Early Consumption of Human Milk Oligosaccharides Is Inversely Related to Subsequent Risk of Respiratory and Enteric Disease in Infants

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ABSTRACT

A pilot study tested the relationship between human milk oligosaccharide consumption, oligosaccharide content of feces, and subsequent disease in breastfed infants. Forty-nine (49) mother–infant pairs provided milk and fecal samples 2 weeks postpartum; infant health was assessed through 2, 6, 12, and 24 weeks. LNF-II (lacto-N-fucopentaose II), a major human milk oligosaccharide, was measured to represent levels of total oligosaccharides consumed in milk and remaining in feces. LNF-II levels in milk at 2 weeks postpartum were associated with fewer infant respiratory problems by 6 weeks ($p = 0.010$), as were LNF-II levels in infant feces ($p = 0.003$). LNF-II levels in milk at 2 weeks were also associated with fewer respiratory problems by 12 weeks ($p = 0.038$), and fewer enteric problems by 6 weeks ($p = 0.004$) and 12 weeks ($p = 0.045$). Thus, consumption of human milk oligosaccharides through breastfeeding, represented by LNF-II, was associated with less reported respiratory and gastrointestinal illness in infants.

INTRODUCTION

THE PROTECTION THAT HUMAN MILK affords against illness was identified as early as 1892.1 Data supporting protection by breastfeeding, an “old” topic, continues to accumulate: Even now, even in the United States, breastfeeding reduces the risk of postneonatal death.2 Breastfed babies have one-sixth the risk of dehydrating diarrhea than bottle-fed infants,3 and are less likely to have asthma, lower respiratory infections,4 upper respiratory infections,5 and ear infections.6 With fewer illnesses, typical health care costs of breastfed infants are $331 to $475 less per breastfed infant during the first year of life.7 The savings would have amounted to a total of 1.3 to 1.9 billion dollars per year if every infant born in the United States in 2002 had been breastfed.

Although the fundamental role in the defense against infections has been attributed to antibodies, oligosaccharides also may contribute to the protective properties of human milk.8,9 Human milk oligosaccharides are thought to interfere with binding by a pathogen to its host cell receptor.10 Oligosaccharides are one of the major components found in human milk, along with lac-

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tose, fat, and protein. Over 100 major milk oligosaccharides have been isolated and their structures determined.\textsuperscript{11,12} Consumed oligosaccharides remain mostly undigested, and human milk oligosaccharide concentrations are higher in feces of breastfed infants than in mother’s milk, and much higher in feces than urine.\textsuperscript{13} They can act as soluble receptor analogs for a variety of enteric pathogens. Thus, as they traverse the alimentary canal, the oligosaccharides become more concentrated than they were in milk, maximizing their potential for protecting breastfed infants against diarrhea.\textsuperscript{14,15} Binding by \textit{Streptococcus pneumoniae}, a respiratory pathogen, is also inhibited by oligosaccharides.\textsuperscript{16} However, a relationship between ingestion of human milk oligosaccharides and incidence of respiratory disease in infants has not been demonstrated.

This pilot study explores the utility of a simple measure of a single oligosaccharide in human milk and feces, the concentration of lacto-N-fucopentaose II (LNF-II) as a determinant of health outcomes in clinical studies. The association between this single oligosaccharide measure, LNF-II, in breast milk and maternal reports of respiratory or gastrointestinal problems in their breastfeeding infants was determined. The association between a single measure of human milk oligosaccharides in feces of breastfed infants (LNF-II) and the same maternal reports of respiratory or gastrointestinal problems in their breastfeeding infants was determined to compare the utility of each measure, and confirm any associations.

**MATERIALS AND METHODS**

Using data from a study that examined the impact of motivational interviewing to promote breastfeeding duration from birth through the first 6 months,\textsuperscript{17} a secondary analysis correlation model was used to explore the association of oligosaccharide levels on infant health outcomes.

**Sample/setting**

A convenience sample of 73 primiparous mothers who intended to breastfeed, ranging between the ages of 19 and 38 (M = 27, SD = 4), was recruited at three western rural community hospital sites in the original study. Of these mothers, the 49 (67\%) who were still breastfeeding (defined as reporting any breastfeeding during the prior 24 hours) at 2 weeks provided breast milk (49) and infant fecal samples (43) for this analysis. Of these mothers, 94\% reported breastfeeding between six and 12 times per day, with a median of nine times per day. This frequency of breastfeeding is consistent with “full or nearly full breastfeeding,” as defined by Labbok et al.,\textsuperscript{18,19} which includes exclusive, almost exclusive, and high partial breastfeeding patterns. In order to provide a stable cohort of infants, mothers were excluded if their infants: (a) were admitted to a neonatal intensive care unit; (b) were born prior to gestational age of 37 weeks; (c) weighed less than 2500 g at birth; or (d) had a bilirubin level above 15 mg/dL.

**Procedure**

After institutional review board approval from the University of Wyoming and the University of Nebraska Medical Center, potential participants were recruited at prenatal classes or during preadmission procedures and consent was obtained. Prior to discharge from the hospital after delivery, mothers completed a baseline questionnaire that included demographics and obstetric histories. Participants were randomly assigned to either an intervention or comparison group for the original study. Data were collected by research nurses before discharge from the hospital and at outpatient visits at infant age: 2 to 4 days, 2 weeks, and 6 weeks, and also during monthly phone calls thereafter until 24 weeks postpartum. Data included in this study were: (a) breast milk oligosaccharide samples at 2 weeks; (b) fecal samples of oligosaccharides at 2 weeks; (c) days of breastfeeding during the first 24 weeks; and (d) monthly infant health outcomes.

**Demographics and obstetric history**

To obtain a comprehensive description of the sample, a demographic and obstetric history questionnaire was completed while the participants were hospitalized during the postpar-
Infant health outcomes

Infant illnesses and health problems were measured by maternal report using selected portions of the National Maternal Infant Health Survey [NMIHS]. The health outcomes measured included fever, upper respiratory infection, wheezing, pneumonia, otitis media, conjunctivitis, listlessness, fussiness, seizures, injuries, colic, vomiting, and diarrhea. The NMIHS has been validated for infant health outcomes in other studies. The infant health outcomes were measured when infants were 2 weeks and 6 weeks of age, then monthly from 8 to 24 weeks.

Twenty-four-week breastfeeding duration

At each data collection time, mothers were asked to report the date that breastfeeding last occurred. Calculation of days of breastfeeding was completed using the birth date through the last date of any feeding. Duration was recorded as 180 days for mothers who were still breastfeeding at the 24-week data collection.

Human milk oligosaccharides

The entire milk content from one breast was collected by pumping with a Lactina Select (Medela #016SC01) prior to an early morning feeding. Fecal samples (1 mL) were collected from the infant’s diaper by lining the inside of the diaper with plastic wrap, and transferring 1 mL of the stool specimen to a test tube. Samples were frozen, shipped, and stored at \(-80^\circ C\) until analysis. LNF-II levels were measured in infant stool and maternal milk samples as follows.

**Extraction of oligosaccharides from milk.** Oligosaccharides were isolated from milk samples by an adaptation of the method of Chaturvedi et al. Samples were centrifuged in a microcentrifuge for 5 minutes. The aqueous layer (100 \(\mu L\)) was diluted to 1 mL with water, brought to 67% ethanol, and left at \(4^\circ C\) overnight. The protein precipitate was removed by centrifugation. The supernatants, which contained the oligosaccharides, were dried under a stream of nitrogen to remove ethanol, lyophilized, and stored at \(-20^\circ C\) until analyzed.

**Extraction of oligosaccharides from feces.** Oligosaccharides were isolated from fecal samples by an adaptation of the method of Chaturvedi et al. Approximately 100 mg of each sample was weighed, suspended in 2 mL distilled water, and passed through a column of 1 mL AGI-X8 anion-exchange resin (acetate). The eluate was collected, frozen, lyophilized, and stored at \(-20^\circ C\) until analyzed.

**Analysis of oligosaccharides by high-performance thin-layer chromatography.** Samples were dissolved in 100 \(\mu L\) water. Two \(\mu L\) were applied to a 10 \(\times\) 10 cm amino propyl silica HPTLC plate (E. Merck, Darmstadt, Germany) using 2 \(\mu g\) LNF-II as standards on two lanes of each plate. The oligosaccharides were resolved using pyridine:ethyl acetate:acetic acid:water (6:2:1:2, v/v) as the mobile phase. Oligosaccharides were visualized with 0.2% orcinol in 2 N sulfuric acid spray followed by heating. Plates were scanned on an Epson scanner, images stored as Adobe Photoshop 7 files, and the optical density of the oligosaccharide bands were quantified using ONE-Dscan for the Macintosh, version 2.2 (Scanalytics, Inc., Fairfax, VA).

**Statistical analysis**

Infant health problems were reported by their mothers on the Infant Health Survey for the period from birth to 2 weeks, and the elapsed period from the previous data collection to 6, 12, 16, 20, and 24 weeks. Occurrences were summed to calculate cumulative occurrences in each category by 2, 6, 12, and 24 weeks. For analysis, three categories of health
problems were considered: (a) respiratory problems, consisting of upper respiratory infections (runny nose or cold), cough, or pneumonia; (b) gastrointestinal tract (GI) problems, which included vomiting, diarrhea, or colic; and (c) ear infections. Distributions of health problems were skewed, with only a few infants experiencing more than one or two problems in any outcome category.

For analysis, infants were categorized as either having no problems in the relevant period or having one or more. Means and standard deviations of LNF-II from human milk and infant fecal samples obtained 2 weeks postpartum were calculated for each of these groups. Three fecal samples in which the level of LNF-II was below the threshold for detection were given a value of zero. Because of the small and disproportionate group sizes, the Mann-Whitney U-test, a nonparametric test of mean ranks, was used to compare the groups (problems/no problems) on level of LNF-II at 2 weeks. Distributions of LNF-II also were evaluated to identify cases with unusual values. If such cases were found, analyses were conducted both with and without these observations and discrepancies used to assess the robustness of the results. A significance level of 0.05 was used, with no adjustment for the number of comparisons. As little work has been done previously on the relationship of this oligosaccharide with health outcomes, investigators considered type I errors to be less serious than overlooking relationships that might merit further investigation (type II errors). All tests were two-tailed.

Because not all of the women in the sample continued to breastfeed for the entire 24-week period (93%, 77%, and 56% reported breastfeeding at 6, 12, and 24 weeks, respectively), a supplemental analysis investigated whether the relationships described in the preceding would still be observed if breastfeeding behavior following the sample acquisition at 2 weeks were taken into account. A logistic regression model was fit at each time (except for 2 weeks, when all women were still breastfeeding) predicting outcome category from both LNF-II level at 2 weeks and percentage of days the mother reported breastfeeding out of the total number of days in the specified time period. Unlike multiple regression, logistic regression has more than one possible test of significance for individual predictors. Two common alternatives, the Wald test and the likelihood ratio chi-square test of the difference between models with and without the predictor of interest, are asymptotically equivalent, but they often differ for analyzing small samples, with the likelihood ratio test having somewhat more desirable properties for small sample sizes. Note that the 95% confidence interval is calculated from the Wald-based standard error, and thus may include 1 despite being judged significant by the likelihood ratio chi-square test.

RESULTS

The sample included women who were predominantly young (25 to 31 years old), white, married, and whose babies were delivered via vaginal delivery after a labor of a range of 0 to 26 hours. These women generally were non-smokers with at least high school educations, who attended childbirth preparation classes and began breastfeeding their babies in the first 4 days postpartum. Family incomes reported by the subjects were documented as: approximately 40% of the families had an income of $29,000/year or less, approximately 25% of the families had an income ranging between $30,000 and $49,000, and approximately 35% of the families had an income of $50,000 or more. Approximately half the infants whose mothers completed the study were born between 38 and 39 weeks of gestation, and 91% of all infants studied had no problems or distress at birth.

Comparing cases with and without health outcome data at 6, 12, and 24 weeks by student’s t-test indicated that the two groups did not differ significantly on LNF-II levels in either milk or fecal samples at 2 weeks, but those with missing data did breastfeed for a lower percentage of days in each time period. The difference was significant only for the 24-week period, with women in the analysis breastfeeding an average of 75% of the days and women with missing data an average of 47% of the days.
Looking descriptively at demographic profiles, the groups were similar in age at all three follow-up times, but a higher percentage of those with complete data were married (80% to 90% compared with 50% of the dropouts), had at least a bachelor’s degree (35% to 38% compared with 17% to 25%), and reported higher household income (a median of $30,000 to $39,000 compared with $10,000 to $19,000). By 24 weeks, 83% of those who had dropped out, but 58% of those who completed the study, reported that they had returned to work. Return to work information was not available for 20% (n = 3) of those mothers who dropped out.

Descriptive statistics and the results of the Mann-Whitney U-tests are presented in Table 1. Samples of human milk obtained at 2 weeks were available for 49 women, with fecal samples for 43 infants. The total number of infants in the analysis decreases to 34 by 24 weeks for human milk LNF-II and 30 for fecal LNF-II because of study attrition.

Infants who experienced respiratory problems by 6 weeks or by 24 weeks had significantly lower levels of LNF-II in their 2-week fecal samples (p = 0.013 and p = 0.045, respectively) than did infants for whom no respiratory problems were reported (see Table 1). The same pattern was found when the LNF-II levels in human milk were analyzed (p = 0.049 and p = 0.028). In the human milk samples, two cases were identified as possible outliers relative to the distribution of LNF-II, having z-scores exceeding 3.0. With these two cases deleted, conclusions remained the same except at 6 weeks, which was no longer significant with a p-value of 0.067.

For GI pathology, LNF-II levels in human milk were significantly lower for the group experiencing problems at least once by 6 weeks (p = 0.019) (see Table 1). If the two outliers are removed from the analysis, this p-value decreases to 0.004 and a significant relationship is found at 24 weeks (the p-value decreases

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**Table 1. LNF-II in Milk or Feces and Reported Health Problems of Breastfed Infants**

<table>
<thead>
<tr>
<th></th>
<th>No symptoms</th>
<th>Symptoms</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SE</td>
<td>n</td>
</tr>
<tr>
<td><strong>LNF-II, µg/mL in Milk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>47</td>
<td>8.0 ± 1.0</td>
<td>2</td>
</tr>
<tr>
<td>6 weeks</td>
<td>38</td>
<td>8.4 ± 1.2</td>
<td>7</td>
</tr>
<tr>
<td>12 weeks</td>
<td>25</td>
<td>9.0 ± 1.5</td>
<td>18</td>
</tr>
<tr>
<td>24 weeks</td>
<td>12</td>
<td>8.0 ± 0.9</td>
<td>22</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>44</td>
<td>8.0 ± 1.1</td>
<td>5</td>
</tr>
<tr>
<td>6 weeks</td>
<td>32</td>
<td>8.1 ± 0.9</td>
<td>13</td>
</tr>
<tr>
<td>12 weeks</td>
<td>26</td>
<td>8.4 ± 1.1</td>
<td>17</td>
</tr>
<tr>
<td>24 weeks</td>
<td>19</td>
<td>7.4 ± 0.8</td>
<td>15</td>
</tr>
<tr>
<td><strong>LNF-II, µg/g in Feces</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>41</td>
<td>690 ± 80</td>
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</tr>
<tr>
<td>6 weeks</td>
<td>33</td>
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<td>12 weeks</td>
<td>21</td>
<td>840 ± 100</td>
<td>17</td>
</tr>
<tr>
<td>24 weeks</td>
<td>9</td>
<td>730 ± 190</td>
<td>21</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>38</td>
<td>720 ± 80</td>
<td>5</td>
</tr>
<tr>
<td>6 weeks</td>
<td>29</td>
<td>730 ± 90</td>
<td>11</td>
</tr>
<tr>
<td>12 weeks</td>
<td>23</td>
<td>780 ± 110</td>
<td>15</td>
</tr>
<tr>
<td>24 weeks</td>
<td>16</td>
<td>770 ± 130</td>
<td>14</td>
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</tbody>
</table>

*p-Values determined by Mann-Whitney U-test; significant differences in bold.
from 0.078 to 0.043). GI health outcomes for any of the time periods do not show a significant relationship with LNF-II levels in the 2-week fecal samples. The category of ear infections is not included in the table because they occurred in very few infants (only 5 by 24 weeks), and none of the relationships were significant at $\alpha = 0.05$.

The results of the logistic regression analyses are presented in Table 2. An analog of Cook’s distance (values $>1.0$) was used to identify outliers for each model. Controlling for breastfeeding behavior, respiratory problems by 6 weeks are inversely related to both milk ($p = 0.010$) and fecal ($p = 0.003$) levels of LNF-II at 2 weeks postpartum. For respiratory problems occurring by 12 weeks, milk LNF-II level is a significant predictor ($p = 0.038$) if a single outlier is omitted from the analysis. Milk LNF-II level at 2 weeks is a significant predictor of GI problems by 6 weeks ($p = 0.004$) and 12 weeks ($p = 0.045$), if a single outlier is deleted. When the outlier was included in the each of these last three analyses, the results were non-significant (all $p > 0.4$). Fecal LNF-II at 2 weeks is not a significant predictor of GI problems at any time period.

**DISCUSSION**

Levels of human milk oligosaccharides consumed by infants at 2 weeks of age, whether measured as milk or fecal concentrations of LNF-II, were inversely related with occurrences of respiratory illness at 6 weeks of age. Similarly, higher milk LNF-II levels at 2 weeks predict lower risk of infant respiratory illness at 12 weeks of age and GI illness at 6 and 12 weeks of age. Consequently, the results support the premise that there is a significant relationship between LNF-II levels in mothers’ milk and lower rates of infant respiratory and GI illness. The LNF-II level was used as a surrogate measure for levels of human milk oligosaccharides, although a specific effect of LNF-II in Milk

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>24 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>45</td>
<td>42</td>
<td>33</td>
</tr>
<tr>
<td>LNF-II in Milk</td>
<td>-0.397</td>
<td>-0.226</td>
<td>-0.126</td>
</tr>
<tr>
<td>B</td>
<td>0.197</td>
<td>0.129</td>
<td>0.120</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odds ratio</td>
<td>0.672 (0.457, 0.989)</td>
<td>0.797 (0.620, 1.026)</td>
<td>0.882 (0.697, 1.115)</td>
</tr>
<tr>
<td>Chi-square</td>
<td>6.56</td>
<td>4.30</td>
<td>1.15</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.01</td>
<td>0.04</td>
<td>0.28</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Gastrointestinal</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>24 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>44</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td>LNF-II in Feces</td>
<td>-0.413</td>
<td>-0.215</td>
<td>0.047</td>
</tr>
<tr>
<td>B</td>
<td>0.176</td>
<td>0.125</td>
<td>0.062</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odds ratio</td>
<td>0.662 (0.468, 0.935)</td>
<td>0.806 (0.632, 1.029)</td>
<td>1.048 (0.928, 1.182)</td>
</tr>
<tr>
<td>Chi-square</td>
<td>8.41</td>
<td>4.02</td>
<td>0.68</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.004</td>
<td>0.04</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*Health outcomes were coded 0 = no problems, 1 = one or more problems. Italicized values are results from analysis after an outlier was removed. Results for these models were not significant (all $p > 0.4$) when all cases were analyzed.

**Chi-square with 1 d.f. testing whether adding 2-week LNF-II value significantly improves prediction beyond a model containing percent days in that interval that infant was breastfed; significant differences are in bold.

***Odds ratios are the change in odds associated with 1 µL LNF-II change/mL milk or 100 µg LNF-II change/g feces.
LNF-II per se cannot be ruled out by this design. The authors propose several mechanisms whereby the human milk oligosaccharides may protect the infant. Because the oligosaccharides are essentially indigestible, they become more concentrated in the gut as the other milk components are digested and absorbed. Thus, their activities as prebiotics and receptor analogs are enhanced in the region in which they are most needed for protection. The superiority of LNF-II measurements in milk to predict risk for health outcomes by the measured levels over the same measurement in feces could result from the technical advantages inherent to milk collection and measurement.

Prebiotics are indigestible dietary carbohydrates that have positive health outcomes through promoting colonization of the gut with beneficial bacteria. For example, human milk oligosaccharides can selectively promote colonization by *Bifidobacterium bifidum*, a bacterial symbiont that resides in the infant colon and is especially prevalent in the guts of breastfed infants. Bifidobacteria produce lactic acid as a product of lactose fermentation, increasing the acidity of the intestinal/colonic environment and affecting gut-associated immune cells, thereby inhibiting infection by pathogenic organisms. Bacterial fermentation also produces short-chain fatty acids, which can be absorbed and used as an energy source, but also have local effects, facilitating the resorption of water, contributing to mucosal integrity, and stimulating the maturation of colonic mucosa. Unused human milk oligosaccharides are excreted through the infant’s feces.

In the intestine, oligosaccharides can act as receptor analogues for cell-surface sites, inhibiting bacterial adhesion to epithelial surfaces. Inhibition of binding by enteric pathogens to their receptors inhibits an obligatory initial stage of the infective process. A mechanism whereby the human milk oligosaccharides might also inhibit respiratory disease is not known. Perhaps there is an indirect effect, possibly through its prebiotic effect, in which healthy colonization of the gut results in immunomodulation that causes other mucosal tissues to become more resistant to pathogens. Other mechanisms could involve intestinal absorption of human milk oligosaccharides into the bloodstream, the source of oligosaccharides in the urine of breastfed infants. If these oligosaccharides migrate to the respiratory airway, they might protect against pathogens. Reflux of milk also could coat mucosal surfaces of the nose, throat, and Eustachian tube with oligosaccharides.

The concentrations of LNF-II in the milks consumed by the infants with no symptoms, about 8 μg/mL, are consistent with earlier observations in a population from Mexico. In contrast, the levels in the milk consumed by infants who exhibit respiratory symptoms early in life seem to include a small subset of milks in which LNF-II is expressed in much lower amounts. By 24 weeks, when the majority of the cohort has experienced symptoms of respiratory infection, the difference in LNF-II expression is no longer apparent between these two groups. These results are consistent with genetically distinct subpopulations, with the dominant trait being expression of full amounts of LNF-II in milk. The recipients of this milk with greater LNF-II may be more protected early in life from the respiratory pathogen that was endemic to this population during this study. By 24 weeks, other factors, in conjunction with multiple exposures, may render both groups eventually susceptible.

**Limitations**

The cohort lacks genetic diversity because of the population homogeneity in the region in which data were collected, raising the possibility that the specific observations may be unique to this population. However, the observations would still support the hypothesis that human milk oligosaccharides could protect against some respiratory disease. Another limitation is the dependence on maternal recall to calculate disease incidence, rather than on independent observations of the infants while they have active symptoms. Also it would be useful to know the severity of symptoms, and which pathogens underlie the symptoms, so that a mechanism of protection could be investigated. Again, this pilot study provides a rationale for a more comprehensive investigation of this phenomenon. Several limitations are associated with the small sample size of this pilot study, especially the attrition of samples. From the original sample of
73, data from only 49 participants were useful for these analyses; most of the losses were in the collection and storage of biological samples. By 24 weeks, the set of complete samples diminished from 49 to 34, and the duration of breastfeeding was lower for mothers who dropped out of the study than for those who remained. Differences in demographic characteristics between the mothers who completed the study and those who dropped out could bias the results if these characteristics influence health outcomes. Perhaps the greatest limitation caused by small sample size is the instability of the data. Results of the logistic regression analysis were greatly influenced by two unambiguous outliers; therefore, caution is advised given that it is unknown whether such values would be either unusual or influential in a larger sample. The wide confidence intervals of the logistic regression coefficients again suggest the utility of a larger follow-up study. Other factors that could potentially influence these results should be measured, such as amount of supplementary feeding and exposure to illness (number of siblings).

CONCLUSION

The results of this study support a relationship between ingestion of human milk oligosaccharides by sustained breastfeeding and putative protection of the infant from enteric and respiratory disease. Confirmation of these findings with a larger sample is warranted. These preliminary results further strengthen endorsement of breastfeeding by individual health care professionals, thereby increasing the acceptance and practice of sustained breastfeeding by mothers and their families.

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