future studies should examine other cellular immune parameters that are also known to be responsive to acute aerobic exercise (e.g., neutrophils, monocytes, T lymphocytes, and B lymphocytes). Future studies should also examine the effect of acute aerobic exercise on mediators of the immune response (e.g., hormones, cytokines, and prostaglandins) as well as investigate the effect of short “microcycles” of aerobic exercise on immune markers in cancer patients and survivors. Because exercise oncology research is ultimately used to construct exercise prescription guidelines for cancer patients and survivors, exercise specialists and clinicians must strive to understand how exercise affects all physiological systems, including the immune system. Thus, the most relevant and specific exercise guidelines can be created, so that cancer patients and survivors can reap the maximum health benefits of exercise.

Conclusions

It was found that 30 minutes of intermittent, moderate-intensity aerobic exercise elicited changes in NK cell counts in recent breast cancer survivors that were similar to those in physically similar women without a history of cancer diagnosis or treatment. Absolute NK cell counts were consistently lower in the breast cancer survivor group compared with the control group, particularly immediately postexercise, a finding that may be related to immune changes stemming from recent cancer treatments. Percentage changes in NK cell count were similar across time in both groups, implying that exercise affected the magnitude of NK cell response similarly in breast cancer survivors and controls. These findings suggest that intermittent aerobic exercise of moderate intensity and duration does not disrupt this aspect of exercise-induced innate immune response in recent breast cancer survivors. Although more research investigating the effect of acute aerobic exercise on the immune system in cancer patients is warranted, this work supports current exercise recommendations for cancer patients and survivors.

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Declaration of Conflicting Interests

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