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Impact of Lactoferrin Supplementation on Growth and Prevalence of *Giardia* Colonization in Children

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Abstract

We conducted a randomized, double-blind, placebo-controlled trial comparing supplementation with bovine lactoferrin versus placebo for the prevention of diarrhea in children. Comparison of overall diarrhea incidence and prevalence rates found no significant difference between the 2 groups. However, there was a lower prevalence of colonization with *Giardia* species and better growth among children in the lactoferrin group.

Lactoferrin, an iron-binding protein with multiple physiological functions (including antimicrobial, anti-inflammatory, and immunomodulatory functions), is one of the major proteins present in milk [1]. By binding to or degrading specific virulence proteins, lactoferrin decreases the virulence of enteropathogens by decreasing their ability to adhere to or to invade mammalian cells [2–4]. Bovine and human lactoferrin are structurally and biochemically similar and have comparable bioactivity, according to in vitro and animal model assessments [1]. Bovine lactoferrin has been safe in multiple studies of iron metabolism in human infants [5,6]. Although efficacy has not been adequately studied in humans, bovine lactoferrin is readily available and is being used for its putative health benefits. Considering what is known regarding its functions, protection against gastroenteritis is the most likely biologically relevant activity of lactoferrin. We hypothesize that lactoferrin given to previously weaned infants will decrease the prevalence of pathogen colonization and/or diarrheal illness and thereby promote child health. We conducted a community-based study to determine the effect of bovine lactoferrin supplementation on prevention of diarrhea during a 9-month trial involving Peruvian children. This study was intended to determine the safety and effects of lactoferrin supplementation and to collect data for sample size calculations for a larger, prospective study.

Patients and methods

The study was conducted in the District of Independencia in Lima, Peru, and included previously weaned children aged 12–36 months. Children with persistent or chronic diarrhea, severe malnutrition, serious previous infections, HIV infection, underlying chronic illness, personal or family history of allergy to cow's milk or infant formula, eczema, moderate-to-severe allergic rhinitis or asthma, or milk intolerance were excluded. Consecutive patients from

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the outpatient clinic of the community medical center who qualified for the study were enrolled. For each child, informed consent was obtained from both parents. The study was approved by Universidad Peruana Cayetano Heredia's Institutional Review Board (Lima, Peru). Patients were randomly assigned to receive lactoferrin (0.5 g; Tatua Nutritionals) or maltodextrin (0.5 g; Maltrin, Grain Processing Corporation). Community health workers visited the children twice daily, 6 days per week for 9 months to give the supplement. Children were evaluated monthly by a physician at the outpatient clinic. The parents and all study personnel were blinded to the intervention.

Diarrhea was defined as ≥3 loose or watery stools in a 24-h period or ≥1 loose stool containing blood. Colonization was defined as the identification of a pathogen (viral, bacterial, or parasitic) from a child without diarrhea for at least 7 days before and after the sample was collected. Stool samples were collected during diarrheal episodes and monthly to detect asymptomatic colonization. Stool sample analysis included culture for the detection of *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio* species; enzyme immunoassay for rotavirus; microscopic examination and staining for ova, cysts, and parasites; and identification of enteropathogenic *Escherichia coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli*, Shiga toxin–producing *E. coli*, diffusely adherent *E. coli*, and enteroinvasive *E. coli* by multiplex real-time PCR [7].

Data were analyzed using Access 2003 (Microsoft) and SPSS, version 7.5 (SPSS). We used a nonparametric 2-sample test (Kruskal-Wallis test) to compare the differences in baseline characteristics and a Yates-corrected χ^2 test to compare differences in diarrhea incidence and mean diarrhea prevalence between treatment and placebo groups. The number of samples positive for *Giardia* species per child was compared by Wilcoxon-Mann-Whitney *U* test.

Results

Twenty-six children were enrolled in each group. There were potentially important, significant differences in baseline characteristics of weight and height that reflected a trend toward a difference in age at enrollment. Other baseline demographic and clinical characteristics, including baseline risk factors for diarrhea, were similar in both groups. Six children in each group dropped out of the study. There were no serious adverse events related to the intervention.

The overall incidence of diarrhea in the study was 1.3 episodes/child-year. In the lactoferrin and placebo groups, the diarrhea incidence was 1.3 versus 1.2 episodes/child-year, and the mean prevalence of diarrhea was 0.7% versus 0.9% (difference was not statistically significant). The mean (±SD) duration of diarrhea episodes was not significantly different in the lactoferrin and placebo groups (1.9 ± 1.6 vs. 2.6 ± 1.7 days). Stool samples were collected during 20 of the 39 diarrheal episodes that occurred during the study period. Of pathogens isolated in the lactoferrin group, there were 2 enterotoxigenic *E. coli*, 1 enteropathogenic *E. coli*, 1 enteroaggregative *E. coli*, 1 *Giardia* species, 1 *Cryptosporidium* species, and 1 rotavirus; in the placebo group, there were 3 enterotoxigenic *E. coli*, 3 enteropathogenic *E. coli*, 2 enteroaggregative *E. coli*, and 2 *Giardia* species.

Stool samples were collected from children without diarrhea (a mean of 8 samples were collected from each child during the 9-month study period). Colonization rates with common pathogens were similar in both groups, with the exception of a lower frequency of *Giardia*-positive samples in the lactoferrin group. Of the 146 samples collected in the lactoferrin group, 14 (9.6%) were positive for *Giardia* species; of the 174 samples collected in the placebo group, 30 (17.2%) were positive ($P = .05$) (table 1). There were 12 children in the lactoferrin group with *Giardia* species detected in at least 1 stool sample; of these 12 children, 11 were asymptomatic. Most of these children (10 of 12 patients) had *Giardia* species found in a single stool sample. In contrast, most of the *Giardia*-positive patients in the control group (10 of 12

patients) had the organism detected in multiple stool samples. The mean number of samples positive for *Giardia* species per child (±SD) was lower in the lactoferrin group (1.2 ± 0.6 samples/child) than in the placebo group (3.2 ± 2.2 samples/child) ($P < 01$). Thus, the mean (±SD) duration of *Giardia* carriage was longer in the placebo group than in the lactoferrin group (4.6 ± 3.1 months vs. 1.6 ± 2.2 months; $P < 01$). The duration of *Giardia* carriage was estimated as the interval from the first positive to the last positive fecal sample, with those patients who had only 1 *Giardia*-positive sample scored as having 1-month duration of carriage.

Anthropometric data (i.e., height-for-age and weight-for-height *z* scores), calculated according to the World Health Organization Child Growth Standards for 2006, were analyzed using a 2-way nested analysis of variance (by group, by month of follow-up, by the interaction of group and month of follow-up, and by child within group). Height-for-age scores were significantly greater in the lactoferrin group when analyzed by group (P=.03) and by the interaction of group and month (P=.03). There was no difference in weight-for-age scores.

Discussion

In this pilot study, several important observations were made related to bovine lactoferrin supplementation. Lactoferrin supplementation was safe. As in other studies, there were no adverse events related to bovine lactoferrin [5,6]. Worldwide, many children in this age group (> 12 months) are given cow's milk as part of their normal diet, so the bovine lactoferrin intervention should not put the children at additional risk. Cow's milk protein allergy is related primarily to the reactivities of α-lactalbumin, β-lactoglobulin, and casein. However, it is important to continue to assess the safety of the intervention in larger studies.

There was a lower mean number of *Giardia*-positive samples per child and a better height-for-age *z* score in the lactoferrin group. The significance of "colonization" or "asymptomatic carriage" in this setting is uncertain because *Giardia* species characteristically cause intermittent or chronic diarrhea. Because stools were sampled only monthly for asymptomatic children, this could have biased the results towards detecting chronic infections, such as those caused by *Giardia* species. Another limitation is the inherent complexity of the study, which is caused, in part, by the inability to control for many factors in an environment where exposure to multiple pathogens is common. *Giardia* species, like other intestinal parasites, have been linked to outcomes associated with poor nutrition, including protein-energy malnutrition and growth deficits in children and reductions in intellectual function [8]. Children who received lactoferrin supplementation had better height-for-age *z* scores by the end of study than did those who did not receive supplementation, although their *z* scores did not differ from those in the control group at baseline. Whether the decrease in the prevalence of *Giardia* colonization is related to improved height-for-age *z* scores will need to be confirmed in a larger study. Of interest, lactoferrin and its derived N-terminal peptides have giardicidal activity and cause ultrastructural changes in *Giardia* trophozoites' plasmalemma, endomembranes, and cytoskeleton [9,10]. The fact that the prevalence of infection and colonization with *Giardia* species decreased in the lactoferrin group suggests that lactoferrin might have therapeutic value in patients infected with *Giardia* species. The importance of this observation is underscored by the huge number of humans infected with this pathogen worldwide.

The overall diarrhea incidence in this study was low; this was probably related to the age of the patients (mean age at enrollment, 26 months) or to an indirect beneficial effect of twice-daily home visits by health workers. Whether bovine lactoferrin supplementation can prevent a significant portion of all diarrheal disease remains to be determined in a larger study. Of interest, a recent study showed that a preparation containing both recombinant human lactoferrin and lysozyme in an oral rehydration solution reduced the duration and recurrence of diarrhea [11]. The morbidity and mortality that result from diarrheal disease after weaning

represent child-health issues of global importance. Prolonging exposure to milk protective factors, such as lactoferrin, may be a cost-effective means of decreasing diarrheal disease burden and its resulting adverse effects on growth and intellectual function.

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Table 1

Enteric pathogens in stool samples obtained from children without diarrhea, by treatment group.

Pathogen	No. (%) of samples	
	Lactoferrin supplementation group (*n* =146)	Placebo group (*n* =174)
Bacteria		
Shigella flexneri	1 (0.7)	2 (1.1)
Salmonella species	0 (0)	2 (1.1)
Campylobacter species	0 (0)	1 (0.6)
Enteroaggregative *Escherichia coli*	16 (11.0)	17 (9.8)
Enteropathogenic *E. coli*	16 (11.0)	16 (9.2)
Diffusely adherent *E. coli*	10 (6.8)	11 (6.3)
Enterotoxigenic *E. coli*	3 (2.1)	6 (3.4)
Shiga toxin–producing *E. coli*	0 (0)	1 (0.6)
Diarrhea-associated parasites		
Giardia lamblia[a]	14 (9.6)	30 (17.2)
Cryptosporidium species	2 (1.4)	0 (0)
Cyclospora cayetanensis	0 (0)	1 (0.6)
Balantidium coli	1 (0.7)	0 (0)
Other parasites[b]	32 (21.9)	37 (21.3)

NOTE. There were multiple mixed infections; therefore, the total number of samples is greater than the specified *n* value.

[a] $P<.05$.

[b] Other parasite included *Blastocystis hominis*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Isospora belli*, *Chilomastix mesnili*, *Endolimax nana*, and *Enterobius vermicularis.*