Exercise Is Associated With Elevated Proinflammatory Cytokines in Human Milk

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ABSTRACT

Objectives: To explore relationships between self-reported exercise in postpartum women and concentrations of cytokines and secretory immunoglobulin A in their milk.

Method: Fifty-eight frozen, unthawed aliquots of human hindmilk were available for analysis from a previous larger study on the influence of lactation on postpartum stress and immunity. The samples were early-morning, hand-expressed, hindmilk that had been collected between 4 and 6 weeks. Milk cytokines were analyzed by a multiplex assay of 20 cytokines, chemokines, and growth factors. Milk secretory immunoglobulin A was analyzed by enzyme-linked immunosorbent assay. Exercise data were extracted from a demographic questionnaire that was used in the original study and approximate metabolic-equivalent tasks assigned to the exercise levels reported. Based on reported frequency of exercise at a particular metabolic-equivalent task, caloric expenditures were calculated for each mother.

Results: With increasing metabolic-equivalent tasks, and thus caloric expenditures, proinflammatory cytokines increased in mothers’ milk. Secretory immunoglobulin A concentrations were not affected by mother’s exercise.

Conclusions: There are several possible interpretations for these results. These data are preliminary, and a larger, longitudinal study with a more structured exercise instrument will clarify if recommendations should be made about heavy exercise in the early postpartum months.

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Cytokines in milk have been measured either singly or in groups. The ranges of proinflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor-α tend to be large across the first 3 postpartum months, whereas the proimmune cytokines such as IL-10 and transforming growth factor-beta (TGF-β) are more constant and change little (Hawkes, Bryan, James, & Gibson, 1999). Milk also contains soluble receptors and cytokine antagonists to proinflammatory cytokines, which may contribute to the anti-inflammatory properties of milk (Buescher & Malinowska, 1996). Interleukin-10 and TGF-β are believed to be immunomodulating on the gastrointestinal epithelium and able to reduce tissue damage associated with necrotizing enterocolitis (Kelleher & Lonnerdal, 2001). These cytokines are reported to correlate with SIgA levels in milk (Bottcher, Jenmalm, Garofalo, & Bjorksten, 2000). Interleukin-1 is found in milk and may be immunostimulatory in the breastfed infant (Soder, 1987). Interleukin-6 and IL-8, both proinflammatory, are in milk and may be produced by mammary epithelium, independent of prolactin levels (Palkowitz et al., 1994).

There is evidence that milk cytokines vary with both infant and maternal states. For example, a recent study found that the breast milk of mothers of infants with bronchiolitis contained lower concentrations of IL-10 (Bryan, Hart, Forsyth, & Gibson, 2007). Breastfeeding appears to protect against atopic illnesses in infants, and the amount of milk TGF-β1, a proimmune cytokine at very high concentration in milk, was inversely associated with wheezing in breastfed infants (Oddy et al., 2003). Allergic mothers produce a different cytokine mix compared with nonatopic mothers, with higher levels of IL-4, IL-8, and regulated upon activation, normal T cell expressed and secreted (Rantes), a chemokine (Bottcher, Jenmalm, & Bjorksten, 2003).

There is little research available on the many potential maternal factors that could alter breast milk immune components such as cytokines. More is known about SIgA, which is the most significant antibody in human milk (Slade & Schwartz, 1987) and acts to bind microbes in the gastrointestinal tract, thereby preventing the microbes from latching onto and penetrating through the mucosa. In developing countries where infections are frequent and sanitation poor, milk is a major protective influence against infectious illnesses for infants. Milk SIgA is reported to vary according to the season and, in Gambian mothers, the plentitude of food (Weaver, Arthur, Bunn, & Thomas, 1998). Others have not found that maternal nutrition impacts milk SIgA (Hennart, Brasseur, Delogne-Desnoeck, Dramaix, & Robyn, 1991).

Another variable thought to potentially alter breast milk composition is exercise. Postpartum women usually have extra weight to lose and may be eager to begin or continue an exercise program. Anecdotal observations and some early research has suggested that after heavy maternal exercise, infants found the milk distasteful, a phenomenon attributed to the possibility of accumulated lactic acid making the milk taste sour (Dewey & Lovelady, 1993; Dewey, Lovelady, Nommsen-Rivers, McCrory, & Lonnerdal, 1994; Lovelady, Lonnerdal, & Dewey, 1990). A more recent study found that even though a small but significant increase in lactic acid occurred in the mothers’ milk after exercise, it did not affect infants’ acceptance of the milk (Wright, Quinn, & Carey, 2002). Others have suggested that heavy exercise in lactating women might have the potential to suppress the immune system and affect the levels of SIgA in milk. Gregory, Wallace, Gfell, Marks, and King (1997) reported that maximal graded treadmill exercise completed by 17 lactating women produced a significant drop in milk SIgA that lasted for 30 minutes after the exercise was completed. In contrast, Lovelady, Hunter, and Giegerman (2003) found that lactating women at 3 months postpartum who reported aerobic exercise of 30 minutes per day, 3 days per week, produced milk that had equivalent amounts of SIgA, lactoferrin, and lysozyme as women who did minimal or no exercise, when the milk was measured 10 and 60 minutes after an exercise session. This research group instructed the women to exercise at 75% of maximal heart rate and found no effect of exercise on milk SIgA. A Canadian guideline sponsored by the Society of Obstetricians and Gynaecologists of Canada endorsed a statement, based on metaanalysis that, “Women should be advised that moderate exercise during lactation does not affect the quantity or composition of breast milk or impact infant growth” (Davies et al., 2003, p. 520). However, none of the studies on exercise and human milk have carefully analyzed the effects of heavy exercise in postpartum women during the very early postpartum period (before 2 months). This is the time when the uterus is involuting and lactation is being established. In addition, mothers are dealing with interrupted sleep and fatigue, are recovering
from the stress of childbirth, and are spending most of their time and energy in nurturing the newborn infant. Nevertheless, a certain percentage of early postpartum women still engage in heavy exercise. The authors had the opportunity to explore the effects of exercise on breast milk immunity in 4 to 6 weeks postpartum women. The research question posed was: What are the relationships between self-reported demographic factors, exercise, and milk SIgA levels and cytokine profiles?

Method

The original study researched stress and immunity in postpartum women and was approved by university and hospital institutional review boards. Women were recruited after giving birth in the postpartum unit of the university hospital in a southern U.S. city, and they gave informed consent at that time. Inclusion criteria required that the women were healthy, had no chronic diseases, were not taking medications that could influence immune function, and had uneventful labors and deliveries, with the delivery of a singleton, healthy, full-term infant, either vaginally or by Caesarean birth. If consent was given, the researchers followed up with the mothers between the 4th and 6th postpartum weeks. A research nurse visited the home between 8 a.m. and 11 a.m., and all data were collected cross-sectionally at this one time period. A questionnaire packet that included a demographic form was sent ahead of the visit, and mothers were asked to complete the form on the day of the visit. Participating mothers were given a gift of $50 for their time. About half of the women approached consented to be in the study. Two hundred women completed the original study, half of whom were exclusive breastfeeding mothers from birth to the time of data collection.

In the original study, blood was drawn and milk and saliva collected and analyzed in relationship to lactation status, stress, and mood. These data are reported elsewhere (Groer, Davis, & Steele, 2004; Groer & Morgan, 2007; Groer, Davis, Casey et al., 2005; Groer, 2005; Groer & Davis, 2006; Groe«r, Davis, & Steele, 2004; Groer & Morgan, 2007; Groe«r, Davis, 2007; Smithet et al., 2005). The milk samples were 5 ml of hand-expressed hindmilk (milk expressed after a feeding on that breast was completed) taken at the end of the first morning feeding on the day of data collection and refrigerated until pick up. Mothers were given a detailed instruction sheet for milk collection. Hindmilk was chosen to produce a uniform sample across participants, as the constituents of milk change from the start to the end of a feeding. As feeding progresses, fat and calories increase, volume and flow decrease, and milk synthesis speeds up. Research nurses brought the milk samples to the laboratory in containers packed with ice, and the tubes were centrifuged at 400 g for 15 minutes and the nonfat whey was extracted by pipette, aliquoted into Eppendorf tubes, and frozen at −80 °C until analysis. Most studies of human milk cytokines have used the nonfat supernatant as was done here (Ustundag et al., 2005). Unthawed milk aliquots of sufficient volume for cytokine analysis remained for only 58 breastfeeding mothers after SIgA measurement; so cytokine data from milk samples were available for a little over half the original sample. Therefore, this article includes data on a sample of 58 postpartum women, drawn from the original sample of 100.

Secretory IgA in milk whey was analyzed by enzyme-linked sandwich immunoassay kits purchased from ALPCO. Cytokines were measured by a Luminex Instrument with multiplex kits purchased from Millipore, which contained polystyrene beads coated with antibodies to 20 cytokines, chemokines, and growth factors. The advantage of this technique is that only 50 μl of sample is needed for the simultaneous measurement of up to 100 analytes in the sample. The milk samples were thawed to room temperature and added in duplicate to 96-well filter-bottom plates. Beads were added to the wells and incubated on a plate shaker at 4 °C overnight. The wells were washed by vacuum through the filter bottoms with buffer, and then antibody conjugate was added, followed by an additional hour of shaking. Then, streptavidin-phycocerythrin was added to the wells, and a final shaking incubation was performed. The plates were washed as previously described, 100 μl of sheath fluid were added to the wells, and the wells were read in a Luminex 200 Multiplex analyzer. Standard curves and quality controls for every analyte were run on the plates. The median fluorescent intensities of the beads with the attached cytokine were converted to concentrations (pg/ml) through the use of a five-parameter logistic model.

The authors explored the effects of exercise on breast milk immunity in 4- to 6-week postpartum women.

Instruments

Demographic Instrument and Exercise Questions

The demographic instrument asked for information on socioeconomic status, current weight, parity, labor and birth history, and smoking and alcohol consumption and contained a section on current...
exercise activities. For exercise, mothers were asked to select the number of minutes per week of exercise currently performed from none, less than 30, 30 to 60, 60 to 90, 90 to 120, or greater than 120. These categories were assigned the scores of 0 through 5. The mothers also described in writing the type of exercise that they currently did, and it was then coded by the researchers into categories of none, minimal, moderate, and vigorous, with four categories used. The categories were derived from U.S. Department of Health and Human Services (1999) guidelines. The minimal category of exercise included activities such as light housework, walking the baby, and playing games with children. This type of activity would be on the order of 1 to 2 metabolic equivalents (METs); 1 MET is the energy expenditure for sitting quietly, which uses 1.2 kCal/minute for a 70 kg individual; Ainsworth et al., 1993). Moderate exercise uses 3 to 6 METs/minute and includes such activities as brisk walking, yoga, and ballroom dancing. The activity was classified as vigorous if it was greater than 6 METs, and this classification included activities such as power walking, aerobic dancing, jazzercise, and running.

The exercise data were converted to METs and then to kilocalories per week. This resulted in approximations, as the categories of time on the demographic instrument were in ranges, so the top of the range for each category was chosen as the possible number of minutes per week that the woman exercised at the level reported. The METS for each category were as follows: none = 1 MET, and, for these women, it was approximated that their minimal activity was 30 kCal/week. The authors made an assumption that even though the woman reported no exercise, it was likely that caring for a new baby involved some additional energy expenditure. For the minimal exercise group, the MET level was 2; for moderate, it was 6; and for vigorous, it was 9. These latter values are in the middle of the ranges for the U.S. Department of Health and Human Services (1999) categories.

Statistical Analysis

The data were examined for normality, and all of the cytokines were log10 transformed to correct for positive skewness. Means, Pearson’s correlations, and hierarchical linear regressions were then performed. SPSS 14.0 was used for analysis.

Results

Demographics

The mean age of the women was 28.5 years, and the majority were married. There was one African American and one Hispanic participant, with the remainder being White. Sixty-seven percent had vaginal birth, with remainder having Caesarean births. The mean length of labor was 91 hours. Sixty-four percent of the sample had two or more children. Forty-one percent had yearly incomes over $40,000. Seventy percent were not working, 8% were working part-time, and 24% were working full-time. The mean time of data collection was Week 5.3 postpartum. None of the demographic data were related to any of the milk cytokines except for parity. Interleukin-10 showed dramatic differences in relation to parity, with the highest levels in primiparas (n = 26; 247.2 ± 101.0 pG/ml), the second highest in mothers who were parity 2 (n = 22; 76.6 ± 46.4 pG/ml), and was absent in milk of mothers with higher than parity 2 (n = 9).

Exercise

Nearly half the women reported essentially no exercise at the time of measurement (49%). The remainder reported fewer than 30 (n = 21%), 30 to 60 (n = 7%), 60 to 90 (n = 6%), and 90 to 120 (n = 17%) minutes per week. The coded intensity of the exercise also varied. Fifty-six percent of the women reported no or very light exercise (gardening, walking the baby, housework). Forty-one percent reported being engaged in moderate exercise such as walking briskly, yoga, or using a treadmill. Only 3% of the sample engaged in vigorous activity (greater than 6 METs).

The mean level of SIgA in the milk samples was 296.4 ± 29.4 mg/dl. The range was 39.5 to 596.5 mg/dl. There was no correlation between exercise scores and milk SIgA (r = .12, p < .39). The only demographic related to milk SIgA was parity (r = .30, p < .03).

The milk cytokine concentrations ranged greatly. The means of the cytokines are listed in Table 1. In terms of minutes per week, there were clear trends in relationship to exercise. Calculated weekly caloric expenditure was correlated with proinflammatory milk cytokines. Statistically significant correlations were found between calories and IL-17 (r = .33, p < .019), IFN-γ (r = .34, p < .017), IL-1β (r = .43, p < .006), and IL-2 (r = .31, p < .03).

Separate hierarchical linear regressions were performed on each of the four milk cytokines that correlated with calories, with caloric expenditure being the predictor variable. Table 2 shows the relationships between these milk cytokines and exercise. Factors that could have influenced milk cytokine levels were considered. Number of cigarettes smoked per day, body mass index, marital
status, income, and delivery type were available from the demographic form. To determine the influence of these variables in the relationship, hierarchical regression analyses on each cytokine were performed. Exercise was entered first, and then the demographic variables. No model changed the percentage of variance due to caloric expenditure alone for any of the proinflammatory cytokines. The percentage of variance was generally small (the largest was 17%).

Discussion

This study suggests the possibility that moderate to vigorous exercise in the early postpartum (Weeks 4-6) is associated with shifts toward proinflammatory cytokine production in milk that exceeds production in those mothers who either did not exercise or engaged in minimal exercise. The level of caloric expenditure produced by the exercise was related to increases in a cluster of proinflammatory cytokines in mothers’ milk. Although demographic data that were collected did not relate to levels of milk cytokines, other possibilities that were not measured need to be considered. Variance in milk cytokines may be influenced by diet, environmental chemicals, medications that the authors were unaware of, lifestyle factors that were not queried, herbal supplements, previous exercise patterns before pregnancy, and so forth. Little is known about how any of these potential factors affect milk immunology, and studies are needed.

The signals that might be responsible for the exercise–cytokine relationship could not be determined adequately in this study. Biochemical changes, nutritional deficits, or sympathetic nervous system activation may also upregulate proinflammatory cytokine secretion. The teleological perspective would argue that exercise at this fairly early point in the postpartum may be physiologically interpreted as a danger signal. The postnatal period is designed to be a stress-resistant, quiet state devoted to nurturance and milk production in all mammals (Carter, Altemus, & Chrousos, 2001). Excessive exercise suggests predation and flight in this perspective, such that the organism responds by upregulating innate immune responses. If this argument is reasonable, then increased proinflammatory milk cytokines are a response to danger for the mother and, thus, also for the infant. Although the functions of most of the milk cytokines are unknown, this cluster of proinflammatory cytokines in human milk may act on the neonatal gut epithelium or mucosal immune system or may provide a host defense signal to the neonate’s immune system. The amounts of these cytokines are small but potentially significant. A newborn infant consumes 400 to 500 ml of breast milk per day, so these cytokines, although highly variable, could nevertheless be in a physiologically active range.

Another interpretation of the results could be that vigorous exercise produces microtrauma in the

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<th>Table 1: Means of Milk Cytokines (pg/ml)</th>
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<td>Cytokine</td>
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<td>GMCSF</td>
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Note. CSF = colony-stimulating factor; GMCSF = granulocyte-monocyte colony-stimulating factor; IL = interleukin; MCP = monocyte chemotactic protein; TNF = tumor necrosis factor.

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<th>Table 2: Milk Cytokine Levels Regressed on Exercise (kCal/week)</th>
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<td>Cytokine</td>
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<td>Interferon-γ</td>
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<td>Interleukin 2</td>
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<td>Interleukin 1β</td>
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<td>Interleukin 17</td>
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Heavy and frequent exercise was related to increases in a cluster of proinflammatory cytokines in mothers’ milk.

breast, with a loosening of tight junctions and an increase in breast inflammation. A way to evaluate this in future research is to measure the sodium concentration of mothers’ milk. It is also important to query when women exercised last. Because milk was collected from the first morning feed, typically collected around 6 to 7 a.m., the women were not queried about whether they had just completed an exercise session. Although unlikely, it is possible, and data should be collected in future studies.

Limitations
A significant limitation to this study is the cross-sectional design, use of self-report data, a nonvalidated exercise instrument, and an approximation of total calories expended by the various type and time of exercise. In addition, only 2 of the 58 women were categorized as vigorous exercisers, and the sample needs to be increased in future work. The purpose of the original study differed, and it was only after data had been collected that the authors became interested in the exercise–immune relationship. The data presented here are preliminary and need to be replicated with a larger sample in a longitudinal design and with a well-validated and structured instrument to evaluate exercise. It is also important in future work to measure other potential influences on milk cytokines. Cytokines as well as their soluble receptors and antagonists should be studied. If confirmed, the evidence would support the development of prescriptive exercise plans for postpartum mothers that take into consideration effects on milk immunology, which in turn may affect infant health. Although it seems logical that heavy exercise might be detrimental during the early postpartum period, considering the fatigue and multiple demands these women face, additional studies are needed before it is suggested that postpartum women limit their time and intensity of heavy exercise.

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