

# Effect of Exercise on Immunologic Factors in Breast Milk

Cheryl A. Lovelady, PhD\*; Christie P. Hunter, MS‡; and Cissy Geigerman, BS§

**ABSTRACT.** *Objective.* Although it is well documented that breast milk provides optimal nutrition and immune benefits to the infant, factors that influence the immunologic composition of breast milk are less understood. A recent study reported that immunoglobulin A (IgA) levels in breast milk are lower after exercise compared with resting concentrations. However, the women exercised until exhaustion. The effect of moderate exercise on immunologic components in breast milk has not been reported. Therefore, the purpose of this study was to 1) compare the levels of immunologic compounds in breast milk of exercising women with the milk of sedentary women and 2) determine whether 30 minutes of moderate exercise affects immunologic properties of breast milk.

*Methods.* Exclusively lactating women were studied at 3 months' postpartum. Women in the exercise group (EG;  $n = 29$ ) reported exercising aerobically at least 30 minutes/d for 3 days/wk, and women in the sedentary group (SG;  $n = 24$ ) had exercised once a week or less during the previous 6 weeks. Cardiovascular fitness levels and concentrations of IgA, lactoferrin, and lysozyme in milk were measured. A subsample of the EG ( $n = 17$ ) participated in a 30-minute exercise session at 75% of maximum heart rate and a rest session of 30 minutes of sitting rest on 2 separate days. Breast milk samples were collected before and 10 and 60 minutes after exercise and rest sessions. IgA, lactoferrin, and lysozyme concentrations were measured.

*Results.* Women in the EG had a higher level of cardiovascular fitness than women in the SG ( $39.7 \pm 1.0$  vs  $32.4 \pm 1.0$  mL O<sub>2</sub>/kg/min). Milk concentrations of IgA, lactoferrin, or lysozyme were not significantly different between groups. In addition, there were no significant differences in the concentrations of IgA, lactoferrin, or lysozyme after moderate exercise compared with sitting rest.

*Conclusion.* Moderate exercise during lactation improves cardiovascular fitness without affecting levels of IgA, lactoferrin, or lysozyme in breast milk. *Pediatrics* 2003;111:e148–e152. URL: <http://www.pediatrics.org/cgi/content/full/111/2/e148>; immunologic compounds in breast milk, lactation, postpartum exercise, IgA, lactoferrin, lysozyme.

ABBREVIATIONS. sIgA, secretory IgA; HRP, horseradish peroxidase; PBS, phosphate-buffered saline.

Breast milk not only provides optimal nutrition to infants but also supplies a range of bioactive factors that are involved in the protection against many invading pathogens. This latter function is reflected by the immunologic composition of breast milk and is vital for newborns as their mucosal and systemic immune systems are primarily undeveloped at birth. The importance of these compounds in breast milk has led the American Academy of Pediatrics to recommend that all infants be exclusively breastfed for the first 6 months and that breastfeeding continue with supplements of solid foods during the next 6 months of life.<sup>1</sup>

Three proteins that are found in relatively high concentrations in breast milk and confer immunologic benefits to infants are secretory immunoglobulin A (sIgA), lactoferrin, and lysozyme. IgA in breast milk is in the molecular form of sIgA and is therefore more resistant to proteolytic activity of the gastrointestinal tract.<sup>2</sup> sIgA prevents the adherence of bacteria to mucosal surfaces and neutralizes toxins from microorganisms.<sup>2</sup> Lactoferrin also works at the mucosal sites and has demonstrated antiinflammatory and antimicrobial activities, such as competing with bacteria for ferric iron and preventing the growth of microorganisms.<sup>3</sup> Lysozyme is a protein that lyses bacteria and may work synergistically with lactoferrin and sIgA in antibacterial functions.<sup>3</sup>

Although the immunologic benefits of lactation are well known, the effect of maternal nutritional status on components in breast milk is questionable. Chang<sup>4</sup> reported that milk concentrations of sIgA and lysozyme from malnourished Chinese women were only half of those of well-nourished women during the first 7 days of lactation. Similarly, Miranda et al<sup>5</sup> reported that colostrum of malnourished Colombian women had significantly lower levels of sIgA, IgG, and complement C4 compared with well-nourished women. However, other researchers have failed to see an effect of maternal nutritional status on immunologic properties of breast milk.<sup>6–8</sup>

Another factor that may affect the immunologic properties of breast milk is maternal exercise. The effects of exercise on the immune system are dependent on the level of fitness of the subjects, the degree of intensity, and the duration of exercise. Heavy exertion usually results in lower resistance, impaired immunity, and increased risk of upper respiratory tract infections, whereas moderate exercise can con-

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fer resistance to infection.<sup>9</sup> A recent study by Klenrou et al<sup>10</sup> found that participants in a 12-week moderate exercise training program reported significantly lower number of days with influenza and light upper respiratory tract infection symptoms compared with sedentary control subjects. In addition, concentrations of salivary IgA increased significantly after training.

There is a paucity of information on the influence of exercise on the immune properties in breast milk. Gregory et al<sup>11</sup> examined sIgA concentrations before and after randomized maximal exercise and resting control periods. They reported that milk samples taken 10 and 30 minutes after exercise were significantly lower in sIgA levels than the corresponding control samples but were similar to control samples by 60 minutes. The authors suggested that breastfeeding immediately after exercise may not be beneficial to the infant. However, this study consisted of exercise to exhaustion, and the results may not be applicable to women who exercise at a moderate intensity level.

During moderate exercise, stress hormones, which can suppress immunity, are not elevated to the same extent as during prolonged or heavy exertion.<sup>12</sup> However, a study by Altemus et al<sup>13</sup> demonstrated an interesting response of stress hormones during intense exercise in postpartum women. Lactating and nonlactating women exercised for 20 minutes on a treadmill, with the speed and grade increasing progressively, reaching 90% of the maximal oxygen uptake during the last 5 minutes. Although the nonlactating women showed the expected increase in stress hormones, the plasma adrenocorticotrophic hormone, cortisol, and glucose responses to exercise were significantly blunted in the lactating women. These investigators concluded that the stress-responsive neurohormonal systems are restrained during lactation and that this inhibition may enhance immune function in lactating women.

McCrary et al<sup>14</sup> reported that short-term (11 days) dieting with aerobic exercise seemed to be safe during lactation. They found no differences in milk volume, lipid, protein, or energy output between the dieting/exercising group and the control group. We recently reported that 10 weeks of moderate exercise and energy restriction did not affect the growth of infants during the first 3 months' postpartum.<sup>15</sup> However, neither study measured any immunologic components in the breast milk to determine whether their concentrations were affected. Therefore, the purpose of this study was to 1) compare the basal levels of immunologic compounds in breast milk of exercising women with the milk of sedentary women and 2) determine whether 30 minutes of moderate exercise affects immunologic properties of breast milk. Although it is acknowledged that there are a host of bioactive compounds that compose the complex immune system in breast milk, the 3 proteins (sIgA, lactoferrin, and lysozyme) described above were selected for the study on the basis of their relatively high concentrations in breast milk and their important immunologic benefits to the nursing infant.

## Participants

Healthy (free from chronic disease or illness in the past week), nonsmoking, exclusively breastfeeding women with a body mass index between 20 and 30 kg/m<sup>2</sup> were studied at 3 months' postpartum. The women delivered healthy, term infants (birth weight: >2500 g; gestational age: 37–42 weeks). All infants were larger than the 10th percentiles of the Centers for Disease Control and Prevention growth charts for weight and length at 3 months of age.<sup>16</sup> Women were recruited through advertisements and announcements of the study in obstetricians' offices and in childbirth and parenting classes offered at the local hospital. Participants were asked about their physical activity in the postpartum period and grouped according to their exercise habits. Women who reported exercising aerobically at least 30 minutes per day for a minimum of 3 days a week for the previous 6 weeks were placed in the exercise group. The sedentary group consisted of women who reported exercising once a week or less. The institutional review board at the University of North Carolina at Greensboro approved the study protocol, and all study participants gave written informed consent.

## Experimental Design

On the day of the laboratory measurements, women expressed 30 mL of breast milk during the first morning feed after 5 AM. Mothers brought the samples to the Human Performance Laboratory, where they were frozen at  $-70^{\circ}\text{C}$  until analysis. Body composition and cardiovascular fitness were then measured on all women.

A subsample of exercising women returned to the laboratory 2 additional times for either a rest or an exercise session to determine the acute effects of exercise on concentrations of sIgA, lactoferrin, and lysozyme in their breast milk. These sessions were approximately 2 days apart, and the order was randomized. The exercise session consisted of brisk walking or jogging for 30 minutes on the treadmill at a speed and grade to elicit an intensity of approximately 75% of predicted maximum heart rate. This workload was determined from the previous measurement of cardiovascular fitness. Heart rate was measured with a heart rate monitor (Polar Inc, Woodbury, NY). The rest session was 30 minutes of sitting quietly. Breast milk samples were collected before and then 10 and 60 minutes after the exercise and rest sessions. Women expressed all milk from both breasts with a Medela electric breast pump (McHenry, IL) at each time point. The milk was weighed, and samples were frozen at  $-70^{\circ}\text{C}$  until analysis.

## Anthropometrics

Women were measured in bathing suits to the nearest tenth of a kilogram on a stationary beam balance scale. Maternal height without shoes was measured with the use of 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.<sup>17</sup> Body density and the percentage of body fat were calculated with the formulas of Brozek et al.<sup>18</sup>

## Cardiovascular Fitness

Cardiovascular fitness was measured by a submaximal graded treadmill test using a modified Balke protocol.<sup>19</sup> Before the test, a seated resting heart rate was measured with a heart rate monitor (Polar, Inc, Woodbury, NY). Women then warmed up with 2 minutes of walking on the treadmill. The speed was then increased to a brisk walk or jog (self-selected by the subjects, depending on their ability) and remained constant for the duration of the test. The women's heart rates and perceived levels of exertion were recorded every minute. Every 2 minutes, the grade of the treadmill was increased by 2.5%. The test continued until the heart rate reached 85% of the predicted maximal heart rate reserve:  $[(\text{maximal heart rate} - \text{resting heart rate}) \times (0.85)] + \text{resting heart rate}$ . The maximum heart rate of each woman was estimated using the equation  $220 - \text{age in years}$ .<sup>19</sup> For example, the maximum heart rate of a 35-year-old would be  $220 - 35 = 185$  beats per minute. The predicted oxygen consumption was calculated for each heart rate after every 2 minutes at each grade level with the following equations<sup>19</sup>:

Walking: (3.5 mL of oxygen/kg/min) + (speed in meters/min × 0.1) + (grade × meters/min × 1.8)

Jogging: (3.5 mL of oxygen/kg/min) + (speed in meters/min × 0.2) + (grade × meters/min × 0.9).

The predicted oxygen consumption at the maximal heart rate was calculated with a linear regression equation, with heart rate as the independent variable and oxygen consumption as the dependent variable.

### Analyses of Immunologic Compounds

Total IgA rather than sIgA was measured in the milk. However, because >95% of the IgA in breast milk is in the secretory form of IgA, total IgA is a reasonable proxy for sIgA.<sup>20</sup> Total IgA, lactoferrin, and lysozyme concentrations were analyzed using a sandwich enzyme-linked immunosorbent assay procedure. Milk samples were defatted by cold centrifugation, and the lipid layer was removed. The polyclonal antibody pairs and standards used were goat anti-human IgA (No. I0844; Sigma Chemical Co, St Louis, MO) and horseradish peroxidase (HRP)-conjugated rabbit anti-human IgA (No. P0216; DAKO, Carpinteria, CA) with breast milk IgA standard (No. BP 148; Binding Site, Birmingham, England); rabbit anti-lactoferrin (No. A0186; DAKO); and HRP-conjugated rabbit anti-human lactoferrin (No. K99172P; Biodesign, Saco, ME) with breast milk lactoferrin standard (No. L0520, Sigma); and rabbit anti-human lysozyme (No. A0099; DAKO), sheep anti-human lysozyme (No. K90073C; Biodesign), and donkey anti-sheep/goat IgG HRP-conjugated (No. W90063P, Biodesign) with breast milk lysozyme standard (No. L6394; Sigma).

Polystyrene microtiter enzyme-linked immunosorbent assay plates were coated with the appropriate antibodies (sIgA, lactoferrin, or lysozyme) and incubated at room temperature overnight. The plates were then washed 4 times with phosphate-buffered saline (PBS) with 0.05% Tween 20 and blocked for 1 hour with PBS/bovine serum albumin. The standards and samples were then added to the wells, and the plates were incubated for 1 hour. After the plates were washed with PBS/Tween, the appropriate anti-human HRP-conjugate was added and the plates were incubated at room temperature for 1 hour. Finally, the plates were washed another 4 times and color was developed with tetramethyl-benzidine (S1599, DAKO) and stopped with H<sub>2</sub>SO<sub>4</sub>. The absorbance was measured at 450 nm using a Powerwave microplate scanning spectrophotometer (Bio-Tek Instruments, Winooski, VT). All samples were measured in triplicate. Each exercise and rest session sample was analyzed on the same plate. For assessing interplate variation, a quality-control sample from a single collection was included on every plate.

### Statistical Analysis

Data were analyzed with the use of SPSS-PC software (SPSS, Chicago, IL). The characteristics of the groups were compared with Student *t* test. Lactoferrin and lysozyme data were log-transformed to normalize distributions. Data from the exercise and rest sessions were analyzed using repeated measures analysis of variance. Statistical significance was set at *P* < .05.

## RESULTS

Fifty-three women participated in the study: 29 in the exercising group and 24 in the control group. There were no significant differences between the 2 groups in baseline characteristics except for their cardiovascular fitness level (Table 1). The mean predicted maximal oxygen consumption of the exercise group was in the 80th percentile of fitness, compared with the 40th percentile for the control group, according to normative values of the American College of Sports Medicine.<sup>21</sup> This confirmed the women's self-reports of physical activity level. The majority of women in the exercise group stated that they exercised 30 to 60 minutes per day 3 to 5 days per week. No women were excluded as a result of conducting exercise to the point of exhaustion.

Despite the higher cardiovascular fitness level of the exercising women, there were no significant differences in body weight or percentage of body fat between the 2 groups. In addition, the breast milk of exercising women had similar basal concentrations of sIgA, lactoferrin, and lysozyme as the milk of sedentary women.

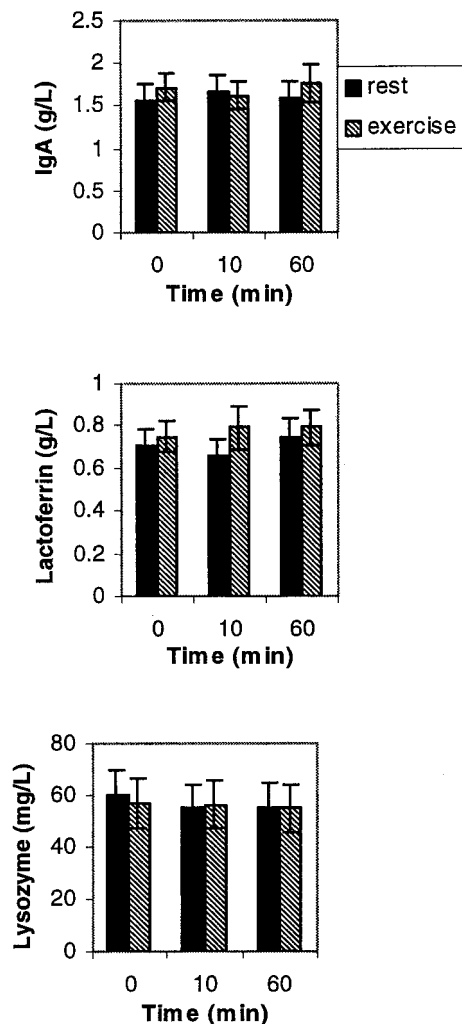
A subsample of 17 women from the exercise group completed the second study to evaluate the acute effects of exercise on concentrations of bioactive compounds in breast milk. They had similar characteristics as the other women in the exercise group except that their baseline lactoferrin levels were significantly higher (1.20 ± 0.07 vs 0.89 ± 0.10 g/L). However, these differences are probably not clinically significant as both means are within the normal value for lactoferrin concentrations at 3 months' postpartum. Concentrations of sIgA, lactoferrin, and lysozyme were not different between rest and exercise sessions (Fig 1). sIgA and lactoferrin levels did not change significantly during each session; however, lysozyme concentrations decreased significantly from baseline to 60 minutes after both rest and exercise sessions (*P* = .04). There was no difference in the amount of milk pumped during the rest or exercise session (data not shown).

TABLE 1. Characteristics of Participants\*

Characteristic	Exercise Group ( <i>n</i> = 29)	Sedentary Group ( <i>n</i> = 24)
Age (y)	31.5 ± 0.6	31.6 ± 1.0
Parity	1.9 ± 0.2	2.3 ± 0.2
Height (cm)	164.8 ± 1.2	163.3 ± 1.1
Weight (kg)	64.1 ± 1.5	66.0 ± 1.6
Body mass index (kg/m <sup>2</sup> )	23.6 ± 0.5	24.8 ± 0.6
Body fat (% of body weight)	25.3 ± 1.1	28.1 ± 1.3
Prepregnancy weight (kg)	61.5 ± 1.5	62.7 ± 1.8
Weight gain during pregnancy (kg)	14.5 ± 0.9	14.8 ± 0.9
Maximal oxygen consumption (mL O <sub>2</sub> /kg/min)	39.7 ± 1.0†	32.4 ± 1.0
Basal levels of		
sIgA (g/L)	1.74 ± 0.11	2.02 ± 0.18
Lactoferrin (g/L)	1.06 ± 0.06	1.08 ± 0.12
Lysozyme (mg/L)	50.5 ± 6.4	45.9 ± 6.2

\* Values are means and ± standard errors of the means.

† Significantly different from sedentary group, *P* < .01.



**Fig 1.** Concentrations of sIgA, lactoferrin, and lysozyme in milk of women before (minute 0) and after (minutes 10 and 60) a 30-minute rest session or exercise session. Values are means  $\pm$  standard error of the means. There were no significant differences between rest and exercise sessions. Lysozyme concentrations decreased after both rest and exercise sessions ( $P = .04$ ); however, there were no significant differences in sIgA or lactoferrin concentrations during either session.

## DISCUSSION

This study demonstrates that moderate exercise during lactation does not affect the sIgA, lactoferrin, or lysozyme concentrations in breast milk. Levels of these immunologic compounds in early-morning milk samples from women who had been exercising regularly for at least 6 weeks were not different from milk of women who had been sedentary during the postpartum period. Furthermore, sIgA, lactoferrin, and lysozyme concentrations were not affected by a 30-minute moderate exercise session. In contrast, Gregory et al<sup>11</sup> reported that breast milk sIgA concentrations were lower 10 and 30 minutes after a graded maximal exercise test (exercising until exhaustion) compared with samples at 10 and 30 minutes after a rest session. By 60 minutes after exercise, the concentrations of sIgA were similar to those after 60 minutes of rest. These authors recommended that mothers discard their milk produced during the first 30 minutes after exercise. However, their study may

be applicable only to lactating women who engage in very strenuous activities at maximal intensities. The moderate intensity of our exercise session is probably more representative of exercise done daily by lactating women. In addition, Gregory et al<sup>11</sup> reported baseline sIgA values of approximately 0.013g/L as compared with our baseline concentrations of 1.56 to 1.72 g/L. Other researchers<sup>6,8,22-26</sup> have reported concentrations ranging from averages of 0.7 to 2.0 g/L in milk from women at approximately 10 to 12 weeks' postpartum. The very low levels of sIgA reported by Gregory et al<sup>11</sup> may be attributable to methodological problems with the assay.

The average concentrations of lactoferrin in our study were 1.07 g/L for exercising and sedentary women, similar to those of healthy US women as reported by Butte et al.<sup>26</sup> Herias et al<sup>24</sup> reported mean lactoferrin concentrations of 0.98 and 1.07 g/L among Guatemalan women who were at 25 weeks' postpartum and receiving high-calorie supplements and low-calorie supplements, respectively. Davidson et al<sup>27</sup> observed lactoferrin concentrations in milk to range from 1.2 to 2.4 g/L among 8 lactating US women between 2 and 10 months' postpartum. However, Filteau et al<sup>22</sup> found higher lactoferrin concentrations (3.17 g/L) in the milk of Bangladeshi women at 3 months' postpartum. They suggested that the high concentration of lactoferrin was attributable to the high prevalence of infection among these women. Furthermore, they found increased mammary permeability, which may have been associated with increased leakage of lactoferrin across the epithelium into the milk.

Mean lysozyme concentrations in this study were approximately 47 mg/L. These values are somewhat lower than the range reported in the literature: 50 to 300 mg/L.<sup>28</sup> Higher concentrations of lysozyme are observed as lactation progresses, as well as in the presence of maternal infection and increased mammary permeability.<sup>22,26</sup> Although the decreases in mean lysozyme concentrations during the rest session from 60 to 56 mg/L and the exercise session from 57 to 55 mg/L were statistically significant, it is doubtful that they were clinically significant.

Women in our exercising group had a cardiovascular fitness level in the 80th percentile of fitness, whereas the sedentary group's average fitness level was in the 40th percentile. These levels of fitness indicate that the self-reports of women regarding their exercise activity in the postpartum period were accurate. The moderate physical activity level of the exercising women is similar to that recommended by the Centers for Disease Control and Prevention and the American College of Sports Medicine to improve cardiovascular fitness and prevent chronic diseases.<sup>29</sup> In previous studies, we have reported that this level of physical activity can improve serum lipids, cardiovascular fitness, and insulin response to a test meal in lactating women.<sup>30,31</sup> We have also found no differences in protein, fat, lactose, calorie, or vitamin B<sub>6</sub> concentrations in milk of exercising mothers compared with milk of sedentary women.<sup>32,33</sup> The findings in this study demonstrate no need to discard

milk produced within the first hour after moderate exercise. In addition, these results suggest that lactating women may engage in regular moderate exercise to improve their cardiovascular fitness level without affecting the immunologic components of their breast milk.

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