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A Double-Blind, Placebo-Controlled, Pilot Study of Bovine Lactoferrin Supplementation in Bottle-fed Infants

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ABSTRACT

Background: Lactoferrin has an array of biological activities that include growth, immune modulation, and antimicrobial effects. The aim of this randomized, placebo-controlled, double-blind study was to examine the impact of bovine lactoferrin supplementation in infants.

Patients and Methods: Healthy, formula-fed infants, ≥34 weeks' gestation and ≤4 weeks of age, enrolled in a pediatric clinic. Infants received either formula supplemented with lactoferrin (850 mg/L) or commercial cow milk–based formula (102 mg/L) for 12 months. Growth parameters and information on gastrointestinal, respiratory, and colic illnesses were collected for the infants' first year. Antibodies to immunizations and hematologic parameters were measured at 9 and 12 months.

Results: The lactoferrin-enhanced formula was well tolerated. There were significantly fewer lower respiratory tract illnesses, primarily wheezing, in the 26 lactoferrin-fed (0.15 episodes/y) compared with the 26 regular formula-fed (0.5 episodes/y) infants ($P < 0.05$). Significantly higher hematocrit levels at 9 months (37.1% vs 35.4%; $P < 0.05$) occurred in the lactoferrin-supplemented group compared with the control formula group.

Conclusions: Lactoferrin supplementation was associated with potentially beneficial outcomes such as significantly fewer lower respiratory tract illnesses and higher hematocrits. Larger, more focused studies in infants are warranted. ***JPGN 44:245–251, 2007.*** **Key Words:** Bovine lactoferrin—Infant formula—Infants. © 2007 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

INTRODUCTION

Lactoferrin is a bilobed, iron-binding glycoprotein found not only in colostrum or milk but also in many other exocrine fluids that bathe mucosal surfaces and in secondary granules of neutrophils (1–3). Each lobe can bind one molecule of iron. Lactoferrin molecules have been demonstrated to be present in an array of mammalian species. Lactoferrin is only scantily represented in commercial cow's milk infant feeding formulas (4). The lactoferrin concentration in bovine milk is ≈20 to 200 mg/L; mature human breast milk contains 1 to 2 g/L (5).

Human and bovine lactoferrin molecules are similar but not identical. They both consist of a single polypeptide chain of ≈690 amino acids with a sequence similarity of ≈69% (6). This high level of sequence homology has been shown to correlate with highly similar 3-dimensional structures between human and bovine lactoferrin (7). Furthermore, it has been shown that intact breast milk–derived lactoferrin can be found in the stool of breast-fed infants (8). Bovine lactoferrin also has been reported to survive stomach passage after oral administration (9). Nevertheless, partial digestion of lactoferrin within the gastrointestinal tract will lead to the release of highly active cationic peptides such as lactoferricin, exhibiting enhanced activity compared with the native molecule (10). It should be noted that bovine lactoferricin has been reported to exhibit higher antimicrobial activity compared with the human analog (11).

In animal and in vitro studies, lactoferrin exhibits an array of biological activities, including enhanced growth,

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antibacterial and antiviral activity, binding of iron and various other metal ions, antioxidant activity, and immunomodulation (3,12–15). However, information on the effects of bovine lactoferrin in human infants is limited. This pilot study assessed the impact of long-term feeding of bovine lactoferrin–enhanced formula on growth, hematologic and immune parameters, and the evaluation of common childhood illnesses in term or near-term healthy infants.

PATIENTS AND METHODS

Population

Study subjects were recruited from the normal newborn nursery and outpatient clinic at the University of Maryland Medical System, Baltimore. Healthy infants were eligible to be in the study if they were 0 to 4 weeks of age, were born at ≥34 weeks of gestation and ≥2000 g, and were strictly bottle fed and if the family planned on staying in the area for at least 1 y and parents/guardians had access to a telephone. Exclusion criteria included intolerance to cow's milk formula, major congenital anomalies, known immunodeficiency, an HIV-infected mother, or parental inability to follow the protocol. Infants were to be followed up until 1 y of age.

The plan was to enroll as many infants as possible over 1 y and study them through their first year of life. This was a practical approach because the bovine lactoferrin study formula was manufactured as a 1-time bulk production and had a projected stability of 2 y. Approximately 200 to 300 newborns are enrolled in our clinic per year. Therefore, depending on parental acceptance rates and breast-feeding prevalence, enrollment was projected to be 50 to 100 infants.

Human subject approval for this study was obtained from the University of Maryland, Baltimore, Institutional Review Board. Informed written consent was obtained from parents/legal guardians.

Formula Randomization

Infants were randomized in a double-blind fashion to receive powdered Similac with iron formula (3 mg/L elemental iron) either with or without added bovine lactoferrin (DMV International, Delhi, NY). The former contained 850 mg/L bovine lactoferrin (treatment group); the latter contained 102 mg/L bovine lactoferrin (control group) (internal communication, DMV International of GRAS notification on Bovine Lactoferrin, FDA GRAS notice no. GRN 000077). The iron content of the lactoferrin additive was 120 μg/g powder added (previous data on file at DMV International). Parents were provided formula at ≈1- to 2-month intervals throughout their infant's first year of life. A record of formula dispensation was kept to monitor compliance.

Baseline Demographic Information

On enrollment, birth information such as gender, gestational age, birth weight, and race was recorded. Additional enrollment information included type of insurance, family history of asthma in a parent or sibling, and proportion of primary caretakers who received more than a high school education. The following household demographic information was obtained at enrollment and repeated at 6 months of age: paternal presence, whether the infant lives with biological parent, type of dwelling, number of sleeping rooms and people who sleep in the home, number of cigarettes smoked by the primary caretaker per day, number of smokers in the home, number of pet dogs and cats, type of heating fuel, number of school-age siblings, and use of day care for the infant.

Clinical Evaluations

Weight, length, and head circumference were measured at birth and during routine well-child visits at ≈1, 2, 4, 6, 9, and 12 months of age. Telephone or face-to-face contact was made with each family about every 1 to 2 weeks to inquire about any recent illnesses of the infant. Specific data were collected about illnesses, including diarrheal, upper respiratory (URI), acute otitis media (AOM), lower respiratory tract (LRTI), and other illnesses. Diagnoses were assigned by the pediatric nurse practitioner (G.E.C.) based on parental recollection of infant symptoms and clinic and hospital records. Illnesses were defined as follows:

Diarrhea: ≥3 looser-than-normal stools in 1 d.
URI: rhinorrhea, cough, sore throat, or conjunctivitis for 2 consecutive days increased from baseline.
AOM: clinician confirmed.
LRTI: clinician-confirmed alteration in respiratory status as manifested by chest retractions, tachypnea, rales, wheezing, barky cough or stridor, or an abnormal chest radiograph.

Endpoints of illnesses were defined as the first of 2 consecutive days without symptoms for diarrhea or a URI and as the date of a normal examination or the first of 2 consecutive days with normalized symptoms for AOM or LRTI episodes. Serious adverse events such as hospitalizations were recorded.

COLIC

For infants under 3 months of age, colic was defined as poorly consolable crying >3 h/d for ≥3 d/week (16). On enrollment, parents were given an information sheet on colic. At each telephone or clinic encounter, parents were queried about any colic symptoms experienced by infants younger than 3 months of age. The endpoint of colic was defined as the first day of a symptom-free 2-week period.

Laboratory Evaluations

Blood samples were obtained at ≈9 and 12 months (±2 months) of age. Hematocrit, hemoglobin, and mean corpuscular volume (MCV) tests were performed at LabCorp (Herndon, VA) with a Beckman Coulter (Fullerton, CA) automated cell counter. Antibodies to diphtheria, tetanus, and *Haemophilus influenzae b* were measured on 9-month sera. Antibodies to hepatitis B surface antigen were measured on the 12-month blood

samples. Commercial enzyme-linked immunosorbent assay kits were used to measure diphtheria antitoxin (catalog no. IVD18, IVD Research Inc, Carlsbad, CA), tetanus antitoxin (catalog no. RP6, IVD Research Inc), and hepatitis B surface antibody (catalog no. P001931, DiaSorin Inc, Stillwater, MN). *H influenzae b* antibody was performed as described by Phipps et al. (17) using Hbo-HA antigen provided by the DMID/NIAID reference laboratory at the University of Alabama at Birmingham per Dr Moon Nahm and reference serum (lot 1983, 70 μg/mL) that was provided by Dr Carl Frasch (CBER-FDA). Both the 9- and 12-month samples were obtained to measure hemoglobin, hematocrit, and MCV.

Data Analyses

As previously mentioned, the sample size for this pilot study was dependent on the number of infants who could be enrolled in the first year after receiving the study formula. Continuous variables were compared between groups using *t* tests. Continuous variables over time such as growth parameters were compared between groups using the hierarchical linear model (18). Data analysis was performed with SAS version 8.2 for Windows.

RESULTS

Of the 79 infants enrolled in the study, 52 completed the full-year study period. Thirteen of the 27 dropouts (48%) received the lactoferrin-enhanced formula. Of the 27 dropouts, 19 withdrew because of parental perception of infant intolerance to the randomized formula (10 received lactoferrin-enhanced formula). Additional reasons for subject disenrollment included withdrawal of consent without further explanation (n = 3) and infant lost to follow-up (n = 5). Disenrollees participated in the study for a median duration of 29 d (range, 1–180 d). Of the 52 infants who finished the study, 26 received the lactoferrin-enhanced formula and 26 received the regular formula. Birthdates did not differ by calendar-year quartiles (i.e., January–March) between infants who did or did not receive lactoferrin-enhanced formula ($P = 0.73$).

Compliance with the formula regimen was noted to be excellent among infants who completed the study. Over the entire year, enough powdered formula was distributed to the parent to result in a mean of 33 oz mixed formula per day for infants in both the treatment and study groups during the study.

Treatment and control groups were similar in terms of perinatal history and demographic characteristics of their families, as shown in Table 1. In addition, there were no statistically significant differences between treatment and control groups in their socioeconomic or exposure characteristics.

No statistically significant differences in growth parameters were noted between the treatment and control groups (Table 2). However, there was a trend toward a greater increase in weight over time for the lactoferrin-enhanced group for the first 6 months ($P = 0.06$) using the hierarchical linear model. This trend disappeared after 6 months of age.

The frequency and duration of common illnesses in these infants are demonstrated in Table 3. There were statistically more LRTIs in the infants fed regular formula

TABLE 1. *Perinatal and demographic characteristics of infants fed lactoferrin-enhanced formula (treatment group, n = 26) and regular formula (control group, n = 26)*

Factors	Study groups at enrolment		Study groups at 6 mo of age	
	Treatment	Control	Treatment	Control
Male, %	50	46		
Gestational age, wk	39.1	38.3		
≤37-wk gestation	23	31		
Birth weight, kg	3.11	3.19		
Black, %	85	96		
Has Medicaid insurance, %	88	92		
Family history of asthma in parent or sibling, %	36	44		
Primary caregiver educated more than high school, %	31	38		
Father lives in home, %	38	38	42	38
Lives in biological home, %	77	88	81	92
Sleeping rooms in home (mean), no.	2.8	2.8	2.8	2.9
People sleeping in home (mean), no.	4.8	4.7	4.5	4.6
Primary caregivers who smoke, %	31	15	38	19
Smokers/home (mean), %	0.8	0.5	0.8	0.5
Homes with dog or cat, %	45*	23*	42	23
Homes with gas or electric heat, %	81	84	92	84
School-age siblings (mean), no.	3	3	2.9	2.7
Study children in daycare, %	4	0	27	19

There were no significant differences between groups for any of the categories.
* $P = 0.145$.

TABLE 2. *Mean (SD) growth parameters of the 52 study infants during their first year of life*

	Weight, kg		Length, cm		Head circumference, cm	
Age	Treatment	Control	Treatment	Control	Treatment	Control
Birth	3.11 (0.48)	3.19 (0.6)	49.8 (2.7)	49.9 (2.7)	33.6 (1.6)	33.2 (1.5)
1 mo	3.65 (0.58)	3.49 (0.61)	51.5 (2.6)	50.5 (2.7)	35.4 (1.4)	35.0 (2.1)
2 mo	5.47 (0.77)	5.28 (0.77)	57.8 (2.4)	56.9 (7.9)	39.1 (1.4)	39.3 (1.5)
4 mo	7.20 (0.96)	6.86 (1.0)	64.6 (2.9)	64.5 (2.4)	42.1 (1.4)	42.0 (1.7)
6 mo	8.24 (1.10)	7.95 (1.02)	69.0 (2.6)	66.7 (2.7)	43.7 (1.0)	43.4 (1.8)
9 mo	8.98 (1.05)	9.36 (1.35)	72.1 (3.2)	73.0 (2.7)	45.0 (1.3)	45.2 (2.1)
12 mo	10.28 (1.33)	10.24 (1.81)	76.4 (3.4)	76.6 (2.9)	46.3 (1.5)	46.7 (2.0)

With the hierarchical linear model, there was a trend toward increased weight over time for the lactoferrin-enhanced group compared to the control group for the first 6 months (noted by dark outline) ($P = 0.06$).

(0.5 episodes per child-year) compared with those fed bovine lactoferrin–enhanced formula (0.15 episodes per child-year). The majority (16 of 17) of these LRTIs were associated with wheezing.

No significant differences were seen in the frequency of diarrhea, URI, AOM, or other illnesses between the treatment groups (Table 3). These other illnesses included skin and mucosal conditions, isolated fevers, teething, a urinary tract infection, and viral meningitis. Additionally, no significant differences in duration of any illnesses were noted. Eight of the study infants, 4 in each randomization group, were hospitalized. All of these hospitalized infants recovered without any serious sequelae. Very few of these infants had colic: 2 infants

TABLE 3. *Mean frequency and duration of illnesses per infant during the 1-year study period (range)*

	Treatment		Control	
Type of illness	Episodes/infant-year	Duration, d	Episodes/infant-year	Duration, d
Diarrhea/gastroenteritis	1.31 (0–5)	10.6 (1–28)	1.35 (0–4)	11.1 (1–40)
URI	3.92 (2–10)	10.65 (2–51)	4.00 (2–9)	11.5 (2–40)
AOM	0.92 (0–3)	12.7 (3–36)	0.92 (0–3)	14.0 (3–35)
LRTI	0.15* (0–1)	10.5 (4–15)	0.5* (0–2)	13.5 (5–28)
Other	0.73 (0–2)	15.8 (1–50)	0.69 (0–5)	15.1 (1–101)
Colic	1 (0–1)	69.069	2 (0–2)	29.0 (9–49)

* $P < 0.05$ between treatment and control groups for LRTIs.

TABLE 4. *Mean antibody levels to routine childhood immunizations and hematologic indexes in the 52 study Infants (range)*

	Data at 9 mo		Data at 12 mo	
Laboratory test	Treatment	Control	Treatment	Control
Hemoglobin, g/dL*	12.1 (8.9–14.2) (n = 17)	11.8 (9.7–15.2) (n = 21)	12.0 (10.2–13.8) (n = 25)	12.1 (10.6–16.6) (n = 23)
Hematocrit, %*	37.1† (33.9–40.9) (n = 18)	35.4 (32.1–41.6) (n = 22)	36.5 (31.8–41.6) (n = 23)	36.5 (32.1–48.1) (n = 23)
MCV, fL*	79.8 (67–88) (n = 16)	77.9 (65–86) (n = 20)	79.7‡ (67–87) (n = 26)	77.1 (67–86) (n = 22)
Diphtheria, IU/Ml§	2.2 (0.1–11.2) (n = 26)	4.2 (0–18.8) (n = 26)		
Tetanus, IU/mL§	3.6 (0.6–6.6) (n = 26)	3.4 (0.1–7.1) (n = 26)		
H influenzae b, μg/mL§	15.6 (0.2–80) (n = 26)	18.1 (0.2–64) (n = 26)		
Hepatitis B, 10 mIU/mL‖			1.4 (0–2.6) (n = 26)	1.5 (0–2.6) (n = 26)

* The first hematologic tests were obtained at 9.4 months (SD, 1.1 months) of age for the treatment group and 9.4 months (SD, 0.6 months) of age for the control group. The second hematologic tests were obtained at 12.8 months (SD, 1.1 months) of age for the treatment group and at 12.7 months (SD, 1.1 months) of age for the control group.

† $P < 0.05$ between the treatment and control groups.

‡ $P = 0.06$ between the treatment and control groups.

§ The mean time between the third DT and Hib vaccine and blood draw was 3.6 months (SD, 1.5 months) for the treatment group and 3.1 months (SD, 1.2 months) for the control group.

‖ The mean time between the third hepatitis B vaccine and blood draw was 3.3 months (SD, 1.6 month) for the treatment group and 3.4 months (SD, 1.2 months) for the control group.

in the control group and 1 in the treatment group ($P = \text{NS}$).

The results of the laboratory studies performed on the infants are shown in Table 4. No significant differences in antibody levels to diphtheria, tetanus, *H influenzae b*, or hepatitis B were detected between the study groups. All specific antibody concentrations were above protective levels. Infants fed the bovine lactoferrin–enhanced formula had significantly higher hematocrit levels at 9 months of age. At 9 months of age, the hemoglobin and MCV values were numerically but not significantly higher in the lactoferrin-enhanced group in this small sample of infants. No significant differences in hematocrit, hemoglobin, or MCV levels were detected at 12 months of age between the treatment groups.

DISCUSSION

Despite the small number of infants enrolled in this pilot study, several potentially beneficial observations were made that are related to bovine lactoferrin enhancement of infant formula. There was a trend toward increased weight gain during the first 6 months of age, significantly fewer LRTIs, and significantly greater hematocrit levels at 9 months of age in infants fed lactoferrin-enhanced formula.

There appeared to be no formula tolerance issues because there were roughly equal numbers of dropouts in both treatment arms. There were also equal numbers of serious adverse events (hospitalizations) of infants in each of the treatment groups, supporting the safety of this nutritional supplement.

The trend toward increased weight gain in the treatment group was slight but consistent throughout the first 6 months of life. This result is in line with a previous study by Hernell and Lönnerdal (14) in which an enhanced weight gain in healthy term infants fed a bovine lactoferrin–supplemented formula was observed. In other studies, bovine lactoferrin has been demonstrated to exhibit growth-promoting effects on the rat intestinal mucosa in vivo and human enterocytes in vitro and to have anabolic effects on liver protein synthesis of formula-fed newborn pigs (19–21). Alternatively, the lactoferrin protein added during the manufacturer's blending process slightly increased the total protein content of the treatment formula by ≈5%, which may have influenced growth or weight gain.

In the present study, the trend toward greater weight gain was not continued after 6 months of age. One explanation is that alternative nutritional sources such as solid foods and juices become increasingly prominent dietary components in infants after 4 months of age. Further studies are indicated to address the weight gain issue, and these studies should include methods to evaluate body fat composition.

The finding of statistically fewer LRTIs, particularly wheezing illnesses, in the bovine lactoferrin–supplemented group is noteworthy. With both bronchiolitis and reactive airway disease, wheezing is associated with an enhanced inflammatory response by the host (22). Lactoferrin has been described as having immunomodulatory effects in animal studies. These effects include the induction of interleukins-4 and -10, which are anti-inflammatory cytokines, and the reduction of tumor necrosis factor-α and interleukin-1β, which are proinflammatory cytokines (1,23–25). In addition, downregulation of the nuclear factor-κB pathway has been observed that could result in decreased cellular inflammatory responses (23).

The frequency of other common childhood illnesses was not observed to be different between study groups. Although lactoferrin has been described to have direct antimicrobial effects on bacteria, viruses, and fungi (3), little absorption of exogenous bovine lactoferrin is expected to occur from the gut. Therefore, the finding of reduced episodes of LRTI may be a spurious result of examining multiple outcomes. Larger studies addressing this issue are needed because reducing wheezing episodes would be a significant benefit in infancy.

Although there were fewer episodes of colic in the treatment group compared with the control group of infants, there were not enough episodes to determine the effects of bovine lactoferrin supplementation on this common infantile malady. The pathogenesis of colic is poorly understood (16). Lactoferrin has been shown to inhibit the growth of pathogenic bacteria and to stimulate the growth of normal intestinal bacterial flora (1). Several reports relate an anti-inflammatory role for lactoferrin in various gastrointestinal disorders, such as inflammatory bowel disease and *Helicobacter*-induced gastritis (24,25). Larger studies are indicated to examine the effect of bovine lactoferrin supplementation on the frequency and severity of colic.

No statistically significant differences in antibody levels were noted between study groups. Lactoferrin has been shown to inhibit antibody synthesis in vitro (26). However, oral lactoferrin supplementation restored humoral responses in immunocompromised mice in vivo (27). Further studies are needed to explore the immunological effects of lactoferrin on immunoglobulin production by B cells. Certainly, the antibody levels in general were observed to be well above putative protective levels in both groups.

The finding of significantly increased hematocrit levels at 9 months of age in the lactoferrin-enhanced fed compared with the control formula-fed infants is noteworthy. Lactoferrins belong to the family of transferrins, which are involved with iron transport (1). Chierici et al. (28) reported that serum ferritin levels were higher in infants fed bovine lactoferrin–supplemented formula. Another study revealed somewhat higher retention of iron in infants fed bovine lactoferrin–supple-

mented formula (29). However, several studies report no enhancement of iron absorption resultant from bovine lactoferrin supplementation (14,30). Furthermore, the bioactivity of lactoferrin may be affected by different processing techniques (31). Larger controlled studies are needed in this area.

Given that most commercial infant formulas are iron fortified, one may not expect large differences in hematologic parameters between infants who are or are not fed lactoferrin-enhanced formulas. In addition, because the contribution of formula to the infant's total diet becomes less prominent as the infant ages, it is not surprising that the hematologic measurements become less different at 12 months of age.

The present study had several strengths and weaknesses. The major strength was the double-blinded, placebo-controlled randomization scheme that resulted in both treatment groups being well matched for perinatal, demographic, socioeconomic, and exposure factors. One coordinator, who was blinded to the study group assignment, followed the infants closely; therefore, the clinical assessments were uniform.

Limitations of this study include the small sample size and the large number of variables studied, thus increasing the likelihood of finding statistically significant outcomes that are not related to lactoferrin enhancement. In addition, illnesses may have been undocumented because of poor parental recall. This limitation was minimized by the close contact established by the coordinator with these study families.

In conclusion, bovine lactoferrin formula supplementation has several apparent benefits in infants, including a trend toward better weight gain up to 6 months of age, decreased LRTIs, and improved hematologic parameters. Lactoferrin-enhanced formula appears to be well tolerated and safe. If the finding of the present study of reduced wheezing episodes is replicated, feeding infants with lactoferrin-enhanced commercial formulas would be beneficial, particularly because bronchiolitis and reactive airway disease are an important morbidity in urban children. We acknowledge that the small sample size used in this study increases the likelihood of type 2 errors, particularly in light of the multitude of variables measured. Larger and more focused studies are indicated to explore the possible benefits of bovine lactoferrin supplementation in infants.

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