Immune Status of Physically Active Women during Lactation

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ABSTRACT

LOVELADY, C. A., C. J. FULLER, C. M. GEIGERMAN, C. P. HUNTER, and T. C. KINSELLA. Immune Status of Physically Active Women during Lactation. *Med. Sci. Sports Exerc.*, Vol. 36, No. 6, pp. 1001–1007, 2004. **Purpose:** The purpose of this study was to provide baseline data on immune status of exercising and sedentary exclusively lactating women. Dietary intake and body composition were also investigated to determine whether they related to immune function. **Methods:** Healthy, exclusively breastfeeding women with a body mass index between 20 and 30 $kg \cdot m^{-2}$ were studied at 3 months postpartum. Participants in the exercise group (EG; $N = 27$) exercised aerobically at least 30 $min \cdot d^{-1}$, 3× wk^{-1}, and women in the sedentary group (SG; $N = 23$) exercised once a week or less during the previous 6 wk. Immune status while at rest was determined by measuring: 1) a complete blood cell count and differential leukocyte count; 2) percentages and absolute counts of peripheral blood T cells (CD3+), cytotoxic T cells (CD3+CD8+), helper T cells (CD3+CD4+), B cells (CD19+), and natural killer cells (CD56+); 3) neutrophil bacterial killing and oxidative burst activity; and 4) *in vitro* mitogenic responsiveness of lymphocytes. Cardiorespiratory fitness, body composition, and dietary intake were also measured. **Results:** Participants in the EG had a significantly higher level of mean predicted cardiorespiratory fitness than women in the SG (39.5 ± 1.1 vs 32.5 ± 1.0 mL $O_2 \cdot min^{-1} \cdot kg^{-1}$; $P < 0.05$); however, there were no significant differences in body composition or dietary intake. There were no significant differences in any of the indicators of immune status between groups. In addition, there were no significant relationships between body composition or dietary intake and immune status. **Conclusions:** The results of this study suggest that women may exercise moderately during lactation and increase their fitness level without impairing their immune function.
Key Words: LEUKOCYTES, LYMPHOCYTES, T CELLS, B CELLS, NEUTROPHILS

Although the immunological benefits of breast milk to the infant are well established, less is known about maternal immune status and the factors that may affect it during lactation. Zimmer et al. (27) reported no significant differences in percentages or counts of CD3+ or CD3+CD8+ between lactating and nonlactating postpartum women. However, Redwine et al. (19) reported that lactating women had a greater lymphocyte proliferation to mitogens compared with nonpostpartum women after stressful tasks of math and public speaking.

Previous research among nonlactating women has found that nutritional status (severe underweight or obesity), weight loss, and/or excessive exercise may compromise immune function (13,16). The effect of acute or chronic exercise is dependent on the intensity and type of physical activity. Heavy exercise may increase the risk of infection, whereas regular moderate exercise may confer resistance to viral and bacterial infections. The current theory of the "inverted J hypothesis" suggests that there is a level of exercise that results in enhanced immune function and reduced risk of cancer and infections, whereas exhaustive exercise is immunosuppressive and increases the risk of disease or infection (26).

This effect of exercise intensity on the immune system may be mediated through hormonal control. Stress hormones, which can suppress immunity, are elevated to a greater extent during prolonged or heavy exertion compared with moderate exercise (13). Altemus et al. (1) compared the effects of intense exercise on stress hormones in postpartum women. Plasma adrenocorticotropic hormone, cortisol, and glucose responses to exercise were significantly lower in the lactating women compared with the nonlactating women. These investigators speculated that the reduced stress response might enhance immune function in lactating women. Unfortunately, they did not measure immune function in this study.

Moderate physical activity is recommended by the Centers for Disease Control and Prevention and the American College of Sports Medicine to improve cardiorespiratory fitness and prevent chronic disease (17). In addition, the American Academy of Pediatrics recommends all women breastfeed their infants during their first year of life (2). However, there is a paucity of research investigating the impact of exercise on the health of exclusively breastfeeding women and the composition of their breast milk.

Although it has been demonstrated that exercise affects the immune system, the impact of exercise on the immune

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status of lactating women has not been investigated. Therefore, the objective of this study was to provide baseline data on immune status of exercising and sedentary exclusively lactating women. Another objective of the study was to determine whether the immune status of women who are physically active is enhanced compared with the immune status of sedentary women during lactation. Dietary intake and body composition were also investigated to see if they related to maternal immune function.

METHODS

Study design and subjects. This research was a cross-sectional study of exclusively breastfeeding women at 12 wk postpartum. Twelve (±2) weeks postpartum was chosen as the period to measure immune status because many women return to work after 3 months of maternity leave. Once returning to work, many women may wean their infants or supplement their breast milk with infant formula because they do not have the time to pump their breast milk. To maximize our recruitment of exclusively lactating women, we measured immune status before mothers returned to work. Six weeks of exercise was selected as the minimum amount of exercise duration because many women do not begin exercising until their medical doctors give them permission at their postpartum visit, which is usually at 4–6 wk postpartum. If women waited until their postpartum visit to begin to exercise, and immune status was measured at 12 wk postpartum, the minimum duration of exercise during exclusive lactation would be 6 wk.

The cross-sectional study design was selected because we were not aware of any previously published reports of immune status of exercising lactating women. This design allows for measuring baseline data in a relatively quicker and less expensive way than an intervention study. These data may be used for developing future longitudinal studies on the effect of exercise on the immune status of exclusively lactating women and the composition of the breast milk they produce. The main independent variable was mean predicted cardiorespiratory fitness level of the participants (mean predicted $\dot{V}O_{2peak}$). Other independent variables of interest were body composition (percent body fat) and dietary intake ($kcal \cdot kg^{-1}$; total vitamin A, C, and E, and total zinc intake). The main dependent variables were: 1) percentages and absolute counts of peripheral blood T cells (CD3+), B cells (CD19+), and natural killer cells (CD56+); 2) neutrophil bacterial killing and oxidative burst activity; and 3) *in vitro* mitogenic responsiveness of lymphocytes. These variables were measured as they are usually the outcomes of interest in exercise and immunity research and our results may be compared with those published in previous reports on men and nonlactating women. Whereas amounts of T, B, and natural killer cells reflect immune status, the ability of neutrophils to kill bacteria and the proliferative response of lymphocytes to mitogens are indicators of immune function.

Healthy (free from chronic disease or acute illness in the week before laboratory measurements), nonsmoking, nonobese (body mass index between 20 and 30 $kg \cdot m^{-2}$), exclusively breastfeeding women who delivered healthy infants (birth weight greater than 2500 g and gestational age between 37 and 42 wk) were studied. Participants were recruited through advertisements and announcements of the study in obstetricians' offices and in childbirth classes offered at the local hospital. The Institutional Review Board at the University of North Carolina at Greensboro approved the study protocol and written informed consent was obtained from all subjects.

Participants were asked about their physical activity in the postpartum period and grouped according to their exercise habits. Those volunteers who reported exercising aerobically at least 30 $min \cdot d^{-1}$ for a minimum of 3 $d \cdot wk^{-1}$ for the past 6 wk were placed in the exercise group (EG). The sedentary group (SG) consisted of women who reported exercising once a week or less. Measurements were made at 3 months postpartum. Each participant came to the Human Performance Laboratory between 8:00 and 9:00 am, after a 12-h fast, for blood collection and measurement of body composition and cardiorespiratory fitness.

Immune status. A blood sample (40 mL) was drawn from an antecubital vein by a trained phlebotomist. Maternal immune status while at rest was determined by measuring: 1) a complete blood cell count (CBC) and differential leukocyte count; 2) percentages and absolute counts of peripheral blood T cells (CD3+), cytotoxic T cells (CD3+CD8+), helper T cells (CD3+CD4+), B cells (CD19+), and natural killer cells (CD56+); 3) neutrophil bacterial killing and oxidative burst activity; and 4) *in vitro* mitogenic responsiveness of lymphocytes. All of the analyses were performed on fresh blood samples.

An EDTA anticoagulated whole blood sample (5 mL) was analyzed for a CBC and differential leukocyte count using an Advia Blood Analyzer (Bayer Corp., Tarrytown, NY). Serum samples (5 mL) were analyzed for C-reactive protein by rate nephelometry (IMMAGE Immunochemistry Systems, Beckman Coulter, Inc., Chaska, MN).

The cellular immunologic profile was determined by flow cytometry. Briefly, whole blood samples were labeled with monoclonal antibodies against CD3+, CD4+, CD8+, CD19+, and CD 56+ cell surface markers. For each of these markers, 100 μL of whole blood was mixed with 10 μL of appropriate fluorochrome labeled antibody (either fluorescein isothiocyanate [FITC] or phycoerythrin [PE], BD BioSciences, San Jose, CA). After incubating in darkness at room temperature for 20 min, RBC were lysed with PharmLyse (BD PharMingen, San Diego, CA) for 15 min, then washed two times with phosphate-buffered saline, 1% fetal bovine serum, and 0.1% Na azide buffer. Cell pellets were resuspended in 500 μL wash buffer and immediately analyzed by flow cytometry (FACSCalibur, BD Biosciences, San Diego, CA).

Neutrophils were isolated by the method of Scaccini and Jialal (21) for the neutrophil killing and oxidative burst assays. Ten milliliters of blood were collected into acid citrate dextran tubes and then layered over Histopaque 1077 (Sigma Chemical, St. Louis, MO). The sample was

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centrifuged and the plasma and mononuclear cells were discarded. Gey's balanced salt solution and 3% dextran were added to the sample to sediment the erythrocytes for disposal. The supernatant was collected and any residual erythrocytes were lysed with hypotonic (0.2%) NaCl solution. Trypan blue dye was added, and viable cells that had not taken up the dye were counted in a hemocytometer. Cell density was adjusted as needed for the killing and oxidative burst assays.

For the bacterial killing assay, an aliquot of Salmonella typhimurium (#14028, ATCC, Manassas, VA) stock was incubated in an orbital shaker at 37°C for 3 h (23). A suspension of 3×10^8 mL^{-1} bacteria in 10% serum was prepared and incubated for an additional 30 min to opsonize the bacteria. A bacterial standard curve was prepared in quadruplicate in a 96-well white microtiter plate. Neutrophils (4.5×10^5) were added to 9×10^6 bacteria in wells. The plate was centrifuged to compress the cells and bacteria, then incubated at 37°C for 20 min to allow the neutrophils time to ingest the bacteria. Sodium deoxycholate was added at a final concentration of 0.07% to lyse the neutrophil plasma membranes. Alamar Blue (AccuMed, Westlake, OH; 8% final concentration) was then added and the plate was incubated for another 15 min. Alamar Blue employs a fluorometric indicator of redox activity. Fluorescence of the wells was read using a LS-50B luminescence spectrophotometer (Perkin-Elmer, Norwalk, CT) set at 530 nm for excitation and 590 nm for emission wavelengths. A reduction in fluorescence was considered proportional to the number of bacteria killed by the neutrophils.

Neutrophil production of superoxide anion was measured by an oxidative burst assay (6). Opsonized zymosan (OZ; 2.5 $mg \cdot mL^{-1}$), formyl-methionyl-leucyl-phenylalanine (FMLP; 10 $\mu mol \cdot \mu L^{-1}$) and phorbol 12-myristate 13-acetate (PMA; 2 $\mu g \cdot mL^{-1}$) were added to neutrophils (2.5×10^5 cells/well) to stimulate superoxide anion production, as measured by the superoxide dismutase (0.11 $mg \cdot mL^{-1}$)-inhibitable oxidation of ferricytochrome c (0.98 $mg \cdot mL^{-1}$). Plates were read with a microplate reader (Power Wave X, Bio-Tek Instruments, Winooski, VT) at 550 nm with a reference wavelength of 620 nm at 0 and 60 min.

The proliferation of lymphocytes in response to mitogens was measured using whole blood cultures as described by Hough et al. (7). The proliferation response was measured by incorporation of ^{3}H-thymidine during DNA synthesis. Blood (10 mL) was collected in heparinized tubes and diluted 1:10 with basal RPMI 1640 medium supplemented with gentamicin and amphotericin B. Cultures were either untreated (control) or subjected to one of three mitogens: phytohemagglutinin (PHA, 10 $\mu L \cdot mL^{-1}$), concanavalin A (ConA, 10 $\mu L \cdot mL^{-1}$), or pokeweed mitogen (PWM, 1 $\mu L \cdot mL^{-1}$). PHA and ConA are lectins that stimulate T cell blastogenesis and pokeweed stimulates both T and B cell blastogenesis. Each treatment was analyzed in quadruplicate. Cultures were incubated at 37°C for 54 h after the addition of the mitogens. Then 0.5 μCi ^{3}H-thymidine diluted with 20 μL of RPMI 1640 was added to each well and cultures were returned to the incubator for an additional 16 h. ^{3}H-Thymidine incorporation into DNA was measured by collecting cellular material on fiber filters using a semi-automated cell harvesting apparatus (Skatron Instruments, Norway). After drying, filters were transferred to vials, 3 mL of liquid scintillation fluid (Ultima Gold, Packard Instrument Company, Meriden, CT) was added, and ^{3}H was measured in a liquid scintillation counter (LS 6000SE, Beckman Instruments Inc., Fullerton, CA). The stimulation index [(^{3}H incorporation in treated cells $-$ ^{3}H incorporation in control cells/^{3}H incorporated in control cells)] was calculated for each mitogen.

Cardiorespiratory fitness. Cardiorespiratory fitness was measured by a treadmill test using a modified Balke protocol (3). Heart rate was measured with a heart rate monitor (Polar, Inc., Woodbury, NY). Before the test, a resting heart rate was recorded. First participants warmed up with 2 min of walking on the treadmill. Then the speed was increased to a brisk walk or jog (approximately 3.4–5 mph, depending on the fitness level of the participants) and remained constant for the duration of the test. Every 2 min the grade of the treadmill was increased by 2.5%. The participants' heart rates and perceived levels of exertion were recorded every min. The test continued until the heart rate reached 85% of the predicted maximal heart-rate reserve: [(maximal heart rate-resting heart rate) $\times$ (0.85)] + resting heart rate. The maximum heart rate of each woman was estimated using the equation: 220 $-$ age in years (3). The predicted oxygen consumption was calculated for each heart rate after every 2 min at each grade level with the following equations (3):

$$\text{Walking: } (3.5 \text{ mL } O_2 \cdot kg^{-1} \cdot min^{-1}) + (\text{speed in } m \cdot min^{-1} \times 0.1) + (\text{grade} \times m \cdot min^{-1} \times 1.8) \quad [1]$$

$$\text{Jogging: } (3.5 \text{ mL } O_2 \cdot kg^{-1} \cdot min^{-1}) + (\text{speed in } m \cdot min^{-1} \times 0.2) + (\text{grade} \times m \cdot min^{-1} \times 0.9). \quad [2]$$

The predicted oxygen consumption at the maximal heart rate ($\dot{V}O_{2peak}$) was calculated with a linear regression equation, with heart rate as the independent variable and oxygen consumption as the dependent variable. Maximal exercise testing is often not feasible for assessing cardiorespiratory endurance of many individuals, so submaximal exercise tests are commonly used. These tests determine the heart rate response to predetermined workloads and use the results to predict $\dot{V}O_{2peak}$. Because heart rate varies linearly with $\dot{V}O_2$, submaximal testing has been validated as a reliable method to estimate $\dot{V}O_{2peak}$ (3).

Anthropometrics and dietary assessment. Participants were weighed in bathing suits on a stationary beam balance scale. Height without shoes was measured using a stationary stadiometer. Body density was measured by underwater weighing. Residual lung volume was measured with an oxygen dilution technique before submersion in the water tank (25). Body density and the percentage of body fat were calculated with the formulas of Brozek et al. (4). Before coming to the laboratory, participants weighed and recorded their dietary intake for three consecutive days, using portable digital gram scales (Ohaus, Florham Park,

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TABLE 1. Characteristics of participants.*

Characteristic	Exercise Group ($N = 27$)	Sedentary Group ($N = 23$)
Age (yr)	31.5 ± 0.6	31.5 ± 1.0
Parity	1.9 ± 0.2	2.2 ± 0.2
Height (cm)	164.9 ± 1.3	163.3 ± 1.1
Weight (kg)	64.5 ± 1.6	65.8 ± 1.6
Body mass index ($kg \cdot m^{-2}$)	23.7 ± 0.5	24.7 ± 0.7
Body composition		
Fat mass		
(% of weight)	25.2 ± 1.2	27.9 ± 1.4
(kg)	16.5 ± 1.1	18.6 ± 1.2
Fat-free mass (kg)	47.8 ± 1.0	47.3 ± 0.9
Cardiorespiratory fitness		
Predicted peak $\dot{V}O_2$ ($mL\ O_2 \cdot min^{-1} \cdot kg^{-1}$)	39.5 ± 1.1**	32.5 ± 1.0
Resting heart rate ($beats \cdot min^{-1}$)	68 ± 2**	75 ± 2
85% of predicted max heart rate reserve ($beats \cdot min^{-1}$)	170 ± 1	171 ± 1
Actual heart rate at end of test ($beats \cdot min^{-1}$)	174 ± 2	177 ± 1
Test duration (min)	11.7 ± 0.5**	10.0 ± 0.4
Test speed ($miles \cdot h^{-1}$)	3.8 ± 0.1**	3.5 ± 0.1
Highest test grade (%)	12.0 ± 0.5**	10.1 ± 0.5

* Values are means and ± SEM.
** Significantly different from the sedentary group, $P < 0.05$.

TABLE 2. Lymphocyte distribution and blood cell indexes in exercising and sedentary women.*

	Exercise Group ($N = 27$)	Sedentary Group ($N = 23$)
T lymphocytes-CD3+		
($\times 10^9\ L^{-1}$)	1.55 ± 0.07	1.49 ± 0.09
(% lymphocytes)	78 ± 2	77 ± 1
T lymphocyte distribution		
CD3+CD4+		
($\times 10^9\ L^{-1}$)	0.96 ± 0.05	0.89 ± 0.06
(% lymphocytes)	49 ± 5	46 ± 6
CD3+CD8+		
($\times\ 10^9\ L^{-1}$)	0.54 ± 0.03	0.57 ± 0.05
(% lymphocytes)	28 ± 1	29 ± 2
B Lymphocytes-CD19+		
($\times\ 10^9\ L^{-1}$)	0.18 ± 0.01	0.18 ± 0.02
(% lymphocytes)	9 ± 1	9 ± 1
Natural killer cells-CD56+		
($\times 10^9\ L^{-1}$)	0.15 ± 0.02	0.19 ± 0.02
(% lymphocytes)	8 ± 1	10 ± 1
C-reactive protein ($mg \cdot L^{-1}$)	3.89 ± 0.40	4.44 ± 0.63
Leukocytes ($\times 10^9\ L^{-1}$)	5.83 ± 0.28	6.14 ± 0.27
Lymphocytes ($\times 10^9\ L^{-1}$)	1.94 ± 0.09	1.90 ± 0.10
Monocytes ($\times 10^9\ L^{-1}$)	0.32 ± 0.02	0.32 ± 0.02
Neutrophils ($\times 10^9\ L^{-1}$)	3.29 ± 0.22	3.63 ± 0.18
Hemoglobin ($g \cdot L^{-1}$)	135 ± 1	138 ± 2
Hematocrit (%)	40.3 ± 0.4	41.3 ± 0.6

* Values are means and ± SEM. Lymphocytes are expressed both as percentages of the total lymphocyte number and as absolute values.

NJ). The Food Processor Analysis Program (version 2.2, Salem, OR) and food composition tables were used to analyze the volunteer's diets (18).

Statistical analysis. Data were analyzed with the use of SPSS-PC software (SPSS, Chicago, IL). The characteristics of the groups were compared with Student's *t*-test or the chi-square test. Pearson's correlation analyses were done to determine relationships between body composition (percent body fat) or dietary intake ($kcal \cdot kg^{-1}$; total vitamin A, C, and E, and total zinc intake) and immune status. Multiple regression analyses were performed to determine whether body composition (percent body fat), dietary intake ($kcal \cdot kg^{-1}$ and total vitamin A), and fitness level (predicted $\dot{V}O_{2peak}$) would predict any immune indicators. Statistical significance was set at $P < 0.05$.

RESULTS

Fifty women participated in the study, 27 in the exercising group and 23 in the sedentary group. Volunteers in the EG exercised an average of 47 $min \cdot d^{-1}$, 4 $d \cdot wk^{-1}$. The majority of participants ($N = 23$) walked briskly and/or participated in aerobic exercise classes. One participant ran and three jogged for their mode of exercise. There were no significant differences between the two groups' characteristics (Table 1) except for their mean predicted cardiorespiratory fitness level (statistical power for the given effect size of mean predicted $\dot{V}O_{2peak}$ was 100%). The mean predicted maximal oxygen consumption of the exercise group was in the 80th percentile of fitness, compared with the 40th percentile for the sedentary group, according to normative values of the American College of Sports Medicine (3). This confirmed the participants' self-reported physical activity level. In addition, the exercise group had significantly lower resting heart rate, significantly longer duration, significantly faster speed, and worked at significantly higher grade than the sedentary group. These results further justify the significant mean predicted cardiorespiratory differences between the two groups.

There were no significant differences in percentages or absolute counts of peripheral blood T cells (CD3+), cytotoxic T cells (CD3+CD8+), helper T cells (CD3+CD4+), B cells (CD19+), and natural killer cells (CD56+); leukocytes; lymphocytes; monocytes; neutrophils; hemoglobin; or hematocrit between groups (Table 2). The statistical power for the given effect size for the main dependent variables of peripheral blood T cells was 8%, B cells was 5%, and natural killer cells was 43%. All participants had C-reactive protein levels less than 8 $mg \cdot L^{-1}$, which indicated that no detectable inflammation and/or infection were present. In addition, there were no significant differences in other CBC data between groups (data not shown).

Immune function was assessed by neutrophil bacterial killing and oxidative burst activity, and mitogenic responsiveness of lymphocytes. Adequate blood was not available for all assays because measurement of lymphocyte proliferation was started after six participants had already completed the study. In addition, the phlebotomist was not able to draw the necessary amount of blood needed for all assays from three other subjects. Therefore, the sample size for: 1) mitogen-induced lymphocyte proliferation was 21 for the EG and 20 for the SG, 2) neutrophil bactericidal activity was 25 for the EG and 22 for the SG, and 3) neutrophil superoxide anion production stimulation was 27 for the EG and 22 for the SG. Figure 1 compares neutrophil bactericidal activity and superoxide anion production stimulated by FMLP of the two groups (statistical power for the given effect size of neutrophil bactericidal activity was 19% and 13% for FMLP stimulation). Results of neutrophil superoxide anion production stimulated by OZ and PMA were similar (data not shown). Figure 2 shows ConA-, PHAP-,

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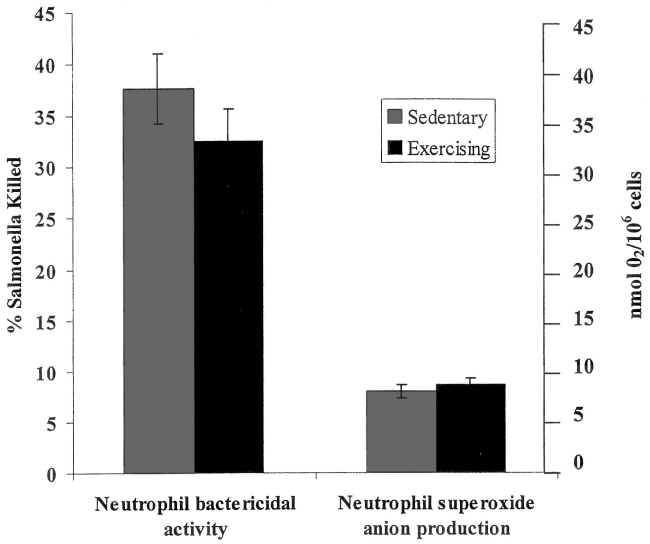

FIGURE 1—Neutrophil bactericidal activity and superoxide anion production after stimulation with formyl-methionyl-leucyl-phenylalanine (FMLP). There were no significant differences between groups for either neutrophil bactercidal activity (EG = 25, SG = 22) or superoxide anion production (EG = 27, SG = 22). Values are means ± standard error of the means.

and PWM-induced lymphocyte proliferation (statistical power for the given effect size for each of these was 6%). There were no significant differences in any of the indicators of immune function between groups.

There were no differences between groups in dietary intake of micronutrients believed to play a role in immune function (zinc, vitamins A, C, and E) or macronutrients (Table 3). All but five participants reported taking vitamin and mineral supplements. Three were in the exercise group and two were in the sedentary group. The results of the data analysis were the same without these five participants.

Because there were no significant differences between groups, data from all subjects were analyzed together to determine whether there were any relationships between body composition (percent body fat) or dietary intake (kcal·kg^{-1}; total vitamin A, C and E, and total zinc intake) and immune status. The only significant relationships were

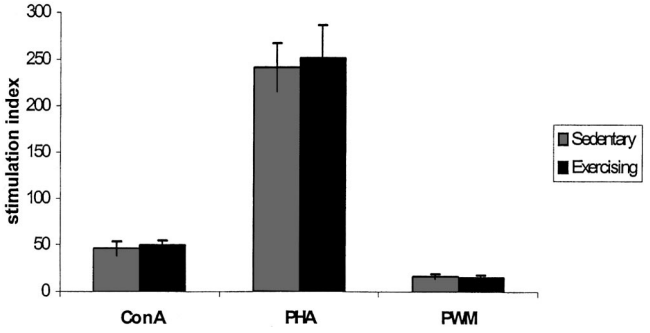

FIGURE 2—Mitogenic reactivity of lymphocytes. Proliferative response of lymphocytes to mitogens concanavalin A (ConA), phytohemagglutinin (PHA), and pokeweed (PWM) for exercising (EG = 21) and sedentary women (SG = 20). Stimulation index defined as (^{3}H incorporation in treated cells-^{3}H incorporation in control cells)/ ^{3}H incorporation in control cells. Values are means and ± error of the means. There were no significant differences between groups.

TABLE 3. Average daily dietary intake of participants.*

Nutrient	Exercise Group (N = 27)	Sedentary Group (N = 23)
Energy		
(kJ)	9232 ± 462	9576 ± 391
(kJ·kg^{-1})	143 ± 8	147 ± 8
Protein (g)	83 ± 4	88 ± 5
Carbohydrate (g)	312 ± 19	315 ± 13
Fat (g)	72 ± 5	79 ± 5
Vitamin A (μg)		
Diet only	2857 ± 1170	1937 ± 505
Diet and supplement	6098 ± 1233	5294 ± 579
Vitamin C (mg)		
Diet only	136 ± 19	131 ± 21
Diet and supplement	225 ± 21	225 ± 22
Vitamin E (mg)		
Diet only	10 ± 1	11 ± 1
Diet and supplement	31 ± 4	29 ± 3
Zinc (mg)		
Diet only	15 ± 4	13 ± 1
Diet and supplement	30 ± 5	29 ± 3

* Values are means and ± SEM.

between total vitamin A intake and neutrophil bactericidal activity (r = 0.30, P = 0.04) and lymphocyte response to PHAP (r = 0.31, $P < 0.05$). Correlation analyses were done between cardiorespiratory fitness (mean predicted $\dot{V}O_{2peak}$) and immune status and no significant relationships were found. Multiple regression analysis was done to determine whether body composition (percent body fat), dietary intake (kcal·kg^{-1} and total vitamin A), and fitness level ($\dot{V}O_{2peak}$) would predict any immune indicators. This model was not predictive of immune status in the subjects.

DISCUSSION

This study demonstrates that moderate exercise does not alter resting immune status during lactation. There were no significant differences in body weight, percent body fat, or dietary intake between the two groups. However, the exercise group did have a significantly higher fitness level, indicating that they were exercising moderately during the postpartum period. These results demonstrate that exercise during lactation does not negatively alter the immune status. These findings support the recommendation of moderate exercise to improve the fitness level of postpartum, breastfeeding women.

The number of leukocytes and their subclasses were similar between exercising and sedentary participants. These results are similar to other studies showing that moderate exercise does not alter the number of T cells (CD3+), T helper/inducer cells (CD4+), T cytotoxic/suppressor (CD8+), or natural killer cells (CD56+) (12,14). Similarly, Scanga et al. (22) reported that the combined effect of energy restriction and moderate exercise did not influence lymphocyte subpopulations including CD3+, CD4+, CD8+, or CD56+ in nonlactating, obese women.

There were no differences in neutrophil bactericidal activity and superoxide anion production between groups. These results are contrary to those of other researchers who have reported that acute, moderate exercise increased the capacity of neutrophils to kill bacteria, as well as their ability to generate reactive oxygen species in both trained

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and untrained males (20,24). However, these studies investigated the acute effects of exercise, whereas our study examined the chronic effects of exercise by measuring resting immune function.

Exercising participants had similar mitogen-stimulated proliferation as sedentary volunteers. These results are similar to those of Nieman et al. (14), who reported that 12 wk of moderate exercise in elderly women did not alter lymphocyte proliferative response to mitogens compared with sedentary women. In another study, acute moderate exercise by young males did not affect lymphocyte proliferative response, whereas high-intensity exercise decreased the response 1 h after exercise (15).

Nieman (13) reviewed the research on exercise and immune functions and found that although there are acute effects of exercise on immune indicators, most studies do not report differences in resting immune function between athletes and nonathletes. He hypothesized that the changes that occur with each bout of exercise have more clinical significance than resting immunity. In addition, Woods et al. (26) cited epidemiological evidence that supports the hypothesis that moderate exercise reduces the risk of infectious and neoplastic diseases.

It is possible that we did not see a difference in immune response in exercising women because the physiological state of lactation may enhance the immune response to a greater extent than exercise by affecting the hormonal response to stress. Altemus et al. (1) compared the effects of intense exercise on stress hormones in postpartum women. Volunteers exercised on a treadmill for 20 min, with the grade and speed of the treadmill increasing progressively, until they were exercising at 90% of their $\dot{V}O_{2max}$ for the last 5 min. Plasma adrenocorticotropic hormone, cortisol, and glucose responses to exercise were significantly lower in the lactating women compared with the nonlactating women. However, resting plasma levels of adrenocorticotropic hormone and cortisol were similar in lactating and nonlactating women. These investigators speculated that the reduced stress response, mediated through prolactin (which is elevated during lactation), might enhance immune function in lactating women.

Redwine et al. (19) measured lymphocyte proliferation to PWM and PHA after stressful tasks (math and public speaking) in lactating, nonlactating postpartum, and nonpostpartum women. The nonpostpartum women had a reduced lymphocyte proliferation to PWM after stress, whereas it did not change in the lactating and nonlactating postpartum women. In addition, lactating women had a greater lymphocyte response to PHA compared with nonpostpartum women after stress. These researchers suggest that the postpartum period and lactation may influence immune function.

Zimmer et al. (27) compared the lymphocyte distribution of lactating, nonlactating postpartum, and nonpostpartum women. They reported no significant differences in percentages or counts of CD3+ or CD3+CD8+ between lactating and nonlactating postpartum women. However, the postpartum women had significantly higher CD3+ and CD3+CD8+ relative percentages and absolute counts than the nonpostpartum women. There were no differences in the CD3+CD4+ percentages or absolute counts between groups. They found that maternal age and parity were the most significant predictors of T cell numbers.

Our results were similar to those reported by Zimmer et al. (27,28). Our lactating women (exercising and sedentary) had higher CD3+ and CD3+CD8+ percentages and absolute counts than nonpostpartum women, but similar to those of lactating and nonlactating postpartum women. However, percentages and absolute counts of CD19+ were similar in all groups (lactating and nonlactating at 2 or 4 months and nonpostpartum women). The average CD3+ percentage of our participants was also higher than that of obese, nonlactating, premenopausal women; however, the percentage of NK cells (CD56+) of our subjects was much lower than those of the obese volunteers (22). In addition, our values of CD4+ and CD8+ were lower than those in mildly obese premenopausal women as reported by Nehlsen-Cannarella et al. (12).

Comparison of results across studies suggests that the postpartum period and lactation may affect immune status. Whereas the results of various studies differed slightly, average percentages or absolute counts of T and B cells and natural killer cells were in normal ranges. The effect of lactation on immune status may be subtle in healthy women. Future studies should include nonlactating women as a comparison group.

The participants in this study were healthy and ate a nutritious diet. Energy intake was close to the RDA of 9715 $kJ \cdot d^{-1}$ estimated for a 30-yr-old sedentary, lactating woman with a BMI of 25 and height of 1.65 m (8). However, it was lower than that recommended for low active (10,634 $kJ \cdot d^{-1}$) or active (11,789 $kJ \cdot d^{-1}$) lactating women. Participants may have been eating less and losing weight during this period or may have under-reported food intake. Vitamin E intake from the diet only was approximately half of the RDA of 19 mg. However, supplement intake increased the total intake to approximately 30 $mg \cdot d^{-1}$. Average vitamin A intake from the diet was above the RDA. Average intake from diet and supplement (6098 μg) was well above the tolerable upper intake level of 3000 $\mu g \cdot d^{-1}$. These results suggest that women should be cautioned about taking supplements containing vitamin A during lactation.

We have previously reported that moderate exercise did not affect resting or postexercise concentrations of secretory immunoglobulin A, lactoferrin, and lysozyme in breast milk (10). This study demonstrated that moderate exercise did not have any negative effects on immune status during lactation in healthy, well-nourished women. However, an exercise program longer in duration than 6 wk may enhance immune status and could result in significant differences in immune functions between exercise and sedentary groups. Other limitations of this study were that the immune status or the cardiorespiratory fitness of the participants before beginning an exercise program was not known. Future research using a randomized clinical trial design is needed to determine the effects of exercise on maternal immune status during the postpartum period.

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The level of exercise done by the lactating women in this study is the amount recommended by the Centers for Disease Control and Prevention and the American College of Sports Medicine to improve cardiorespiratory fitness and prevent chronic disease (17). We have previously reported that aerobic exercise during lactation improved cardiorespiratory fitness, increased high density lipoprotein-cholesterol concentrations, and reduced insulin response to a test meal (5,11). In addition, if exercise is combined with a reduction of approximately 500 calories per day in dietary intake, overweight lactating women may safely lose 0.5 $kg \cdot wk^{-1}$ without affecting the growth of their infant (9). The results of our past studies and this study suggest that women may exercise moderately during lactation and increase their fitness level without impairing their immune function.

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