Breast milk immune factors in Bangladeshi women supplemented postpartum with retinol or β-carotene

Suzanne M Filteau, Amy L Rice, Jennifer J Ball, J Chakraborty, Rebecca Stoltzfus, Andres de Francisco, and Juana F Willumsen

ABSTRACT
Background: Vitamin A supplementation of mothers postpartum may improve infant health, not only by increasing vitamin A delivery to the infant through breast milk but also by increasing delivery of milk immune factors. Our hypothesis was that postpartum supplementation with vitamin A increases milk concentrations of certain soluble immune factors.

Design: In a double-blind trial conducted in Matlab, Bangladesh, women at 1–3 wk postpartum were randomly assigned to receive until 9 mo postpartum 1) a single dose of 60 mg retinol as retinyl palmitate followed by daily placebos (n = 69), 2) daily doses of 7.6 mg β-carotene (n = 72), or 3) daily placebos (n = 71). Milk samples collected at baseline and 3 mo postpartum were analyzed by enzyme-linked immunosorbent assay for secretory immunoglobulin A, lactoferrin, lysozyme, and interleukin 8; by HPLC for total retinol; and by atomic assay for secretory immunoglobulin A, lactoferrin, lysozyme, and interleukin 8; by HPLC for total retinol; and by atomic absorption spectroscopy for sodium and potassium.

Results: After mammary epithelial permeability (defined as an elevated Na:K) and baseline immune factor concentrations were controlled for, there were no significant treatment effects on immune factors at 3 mo. Increased mammary permeability was common (25% of women at baseline and 12% at 3 mo) and was associated with higher concentrations of milk immune factors. Low body vitamin A stores at baseline, as assessed by the modified-relative-dose-response test, were associated with a higher concentration of milk immune factors. Postpartum vitamin A supplementation does not increase milk concentrations of immune factors. The causes of increased mammary epithelial permeability in this population require further study.

Conclusions: Postpartum vitamin A supplementation does not improve the vitamin A status of younger infants. The benefits of programs for improving the vitamin A status of younger infants are less clear, with one study of infant supplementation at birth showing decreased mortality in the postneonatal period (3), but other studies of early supplementation showing no benefit on mortality (4–6).

Possible strategies for improving the vitamin A status of young infants include supplementing infants themselves, either at birth (3) or the time of immunization (2), or supplementing lactating mothers (7). Vitamin A supplementation of mothers has several advantages over supplementation of young infants. It improves the vitamin A status of the mothers, many of whom are deficient (8), as well as of the infants, with no apparent adverse side effects (7) as long as high-dose supplements are not given beyond 8 wk postpartum, to avoid teratogenic effects on a possible subsequent pregnancy (9). Another potential benefit of maternal vitamin A supplementation during lactation, which has not been investigated, is a possible increase in breast milk immune factors. These components of breast milk provide passive protection to young infants and stimulate development of the infants’ own immune systems (10, 11). Vitamin A is known to be important for immune function (12) and it is conceivable that increased intakes by deficient, lactating women would have beneficial effects on immune factors in their breast milk. There may also be immunologic benefits to the woman’s breast health because vitamin A supplementation was shown to decrease the incidence and severity of mastitis in mice (13). Mastitis greatly alters immune factors in the milk of cows and women; inflammatory mediators increase in particular (14). Because it

INTRODUCTION

The beneficial effects on morbidity and mortality of improving the vitamin A status of preschool children are well established (1). The World Health Organization thus recommends that efforts be made to improve vitamin A status, through dietary modification, fortification, or supplementation of all children from 6 mo of age (2). The benefits of programs for improving the vitamin A status of younger infants are less clear, with one study of infant supplementation at birth showing decreased mortality in the postneonatal period (3), but other studies of early supplementation showing no benefit on mortality (4–6).

Possible strategies for improving the vitamin A status of young infants include supplementing infants themselves, either at birth (3) or the time of immunization (2), or supplementing lactating mothers (7). Vitamin A supplementation of mothers has several advantages over supplementation of young infants. It improves the vitamin A status of the mothers, many of whom are deficient (8), as well as of the infants, with no apparent adverse side effects (7) as long as high-dose supplements are not given beyond 8 wk postpartum, to avoid teratogenic effects on a possible subsequent pregnancy (9). Another potential benefit of maternal vitamin A supplementation during lactation, which has not been investigated, is a possible increase in breast milk immune factors. These components of breast milk provide passive protection to young infants and stimulate development of the infants’ own immune systems (10, 11). Vitamin A is known to be important for immune function (12) and it is conceivable that increased intakes by deficient, lactating women would have beneficial effects on immune factors in their breast milk. There may also be immunologic benefits to the woman’s breast health because vitamin A supplementation was shown to decrease the incidence and severity of mastitis in mice (13). Mastitis greatly alters immune factors in the milk of cows and women; inflammatory mediators increase in particular (14). Because it

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appears that immune factors in milk increase infants’ specific immune protection and decrease the potential for nonspecific inflammation (11), the immunologic changes associated with mastitis may affect infant as well as maternal health.

The present study investigated the effect of maternal supplementa-
tion with retinol or β-carotene on amounts of immune factors in breast milk. Supplementation with synthetic β-carotene capsules was included to represent a type of treatment intermediate between retinol supplementation and dietary intake of β-carotene from fruit and vegetables and to determine whether any of the effects seen were retinol specific. The immune factors chosen for analysis were secretory immunoglobulin A (sIgA), lactoferrin, lysozyme, and interleukin 8 (IL-8), which are present in milk in high quantities (except IL-8), are fairly stable during storage, and benefit the infant through a variety of mechanisms (10, 11).

SUBJECTS AND METHODS

Subjects

The study used breast milk samples from the RETIBETA project on maternal vitamin A supplementation conducted at the Matlab field site of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Centre for Health and Population Research. The RETIBETA project was designed to test the effects of retinol or β-carotene supplementation on maternal and infant vitamin A status, details of which are described elsewhere (15). Ethical approval was received from Johns Hopkins University and the ICDDR,B. The study was a randomized, double-blind, placebo-controlled trial in which women at 1–3 wk postpartum received 1 of 3 treatments until 9 mo postpartum: 1) a single large dose of retinol (60 mg) as retinyl palmitate, followed by a daily placebo (n = 74); 2) a daily dose of 7.6 mg β-carotene (n = 73); or 3) a daily placebo (n = 73). The retinol dose was that currently recommended by the World Health Organization for women postpartum (9) and the β-carotene dose was equivalent to the recommended dietary allowance of vitamin A for lactating women (16). All 3 treatments were administered as capsules. Consumption of the first capsule was observed by a project fieldworker. Subsequent capsules were regularly delivered to each woman’s home. Routine and random spot counting of unused capsules determined that compliance was >90% for most women.

Women continued to receive health and family planning services from their regular community health workers every 14 d. In addition, project-specific visits occurred at ~2 wk and 3, 6, and 9 mo postpartum. For the project-specific visits, mother-infant pairs were randomly assigned to 2 field and 2 clinic visits each, equally distributing the number of pairs from the 3 treatment groups to field and clinic visits at each follow-up point. During field visits, mothers gave a casual sample of breast milk (<5 mL collected without respect to the time since the last breast-feeding episode) but no blood sample. During clinic visits, women provided blood samples and a full sample of breast milk (ie, the complete contents of one breast were expressed with a manual breast pump after a 2-h period in which the breast was not used for feeding). Both the women and their infants were weighed by using standard methods and examined (and treated if necessary) by the study physician.

Samples

Milk samples from the baseline and 3-mo follow-up visits were used. Only women for whom samples from both time points were available were included, ie, 69, 72, and 71 women from the retinol, β-carotene, and placebo groups, respectively. Samples were collected between July and November 1994, frozen in plastic containers at 220 °C at Matlab, then transported to Dhaka, where they were stored at 270 °C. The samples were thawed, split, and refrozen. Frozen aliquots were transported to London on dry ice, where they were stored at 260 °C until analyzed. Although sample handling conditions for these initially unplanned analyses were unorthodox, all samples were treated in the same way. Analyses were selected partly for their stability, and results, which were generally high, suggested that loss during storage was not a major problem.

Laboratory analyses

Immune factors were analyzed by using sandwich enzyme-linked immunosorbent assay (ELISA) techniques. The antibody pairs, polyclonal unless otherwise indicated, and standards used were as follows: anti-IgA (no. A0262; Dako, High Wycombe, United Kingdom) and anti-secretory component horseradish peroxidase (HRP) conjugate (no. P0166; Dako) with human milk IgA standard (no. BP148; The Binding Site, Birmingham, United Kingdom); anti-lactoferrin (no. A0186; Dako) and anti-lactoferrin-HRP conjugate (no. AHP13P; Serotec, Oxford, United Kingdom) with human milk lactoferrin as a standard (no. L0520; Sigma, Poole, United Kingdom); anti-lysozyme (no. A0099; Dako) and anti-lysozyme-HRP conjugate (Dako) with human milk lysozyme as a standard (no. L6394; Sigma); and monoclonal anti-IL-8 (no. MAB208; R+D Systems, Abington, United Kingdom) and biotinylated polyclonal anti-IL-8 (no. BAF208; R+D Systems) and avidin-HRP (no. P0347; Dako) with human IL-8 reference preparation no. 89/520 (National Institute for Biological Standards and Control, Potters Bar, United Kingdom). Bovine serum albumin as a blocking agent and bovine serum albumin in phosphate-buffered saline with 0.05% Tween 20 as a diluting agent were used for all analytes except IL-8. For the IL-8 assays, commercial blocking and diluting agents (CLB; Eurogenetics, Middlesex, United Kingdom) were used to decrease background absorbance. A quality-control milk sample from a British woman was included in duplicate on every plate, which permitted calculations of interplate CVs of 22% for sIgA (n = 20), 31% for lactoferrin (n = 20), 18% for lysozyme (n = 26), and 8.6% for IL-8 (n = 11). Although large, the CVs were similar to those found by others with ELISAs of milk proteins (17). The mean (±SD) recovery of IL-8 added in different amounts to the same milk sample was 141 ± 46% (n = 12). Recoveries >100% likely resulted from assay variability, which was in line with the variability of our other milk protein ELISAs but higher than the variability with the IL-8 assays in which the actual samples were run.

Serum retinol and didehydroretinol for the modified-relative-dose-response (MRDR) test and milk total retinol were measured by HPLC with retinyl acetate as an internal standard for serum and β-apo-8’-carotenal-methoxime for milk (15). Milk retinol concentrations were expressed per gram fat to control for the variation introduced by using 2 different sampling procedures (18). For similar reasons, aqueous milk components were expressed either per volume or per mg total protein, measured with a microtiter version of a commercial bicinchoninic acid
method (Pierce Chemical Co, Rockford, IL). However, because results were not affected, only concentrations per volume are presented.

Sodium and potassium were analyzed by flame atomic absorption spectroscopy (Corning 480 flame photometer; Instrumentation Laboratory, Warrington, United Kingdom). Calibration was done with a Corning Multical and interassay CVs for a milk sample containing 55 mmol Na and 22 mmol K were 2.5% for sodium and 1.6% for potassium. Na-K ratios were used to determine the degree of mammary epithelial permeability. On the basis of the observed distribution of Na-K ratios, the following permeability categories were defined: Na:K ≤0.6, Na:K >0.6 to ≤1.0, and Na:K >1.0. Na:K rather than the sodium concentration alone was used to account for 1) the variation due to the different proportions of aqueous and fat fractions as a result of different sampling methods, and 2) the modest parallel decreases in both electrolytes that occur as a result of months of lactation (19). The highest ratio category corresponds to a sodium concentration of ≈18 mmol/L, which, after about the first 3 d of lactation, is considered to indicate the opening of mammary epithelial tight junctions in association with mastitis or weaning (20). Infants were not weaned before the last samples were taken at 3 mo postpartum; therefore, we consider high Na-K ratios to indicate inflammation.

**Statistical analyses**

The sample size selected for the main study (n = 220) was based on numbers required to detect a difference between either the retinol or the β-carotene group and the placebo group in the proportion of infants classified as being vitamin A–deficient at 6 mo of age. This number was sufficient, at 95% significance and 80% power, to show 20–30% differences between group means for sIgA, lactoferrin, and lysozyme on the basis of their concentrations in casual milk samples from Zairean women (21). Published data were not available on which to base IL-8 calculations. The software package SPSS for MS WINDOWS (version 6.1; SPSS Inc, Chicago) was used for the analyses. Data were log-transformed to normalize distributions, and geometric means and 95% CIs are given. In the analysis of variance of treatment group differences at 3 mo, the following covariates were considered: concentrations of the particular immune factor at baseline, Na-K, type of sample (casual or full), the number of days postpartum at which the sample was collected, maternal age, body mass index, parity, initial infant weight, and infant weight gain between baseline and 3 mo.

**RESULTS**

Geometric mean concentrations of the immune factors at both baseline and 3 mo are shown in Table 1. There were no significant differences in any of the immune factors between treatment groups at either time point. However, total (means of the 3 groups) lactoferrin, sIgA, and IL-8 concentrations decreased between baseline and 3 mo, significantly so for lactoferrin and sIgA. Lysozyme concentrations increased with time, as was shown by others (21, 22). In addition, there were weak but significant negative correlations between the number of days postpartum that the baseline sample was collected (7–21 d) and baseline concentrations of all immune factors, except lysozyme (data not shown). At both baseline and 3 mo, concentrations in individual milk samples of all the immune factors except lysozyme were significantly correlated with each other and with total retinol in milk (data not shown). Infant weight gain between baseline and 3 mo, after the various times postpartum at which the samples and data were collected were controlled for, was negatively correlated with concentrations of IL-8 (P = 0.007), lysozyme (P = 0.02), lactoferrin (P < 0.001), and sIgA (P = 0.051) at 3 mo.

At 3 mo, treatment group, type of sample (casual or full), maternal age, body mass index, and parity did not significantly affect concentrations of immune factors. In each case, the concentration of the factor at baseline was significantly related to its concentration at 3 mo (P < 0.001 for sIgA, lactoferrin, and lysozyme; P = 0.012 for IL-8). Na:K was significantly correlated with concentrations of sIgA (P = 0.049), lactoferrin (P < 0.001), and IL-8 (P = 0.001), but not with lysozyme (P = 0.54). The relations between infant weight gain and immune factor concentrations seen in the simple correlations were no longer significant once the Na-K ratio was included in the analysis of variance. When the effects of supplementation were examined in only women with the lowest quartile of initial immune factors, there was still no significant effect of vitamin A.

Because of the unexpectedly large effect of increased mammary permeability, in terms of the number of affected women (baseline: 52/212, or 25%; 3 mo: 25/212, or 12%) and the concentrations of most of the immune factors measured, we calculated geometric mean values on the basis of Na-K category rather than on the basis of treatment group and included the number of days postpartum that the sample was collected as a covariate (Table 2). IL-8 and sIgA increased at both time points with increasing Na-K ratios; lactoferrin and lysozyme showed similar trends that were not always significant and total retinol

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**TABLE 1**

Concentrations of breast milk immune factors at baseline and 3 mo after supplementation with placebo, retinol, or β-carotene

<table>
<thead>
<tr>
<th></th>
<th>sIgA (g/L)</th>
<th>Lactoferrin (mg/L)</th>
<th>Lysozyme (μg/L)</th>
<th>IL-8 (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n = 212)</td>
<td>1.15 (1.09–1.21)</td>
<td>8.04 (7.56–8.54)</td>
<td>48.9 (44.4–53.8)</td>
<td>35.8 (29.0–44.1)</td>
</tr>
<tr>
<td>3 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n = 212)</td>
<td>0.79 (0.75–0.85)²</td>
<td>3.17 (2.92–3.44)²</td>
<td>116 (106–128)²</td>
<td>29.7 (25.9–33.9)</td>
</tr>
<tr>
<td>Placebo group (n = 71)</td>
<td>0.86 (0.81–0.91)</td>
<td>3.40 (3.16–3.67)</td>
<td>109 (100–119)</td>
<td>33.6 (29.6–38.3)</td>
</tr>
<tr>
<td>Retinol group (n = 69)</td>
<td>0.74 (0.70–0.79)</td>
<td>3.06 (2.84–3.30)</td>
<td>118 (108–129)</td>
<td>29.6 (26.0–33.7)</td>
</tr>
<tr>
<td>β-Carotene group (n = 72)</td>
<td>0.79 (0.74–0.84)</td>
<td>3.04 (2.82–3.28)</td>
<td>124 (114–136)</td>
<td>26.2 (23.0–29.8)</td>
</tr>
</tbody>
</table>

¹ Geometric mean; 95% CI in parentheses. Means for individual treatment groups at 3 mo were adjusted for baseline immune factor concentration and Na-K at 3 mo. There were no significant differences among treatment groups at either time point. sIgA, secretory immunoglobulin A; IL-8, interleukin 8.

² Significantly different from baseline, P < 0.001 (paired t test).
increased with increasing Na-K ratios at baseline.

Because blood samples were taken from only half the women at each time point, sample size was reduced for analyses of the effects of baseline vitamin A status on milk immune factors and significant initial or treatment differences were not seen. However, women with poor vitamin A status at baseline, as indicated by MRDR test results > 0.06, had higher geometric mean Na-K ratios [0.63 (95% CI: 0.51, 0.76); n = 23] than those with better vitamin A status [0.50 (0.46, 0.55); n = 83; P = 0.04]. There was no significant effect of retinol or beta-carotene supplementation on the proportion of women with high Na-K ratios at 3 mo, possibly because of the small number of subjects; 6 women in each of the retinol and beta-carotene groups and 7 in the placebo group had intermediate Na-K ratios; 1 in the retinol group, 2 in the beta-carotene group, and 3 in the placebo group had high ratios.

DISCUSSION

Our primary aim was to determine whether or not maternal supplementation with retinol or beta-carotene influences the concentrations of immune factors in breast milk. The results showed that vitamin A supplementation had no effect on immune factors even though initial vitamin A status in the population was poor and improved with supplementation (15). When we conducted a retrospective analysis using our own data, we had statistical power to detect differences of ~30% between groups for lactoferrin and lysozyme, but only differences of ~60% for sIgA and IL-8. Others have shown only modest effects of food supplements on milk immune factors in women (22, 23). To our knowledge, there are no other data on micronutrient status and milk immune factors in humans; however, vitamin E and selenium supplementation of cows was shown to increase the number of and to improve the function of milk neutrophils, with consequent benefits for mammary gland health (24). We found a slight effect of initial vitamin A stores, as estimated from the MRDR results, on mammary epithelial permeability, as indicated by Na-K ratios. Because an elevated Na-K may indicate mammary inflammation, this result is similar to results from rodent studies in which the incidence and severity of experimental mastitis decreased with increasing dietary retinol (13). However, neither retinol nor beta-carotene supplementation affected the prevalence of elevated Na-K ratios.

The major contributors to milk immune factor concentrations were mammary permeability and individual variation, as evidenced by the large effect of baseline concentrations on 3-mo concentrations of each factor. An increased Na-K was associated with an increase in all immune factors, as was shown by others (25), as well as in total retinol concentrations at baseline. Concentrations of the inflammatory cytokine IL-8 were most strongly affected, suggesting that an elevated Na-K represents true inflammation, not just a “leakiness” of the mammary epithelium due to other factors. In addition, this observation and the association of an elevated Na-K with reduced infant growth support in vivo production of IL-8, possibly by mammary epithelial cells (26), rather than ex vivo stimulation of cells during sample collection or processing.

We found a high proportion of women, especially at baseline, with elevated milk sodium concentrations characteristic of mastitis (20, 25) or breast engorgement (27). Na-K decreased as the days postpartum at which the baseline sample was collected increased, suggesting that this was partly a normal physiologic change as milk production adjusted to meet infant demand and as breast engorgement was relieved. Nevertheless, baseline concentrations of immune factors were still significantly correlated with Na-K even after the number of days postpartum at which the sample was collected was accounted for, indicating a possible residual effect of inflammation. Breast pain was not recorded during the study, but there was little obvious mastitis. We are unaware of comparable published studies of Na-K ratios in milk from a large unselected population of mothers from a developed country, but recently found similarly high Na-K ratios in milk samples from Tanzanian (28) and South African women (SM Filteau, JF Willumsen, unpublished observations, 1998). Vitamin A deficiency may have contributed slightly to the high prevalence because women with abnormal MRDR results had higher milk Na-K ratios.

Lysozyme and sIgA concentrations in the present study were comparable with those commonly found in the mature milk of

TABLE 2
Effect of mammary permeability (determined on the basis of Na-K ratios in breast milk) on milk immune factors

<table>
<thead>
<tr>
<th>Na-K</th>
<th>Baseline</th>
<th>Na-K &gt; 0.6 to ≤ 1.0</th>
<th>Na-K &gt; 1.0</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>158</td>
<td>31</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>sIgA (g/L)</td>
<td>1.10 (1.05–1.15)a</td>
<td>1.20 (1.15–1.26)a</td>
<td>1.64 (1.57–1.71)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lactoferrin (g/L)</td>
<td>8.01 (7.54–8.50)a</td>
<td>8.26 (7.78–8.77)b</td>
<td>9.79 (9.23–10.40)b</td>
<td>0.151</td>
</tr>
<tr>
<td>Lysozyme (mg/L)</td>
<td>47.3 (42.9–52.1)a</td>
<td>46.8 (42.5–51.6)b</td>
<td>69.8 (63.3–76.9)b</td>
<td>0.059</td>
</tr>
<tr>
<td>IL-8 (ng/L)</td>
<td>24.6 (20.7–29.2)a</td>
<td>66.4 (56.0–78.9)b</td>
<td>397 (319–450)c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinol (µg/g fat)</td>
<td>8.43 (7.92–8.98)a</td>
<td>9.11 (8.56–9.70)a</td>
<td>12.8 (12.0–13.7)b</td>
<td>0.001</td>
</tr>
<tr>
<td>n</td>
<td>186</td>
<td>19</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>sIgA (g/L)</td>
<td>0.78 (0.73–0.83)a</td>
<td>0.86 (0.81–0.92)a</td>
<td>1.54 (1.45–1.64)b</td>
<td>0.001</td>
</tr>
<tr>
<td>Lactoferrin (g/L)</td>
<td>2.99 (2.76–3.23)a</td>
<td>3.28 (3.03–3.55)a</td>
<td>9.05 (8.37–9.78)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lysozyme (mg/L)</td>
<td>115 (104–128)a</td>
<td>108 (92–113)b</td>
<td>218 (197–242)b</td>
<td>0.063</td>
</tr>
<tr>
<td>IL-8 (ng/mL)</td>
<td>27.0 (23.7–30.8)a</td>
<td>42.5 (37.3–48.4)a</td>
<td>160 (140–182)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinol (µg/g fat)</td>
<td>6.18 (5.80–6.58)a</td>
<td>6.76 (6.36–7.20)a</td>
<td>6.94 (6.52–7.39)a</td>
<td>0.61</td>
</tr>
</tbody>
</table>

1 Geometric mean; 95% CI in parentheses. Confidence intervals were calculated from the pooled error terms of the ANOVAs. sIgA, secretory immunoglobulin A; IL-8, interleukin 8.
2 The main effect of Na-K category from analyses that included the number of days postpartum the sample was collected as a covariate. The superscripts are included for illustrative purposes and are from the ANOVAs without the covariate. Values in a row with different superscript letters are significantly different, P < 0.05 (Duncan’s multiple range test).
Western women, whereas lactoferrin concentrations were somewhat higher (10, 11). Although the normal to high concentrations of immune factors in this population may have contributed to the lack of effect of vitamin A supplementation, there was similarly no effect in the quartile with the lowest initial immune factor concentrations. Several studies have shown a tendency toward higher milk concentrations of slgA and lactoferrin in African than in European women (21, 22, 29), suggesting that concentrations are higher in areas with a high prevalence of ambient infection. Our results suggest that this may not only be a result of increased maternal synthesis due to general stimulation of immune responses in the mucosal immune system, but also a result of increased mammary permeability causing increased leakage of many compounds across the epithelium into the milk.

Several factors that could not be assessed within the context of this study may affect immune compounds and sodium in milk, including the amount of breast milk consumed by infants and gestational age at birth. Weaning or poor suckling by infants might result in breast engorgement, with resultant increases in milk sodium, but, in this area of rural Bangladesh, exclusive or predominant breast-feeding is virtually universal until ≥3 mo of age and infant formula is not used. Because mother-infant pairs were recruited at 2 wk postpartum, accurate measures of gestational age were unavailable. Because the treatment allocation was random, these confounders (ie, the amount of breast milk consumed by infants and gestational age at birth) would have been similar across groups and, at most, would have increased our variability. Because we found no important trends, the increased variability that resulted from the confounders likely had little effect on our conclusions.

Although samples were collected by using 2 different methods, a casual manual sample or a full breast sample obtained with a pump, this had no significant effect on immune factor concentrations. Similarly, the results of analyses of immune factors did not differ significantly when factors were expressed per mg total protein or per volume. These observations likely reflect the fact that the aqueous part of milk, presumably the major fraction containing these protein immune factors, is the largest component of the milk and, as has been shown by others (30), has a fairly constant composition throughout a feeding. Thus, it appears appropriate to use whatever method is easiest to collect and analyze milk for immune factor measurements.

In conclusion, neither maternal retinol nor β-carotene supplementation of lactating Bangladeshi women significantly affected milk immune factors at 3 mo postpartum. However, even in the unsupplemented group, the concentrations of most factors were within the normal range. Milk samples with high immune factor concentrations also had high sodium concentrations, which are associated with breast inflammation. Because a relatively large proportion of women were identified with high milk sodium and IL-8 concentrations—and inflammatory stimuli in the gut of young infants can be harmful (11) and breast inflammation can be painful—the underlying causes of this condition and its potential association with infant growth in other populations deserve further investigation. Most problems with lactation can be remedied with breast-feeding counseling (31) and it is possible that low-cost education programs on good breast-feeding practices may have important health benefits for these women and their infants.

We thank Gina Coates and Rina Naik, Institute of Child Health, for technical assistance; Ann Prentice, Dunn Nutrition Laboratory, Cambridge, for advice; the Bangladeshi women who participated in the study; the London woman who generously donated her milk for our use in assay development and as a quality control; and the milk bank staff at St Georges Hospital, London.

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