Oligofructose-supplemented infant cereal: 2 randomized, blinded, community-based trials in Peruvian infants[1–3]

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ABSTRACT

Background: Prebiotics are nondigestible food ingredients that stimulate the growth of *Bifidobacterium* and other bacteria in the gastrointestinal tract. Improved gastrointestinal and other health effects have been attributed to them.

Objective: The objective of this study was to evaluate the effects of dietary supplementation with the prebiotic oligofructose with and without zinc on the prevalence of diarrhea in a community with a high burden of gastrointestinal and other infections.

Design: Two consecutive randomized, blinded, controlled clinical trials were performed in a shantytown community near Lima, Peru. The first trial compared an infant cereal supplemented with oligofructose (0.55 g/15 g cereal) with nonsupplemented cereal. During the second trial, zinc (1 mg/15 g cereal) was added to both oligofructose-supplemented and control cereals.

Results: We enrolled 282 infants in the first trial and 349 in the second. In the first trial, mean (± SD) days of diarrhea were 10.3 ± 9.6 in the nonsupplemented cereal group and 9.8 ± 11.0 in the prebiotic-supplemented cereal group ($P = 0.66$). In the second trial, mean days of diarrhea were 10.3 ± 8.9 in the group consuming cereal fortified only with zinc and 9.5 ± 8.9 in the group consuming cereal containing both zinc and prebiotics ($P = 0.35$). Postimmunization titers of antibody to *Haemophilus influenzae* type B were similar in all groups, as were gains in height, visits to clinic, hospitalizations, and use of antibiotics.

Conclusions: Cereal supplemented with prebiotics was not associated with any change in diarrhea prevalence, use of health care resources, or response to *H. influenzae* type B immunization. Infants and young children who continue to breast-feed may not benefit from prebiotic supplementation. *Am J Clin Nutr* 2003;77:937–42.

KEY WORDS Prebiotics, oligofructose, randomized trial, infants, gastroenteritis, diarrhea, zinc, Peru

INTRODUCTION

Dietary supplementation with probiotics [live microorganisms such as *Lactobacillus* or *Bifidobacterium* that promote health through establishing an improved balance of intestinal microflora (1)] can apparently reduce gastrointestinal infections and other symptoms in infants and young children (2–5). Prebiotics, in contrast, are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or more bacteria in the colon (1), including *Bifidobacterium* (6, 7). Oligofructoses are naturally occurring plant carbohydrates consisting of fructose polymers with a terminal glucose molecule (8). Preliminary studies suggested that dietary supplementation with prebiotics may provide beneficial effects similar to those seen in probiotic supplementation trials (9).

Small children and infants have also been shown to benefit from zinc supplementation. Zinc supplementation reduces the severity of acute diarrhea (10, 11) and the incidence of persistent diarrhea (10) and respiratory infections (12, 13). These effects may be mediated through the immune system–enhancing effect of zinc or through local effects on the gastrointestinal or respiratory mucosa (14). These mechanisms of action are similar to those postulated to explain the effects of prebiotics, and possible interactions between prebiotics and zinc have not been widely studied (15).

To assess the possible roles of prebiotics and zinc in reducing diarrheal disease among children living in a community with a high burden of gastrointestinal illness, we performed 2 consecutive randomized trials of infant cereal in Peruvian infants. The first trial compared prebiotic-supplemented cereal with standard cereal, and the second compared cereal fortified with both prebiotics and zinc with cereal containing zinc alone.

SUBJECTS AND METHODS

Study population

The trials took place in Canto Grande, in the district of San Juan de Lurigancho, a shantytown community near Lima, Peru. Infants aged 6–12 mo were eligible for enrollment, provided that they had no known chronic illnesses or congenital anomalies that would interfere with the study outcomes, their family intended to reside in Canto Grande for the next 6 mo, and they were already consuming solid food (eg, purées, puddings, or cereals). Excluded

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were those with severe acute malnutrition (weight-for-height *z* score < -2), those who were exclusively breast-fed (ie, not yet consuming solid foods), or those who were receiving chronic corticosteroids or antibiotics (as defined by > 6 consecutive weeks of therapy before enrollment). The trial was approved by the Human Research Committee of the Massachusetts General Hospital and the Ethics Committee of the Instituto de Investigación Nutricional. All parents provided written informed consent.

Study endpoints

The primary study outcome was the prevalence of diarrhea (as defined by the proportion of total observed days in which subjects had ≥ 3 loose or watery stools within 24 h). Secondary outcomes included *1*) the number of episodes of diarrhea (defined as an occurrence of diarrhea, as specified above, followed by the passing of 2 consecutive days without diarrhea), *2*) the incidence of severe diarrhea [> 5 loose or watery stools in 24 h plus ≥ 1 incident of vomiting, documented fever (body temperature > 38 °C), office visit for evaluation, or dehydration], *3*) the incidence of persistent diarrhea (≥ 3 loose bowel movements each day for ≥ 14 d, with no span of > 48 h without diarrhea during the 14 d), *4*) nutritional status (change in weight and height *z* scores), and *5*) postimmunization titer of antibody to *Haemophilus influenza* type B (Hib).

Study protocol

Identical protocols were followed for the 2 trials. Trained field workers recruited eligible subjects from the community and referred them to the central study site. Eligibility was then confirmed with a screening history, physical examination, and anthropometric measurements. Body weight was measured with the use of a digital scale with 10-g precision (Electronic Baby Scale Model 7726; Soehnle-Waagen GmbH, Murrhardt, Germany), and length was measured with a rigid length board with movable footpiece with 1-mm precision. The mean of 3 lengths was recorded. Two fieldworkers performed all anthropometric measures. Enrolled infants returned to the study clinic at 2, 4, and 6 mo after enrollment for repeat anthropometric measurements.

The intervention under study was the provision for 6 mo of infant cereal supplemented with oligofructose (0.55 g/15 g cereal; Orafti Group, Tienen, Belgium). Both rice- and oat-based cereals were used (Gerber Products Co, Fremont, MI), with family and child preference determining the allocation of cereal type. During the second trial, zinc (1 mg/15 g cereal) was added to both the oligofructose-supplemented cereal and the nonsupplemented cereal. Because of the logistics of cereal production, 2 consecutive trials were undertaken. Each standard lot of cereal underwent quality assurance procedures at Gerber and then was exported directly to Lima. The cereals were then inventoried and stored in an insect-free, rodent-proof, dehumidified, and temperature-controlled storeroom at the Instituto de Investigación Nutricional. Cereals were supplied in identical-appearing bleached paperboard boxes with a polypropylene overwrap and were made for immediate consumption after being mixed with liquid (milk, breast milk, or water).

Before the start of the trials, personnel not involved in the study generated the list of random assignments (using a permuted block design) and prepared envelopes containing the cereal assignments. On enrollment of an infant, study staff opened the next study envelope to determine which cereal was to be dispensed to that infant. Families were given instructions on safe and hygienic cereal storage.

Study fieldworkers visited the family 5 times/wk during the first 2 mo of the study and 2 times/wk during months 3 through 6. These visits occurred at a time of day when the infant would normally eat the cereal. The fieldworker weighed the box by using a portable digital scale accurate to 1 g (Electronic Scale Magnum; Soehnle-Waagen GmbH) and either prepared the cereal according to the caretaker's instructions or observed the caretaker doing so. The box was weighed after the preparation of each serving of cereal. The prepared cereal mix was also weighed before and after the meal, thereby allowing calculation of the amount of cereal consumed. The mother was asked about any cereal consumed by the infant between visits. The box weight between visits was recorded.

Each infant's caretaker was asked about the frequency and consistency of the infant's stool, the presence of visible blood in any stool, vomiting (defined as forceful regurgitation of food), cough and other signs of upper respiratory infection, appetite, reported fever, and any visit to health facilities. A physical examination [axillary temperature, respiratory rate, and assessment of hydration status (16)] was performed if any new symptoms occurred or if an existing symptom worsened. Baseline socioeconomic and demographic data (17) were obtained, and a brief hygiene survey was completed at the first home visit. Dietary intake was measured with two 24-h food-recall surveys.

Passive data collection occurred through an ongoing survey of patient visits at the study clinic in Canto Grande. The reasons for referral, examination findings, discharge diagnoses, and treatment plans for all clinic referrals were recorded. Treatment of common illnesses followed standardized protocols in keeping with World Health Organization or Peruvian Ministry of Health guidelines.

Laboratory methods

During home visits, fresh stool samples were collected from all episodes of severe diarrhea and dysentery and from 10% of all diarrhea episodes identified within 3 d of onset. Samples were obtained from fresh stool or diaper and immediately plated on appropriate transport media; they were then kept at the central study site in Canto Grande before being transported to the microbiology laboratory at the Instituto de Investigación Nutricional. Blood samples were obtained on enrollment and 5 and 6 mo after enrollment. After the 5-mo blood sample was drawn to establish the preimmunization Hib antibody titer, one dose (0.5 mL) of Hib vaccine (ActHib; Pasteur Merieux Serums and Vaccines, Lyon, France) was given intramuscularly. Postvaccination titers were measured at 6 mo by enzyme-linked immunosorbent assay (18). Hemoglobin and serum zinc were also measured at enrollment and at 6 mo.

Data management and statistical analysis

The first study was powered to detect a 25% reduction in the occurrence of diarrhea, assuming a mean ($\pm$SD) prevalence in the control group of 8.5 ± 6 d of diarrhea/100 d at risk (19 and M Penny, unpublished observations, 1997). With the use of a two-sided α of 0.05, a power of 80%, and a 1-to-1 treatment allocation ratio, 127 infants were required per group (254 total) to observe a reduction in diarrhea prevalence from 8.50 to 6.38 d/100 d at risk.

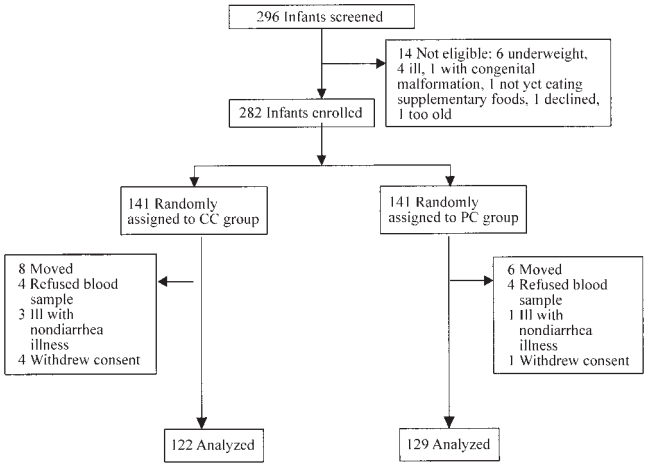

FIGURE 1. Profile of trial 1: control cereal (CC group) compared with prebiotic-supplemented cereal (PC group).

Assuming a 10% loss to follow-up, we required a total of 282 infants for the first trial.

The second trial was powered to detect a further 25% reduction in mean prevalence of diarrhea, ie, a reduction from 6.38 to 4.78 d/100 d at risk. With the use of a two-sided α of 0.05, a power of 80%, and a 1-to-1 treatment allocation ratio, we required 156 infants per group (312 total). Assuming a 10% loss to follow-up, we required a total of 348 infants for the second trial.

Sample sizes for both trials provided adequate power to detect a small increment in mean titers of anti-Hib as follows. The geometric mean titer of anti-Hib in 7–12-mo-old infants in the United States 1 mo after the first dose of Hib conjugate vaccine is 2.91 μg/mL (20). With a sample size of 77 patients per group, we had 95% power to detect a twofold difference in mean antibody titer between an estimated maximal response of 2.91 and 1.46 μg/mL. This sample size was calculated with the use of log-transformed means of 1.07 and 0.38 with an SD of 1.51 (20).

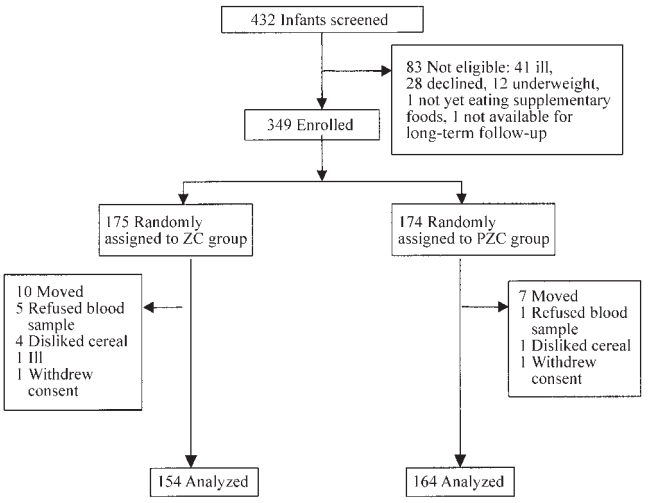

FIGURE 2. Profile of trial 2: zinc-fortified cereal (ZC group) compared with zinc- and prebiotic-supplemented cereal (PZC group).

Therefore, randomly selected subsamples of sera from both trials (n = 154) were tested for Hib antibodies before and after immunization.

Home visit data were collected by fieldworkers on forms that were reviewed by supervising staff for completeness and legibility. Data were then entered in a relational database (Visual FoxPro; Microsoft Inc, Seattle) using range and logic checks. Further data cleaning and analysis were performed with the use of SPSS for Windows software, version 10.0 (SPSS Inc, Chicago). Within each trial, a chi-square or Fisher's exact test was used to compare categorical data between cereal groups. Continuous data were compared with Student's *t* test for normally distributed data or with the Mann-Whitney *U* test for nonparametric data. Continuous nonnormal data (eg, antibody titers) were log transformed before analysis. Post hoc analyses of the main study outcomes were performed in the subgroups of subjects in the highest quartile for cereal intake and subjects in the lowest quartile of weight-for-age *z* score (WAZ) on enrollment.

If > 7 d elapsed between home interviews, data were recorded only for the previous week and the remaining data were coded as missing. To enable us to conduct an intent-to-treat analysis, we used a planned approach to missing information on diarrhea. If the data were missing because the infant was hospitalized for treatment of diarrhea, missing days were coded as days with diarrhea. If data were missing because the infant was hospitalized for another reason, the days were coded as days without diarrhea. For all other missing data, we analyzed the data in 2 ways. First, we conservatively assigned the other treatment group's mean values of diarrhea prevalence for each missing day in an attempt to generate a bias toward the null hypothesis. Second, we assumed that missing days were days without diarrhea.

RESULTS

The patients screened and enrolled in the 2 trials are shown in **Figures 1** and **2**. Enrollment for study 1 lasted from 5 July 1999 to 4 October 1999, and that for study 2 lasted from 3 January 2000 to 29 February 2000. Baseline characterizations of the 4 groups are shown in **Table 1**. Infants in the group receiving cereal with prebiotic alone (PC group) were slightly older than were those receiving the nonsupplemented (control) cereal (CC group) and had lower WAZ and height-for-age *z* scores (HAZ), but they did not have different hematologic and socioeconomic characteristics. Infants receiving the zinc-fortified cereal (ZC group) weighed less than did infants receiving cereal containing both prebiotics and zinc (PZC group), but they did not have significantly different WAZ, HAZ, and socioeconomic characteristics. During the trials, daily cereal intake did not differ among the groups (18.4 ± 10.4 g/d). Breast milk was used on 88.4% of all study days in the CC group, 86.5% in the PC group, 87.6% in the ZC group, and 86.1% in the PZC group.

Subjects in the PC group were followed for an average of 8 d less than were those in the CC group (**Table 2**). There was no significant difference in the number of days with diarrhea between the treatment groups in either study with the use of either imputed or raw data. There were no significant differences between the treatment groups in either study in the numbers of days of severe diarrhea, dysentery, or vomiting or in the frequency with which diarrheal pathogens were isolated.

TABLE 1
Baseline characteristics of infants enrolled in 2 trials

	Trial 1		Trial 2	
	Control cereal (n = 141)	Prebiotic-supplemented cereal (n = 141)	Zinc-fortified cereal (n = 175)	Prebiotic- and zinc-supplemented cereal (n = 174)
Age (mo)	8.5 ± 1.9[1]	9.2 ± 2.0[2]	8.5 ± 1.7	8.7 ± 1.7
Males (%)	45.4	51.8	47.4	52.3
Weight (kg)	8.7 ± 1.1	8.8 ± 1.1	8.5 ± 1.0	8.8 ± 1.1[3]
Weight-for-age (z score)	0.18 ± 0.99	−0.09 ± 0.97[2]	−0.08 ± 0.89	0.05 ± 1.1
Height-for-age (z score)	−0.45 ± 0.87	−0.67 ± 0.78[2]	−0.54 ± 0.75	−0.51 ± 0.78
Hemoglobin (g/dL)	10.2 ± 1.1	10.2 ± 1.1	10.1 ± 1.0	10.0 ± 1.1
Plasma zinc (μg/dL)	73.6 ± 18.8	74.2 ± 18.5	76.6 ± 10.5	78.3 ± 12.3
Maternal age (y)	25.1 ± 6.1	25.3 ± 7.1	25.6 ± 6.5	25.1 ± 5.8
Other children aged <5 y at home	1.5 ± 0.7	1.6 ± 0.7	1.5 ± 0.7	1.4 ± 0.7
Regular job for father (%)	63.3	66.9	63.9	69.7
Dirt floor in home (%)	47.5	42.6	34.9	38.2

[1] $\bar{x}$ ± SD.
[2] Significantly different from control cereal, $P < 0.05$.
[3] Significantly different from zinc-fortified cereal, $P < 0.03$.

Nutritional outcomes of the groups are presented in **Table 3**. In study 1, HAZ decreased in both the PC and CC groups. WAZ also declined over the course of the trial, but significantly ($P < 0.05$) more slowly in the PC group than in the CC group. At the end of the study, PC and CC group subjects had similar WAZ values, because the infants in the CC group were heavier for their age at enrollment than were infants in the PC group. When WAZ at baseline was taken into account to evaluate change in WAZ, the difference in treatment groups disappeared. In study 2, no effect of prebiotic supplementation was observed on changes in either WAZ or HAZ over the course of the trial.

The prevalence of other clinical outcomes did not differ significantly in the 2 trials of prebiotic supplementation (**Table 4**). Among the subgroup of subjects with the highest quartile of cereal intake (> 22.5 g/d), there were no significant differences in days of diarrhea, number of diarrhea episodes, visits to the health center, antibiotic use, or rates of WAZ or HAZ changes between trial 1 subjects and trial 2 subjects (data not shown). In addition, no substantial differences were found in the subgroup of subjects in the lowest quartile of WAZ score on study entry (data not shown).

DISCUSSION

We report the results of 2 large, randomized, blinded trials of prebiotic supplementation in children with detailed morbidity data, in which we were unable to document any effect on the occurrence or severity of diarrhea in free-living Peruvian infants. In addition, there was no effect of supplementation on antibody response to Hib vaccination or on the occurrence of respiratory tract symptoms, antibiotic use, or use of health care resources.

Scientific evaluation of the effects of prebiotics and probiotics on human health has been limited. There is a growing literature supporting a variety of health benefits of probiotics (21, 22), but fewer studies have been performed to evaluate the effects of prebiotics. Further, these studies have been generally been limited to small numbers of healthy adults or have restricted their outcomes to bacterial colonization of the fecal flora. Saavedra et al (9) conducted a randomized, blinded clinical trial of infant cereal supplemented with oligofructose and found that children consuming the supplemented cereal had fewer symptoms associated with loose stools, fewer physician

TABLE 2
Principal gastrointestinal outcomes of 2 trials[1]

	Trial 1		Trial 2	
Variable	Control cereal (n = 141)	Prebiotic-supplemented cereal (n = 141)	Zinc-fortified cereal (n = 175)	Prebiotic- and zinc-supplemented cereal (n = 174)
Time observed (d)	174 ± 25	166 ± 39[2]	164 ± 37	169 ± 26
Time of diarrhea (d)				
Raw data	10.3 ± 9.6	9.8 ± 11.0	10.3 ± 8.9	9.5 ± 8.9
Imputed data	10.7 ± 9.4	10.7 ± 10.7	11.2 ± 8.8	10.1 ± 7.9
Episodes of diarrhea (n)	4.0 ± 2.9	4.0 ± 3.5	3.7 ± 2.6	3.7 ± 2.3
Episodes of severe diarrhea (n)	1.3 ± 1.5	1.1 ± 1.2	1.5 ± 1.4	1.3 ± 1.3
Episodes of dysentery (n)	0.2 ± 0.6	0.1 ± 0.4	0.2 ± 0.4	0.1 ± 0.4
Time with vomiting (d)	5.3 ± 6.2	4.4 ± 4.7	4.7 ± 4.3	4.3 ± 4.1
Time with 0 stools (d)	13.0 ± 14.1	10.5 ± 13.4	8.3 ± 9.6	10.9 ± 11.9[3]
Rotavirus-positive episodes (n)	0.03 ± 0.2	0.02 ± 0.1	0.2 ± 0.4	0.2 ± 0.3

[1] $\bar{x}$ ± SD.
[2] Significantly different from control cereal, $P < 0.05$.
[3] Significantly different from zinc-fortified cereal, $P < 0.05$.

TABLE 3
Nutritional outcomes of 2 trials[1]

Variable	Trial 1		Trial 2	
	Control cereal (n = 120 of 141)	Prebiotic-supplemented cereal (n = 118 of 141)	Zinc-fortified cereal (n = 152 of 175)	Prebiotic- and zinc-supplemented cereal (n = 157 of 174)
Final weight (kg)	9.9 ± 1.2	9.9 ± 1.2	10.1 ± 1.1	10.3 ± 1.3
Final WAZ	−0.47 ± 0.98	−0.65 ± 0.94	−0.26 ± 0.83	−0.18 ± 1.13
WAZ change from baseline to 6 mo	−0.67 ± 0.59	−0.53 ± 0.50[2]	−0.21 ± 0.54	−0.25 ± 0.62
Final height (cm)	75.9 ± 3.1	75.9 ± 3.5	75.9 ± 3.0	76.2 ± 3.0
Final HAZ	−0.69 ± 0.90	−0.97 ± 0.85[2]	−0.69 ± 0.79	−0.71 ± 0.85
HAZ change from baseline to 6 mo	−0.25 ± 0.42	−0.27 ± 0.34	−0.17 ± 0.37	−0.21 ± 0.42
Non-breast milk energy intake ($kcal \cdot kg^{-1} \cdot d^{-1}$)	77.9 ± 30.7	88.9 ± 37.8[2]	83.5 ± 36.7	82.6 ± 34.1
Plasma zinc at 6 months (μg/dL)	66.1 ± 16.6	66.8 ± 17.7	73.4 ± 10.8	75.0 ± 10.0

[1] $\bar{x}$ ± SD. WAZ, weight-for-age z score; HAZ, height-for-age z score.
[2] Significantly different from control cereal, $P < 0.05$.

visits for diarrhea, and fewer missed days of daycare because of diarrhea than did children consuming nonsupplemented cereal, but there were no differences between groups in the incidence of diarrhea or other infections. A recent trial of galacto-oligosaccharides and fructose oligosaccharides added to infant formula showed a bifidogenic effect at a dose of 0.8 g/dL (23). Clinical outcomes were not reported.

Several factors could explain our finding of the lack of treatment effect of prebiotics. First, prebiotics may not provide the same clinical benefits as probiotics, despite their ability to stimulate colonic growth of *Bifidobacterium* and other species. Second, the prebiotic supplement may not have been given in a sufficient dose to influence the colonic bacterial flora. We chose a concentration of 0.55 g/15 g cereal (one serving size) on the basis of assumptions about dietary intake and the bifidogenic effect of the prebiotic. Prebiotic consumption in adults in the range of 3.5–15 g/d is sufficient to have a prebiotic effect on stool microflora (24, 25), whereas doses as high as 30 g/d (roughly 0.5 g/kg) have been associated with gastrointestinal side effects (26). Data from North American infants and children aged 2–18 mo show a median daily intake of infant cereal of 14 g, with 90th percentile intake at 41.1 g (F Colletta, personal communication, 2001). With a prebiotic concentration of 0.55 g/15 g cereal, a 6-kg infant consuming cereal at the 90th percentile would ingest 41 g cereal/d, or 0.25 g prebiotic $\cdot kg^{-1} \cdot d^{-1}$. Because this is approximately one-half of the dose associated with side effects in healthy adults, the dose of 0.55 g/15 g cereal was considered appropriate to avoid side effects. On average, subjects in our study consumed 0.67 g prebiotic/d, or 0.11 $g \cdot kg^{-1} \cdot d^{-1}$, a dose well within the range of 0.05–0.11 $g \cdot kg^{-1} \cdot d^{-1}$ consumed by adults in studies that documented a bifidogenic effect on stool flora (24, 25). Although data on the minimum daily dose needed for a bifidogenic effect in childhood are not to our knowledge available, we suspect that the dose used and cereal intake observed in this study were not responsible for the lack of an observed treatment effect.

A third possible reason for a negative study would be inadequate statistical power. Our trials were powered to detect successive 25% reductions in diarrhea occurrence in a population with a high burden of gastrointestinal illness. With our observed frequency of illness, our first trial study had 57% power to detect a 25% reduction in diarrhea prevalence (from 5.9 to 4.73 d/100 d) and an 85% power to detect a 35% decrease in diarrhea prevalence. The second trial had a 68% power to detect a 25% reduction in diarrhea prevalence. It therefore seems unlikely that our trials missed a clinically important treatment effect.

The fourth and final possible reason for the finding of no effect of prebiotics could be the widespread practice of breast-feeding among the population studied. Oligosaccharides in human milk may play a role in intestinal host defense against microbial pathogens, because they compete with gastrointestinal epithelial cell binding of numerous bacterial pathogens or their toxins (27). Prebiotic supplementation of children who are already receiving

TABLE 4
Other health outcomes of 2 trials[1]

Variable	Trial 1		Trial 2	
	Control cereal (n = 141)	Prebiotic-supplemented cereal (n = 141)	Zinc-fortified cereal (n = 175)	Prebiotic- and zinc-supplemented cereal (n = 174)
Post-Hib vaccine antibody titer (μ/mL)[2]	10.8 (0.25–117) [78]	13.4 (0.22–173) [76]	10.7 (0.17–690) [71]	9.3 (0.34–1455) [84]
Days irritable (n)	17.4 ± 13.3[3]	17.6 ± 14.1	15.4 ± 13.8	15.0 ± 11.2
Days with rhinorrhea (n)	107.8 ± 39.4	102.7 ± 39.9	92.6 ± 43	93.6 ± 39
Days with anorexia (n)	3.1 ± 4.4	3.4 ± 5.7	1.9 ± 2.8	2.1 ± 3.3
Days not breast-fed (n)	20.2 ± 52.2	22.4 ± 51.7	20.3 ± 49	23.5 ± 53
Visits to study clinic (n)	13.3 ± 6.9	13.0 ± 5.8	12.1 ± 6.4	12.6 ± 5.4
Days of antibiotic use (n)	18.6 ± 12.6	17.8 ± 13.2	18.7 ± 14	19.9 ± 12

[1] Hib, *Haemophilus influenzae* type B. There were no significant differences between the groups in either trial.
[2] Geometric $\bar{x}$; range in parentheses; n in brackets.
[3] $\bar{x}$ ± SD.

oligosaccharides through breast milk may offer no further clinical benefit. In a trial of the probiotic *Lactobacillus* GG performed in an urban slum near Lima, those children who were not breast-fed were found to have derived the most benefit from this probiotic (5).

In summary, we performed 2 consecutive trials of oligofructose-supplemented infant cereal in a shantytown community near Lima, Peru. Prebiotic supplementation had no effect on the occurrence or severity of gastrointestinal infections, growth, immune response, or use of health care resources. Infants and young children who continue to breast-feed may not benefit from prebiotic supplementation.

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CD, MP, PH, and RK designed the study, took part in data analysis, and wrote the report. MP was responsible for all aspects of fieldwork. AG oversaw the collection and analysis of biochemical and microbiologic specimens. AH, AC, and PH performed data analysis. FC and CE assisted in the study design and oversaw cereal production, quality control, and shipping. All authors contributed to the final report. FC and CE are employees of Gerber Products Company. RK is a member of the Gerber Products Company Scientific Advisory Committee.

REFERENCES

1. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995;125:1401–12.
2. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human Lactobacillus strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991;88:90–7.
3. Elmer GW, Surawicz CM, McFarland LV. Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. JAMA 1996;275:870–6.
4. Vanderhoof JA, Young RJ. Use of probiotics in childhood gastrointestinal disorders. J Pediatr Gastroenterol Nutr 1998;27:323–32.
5. Oberhelman RA, Gilman RH, Sheen P, et al. A placebo-controlled trial of Lactobacillus GG to prevent diarrhea in undernourished Peruvian children. J Pediatr 1999;134:15–20.
6. Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. Br J Nutr 1999;82:375–82.
7. Menne E, Guggenbuhl N, Roberfroid M. Fn-type chicory inulin hydrolysate has a prebiotic effect in humans. J Nutr 2000;130: 1197–9.
8. Roberfroid MB, Delzenne NM. Dietary fructans. Annu Rev Nutr 1998;18:117–43.
9. Saavedra J, Tschernia A, Moore N, et al. Gastrointestinal function in infants consuming a weaning food supplemented with oligo-fructose, a prebiotic. J Pediatr Gastroenterol Nutr 1999;29:513 (abstr).
10. Sazawal S, Black RE, Bhan MK, Bhandari N, Sinha A, Jalla S. Zinc supplementation in young children with acute diarrhea in India. N Engl J Med 1995;333:839–44.
11. Zinc Investigators Collaborative Group. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. Am J Clin Nutr 2000;72:1516–22.
12. Sazawal S, Black RE, Jalla S, Mazumdar S, Sinha A, Bhan MK. Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: a double-blind, controlled trial. Pediatrics 1998;102:1–5.
13. Zinc Investigators' Collaborative Group. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. J Pediatr 1999; 135:689–97.
14. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 1998; 68(suppl):447S–63S.
15. Mengheri E, Nobili F, Vignolini F, Pesenti M, Brandi G, Biavati B. *Bifidobacterium animalis* protects intestine from damage induced by zinc deficiency in rats. J Nutr 1999;129:2251–7.
16. Division of Diarrhoeal and Acute Respiratory Disease Control. The treatment of diarrhoea: a manual for physicians and other senior health workers. WHO/CDR/95.3 1995:1–54.
17. Yeager B, Lanata C, Lazo F, Verastegui H, Black R. Transmission factors and socioeconomic status as determinants of diarrhea incidence in Lima, Peru. J Diarrhoeal Dis Res 1991;9:186–93.
18. Molrine DC, George S, Tarbell N, et al. Antibody responses to polysaccharide and polysaccharide-conjugate vaccines after treatment of Hodgkin disease. Ann Intern Med 1995;123:828–34.
19. WHO/CHD Immunization-Linked Vitamin A Supplementation Study Group. Randomized trial to assess benefits and safety of vitamin A supplementation linked to immunization in early infancy. Lancet 1998;352:1257–63.
20. Rothstein E, Schiller R, Girone J, et al. Response of 7- to 15-month old infants to sequential immunization with *Haemophilus influenzae* type b-CRM197 conjugate and olysaccharide vaccines. Am J Dis Child 1991;145:898–900.
21. Vanderhoof JA. Probiotics: future directions. Am J Clin Nutr 2001; 73(suppl):1152S–5S.
22. Duggan C, Gannon J, Walker W. Protective nutrients and functional foods for the gastrointestinal tract. Am J Clin Nutr 2002;75: 798–808.
23. Moro G, Minoli I, Fanaro S, et al. Dosage related effect of oligosaccharides on faecal flora and stool characteristics in term infants. Am J Clin Nutr 2002;75(suppl):418S (abstr).
24. Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 1995;108:975–82.
25. Buddington RK, Williams CH, Chen SC, Witherly SA. Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. Am J Clin Nutr 1996;63: 709–16.
26. Rumessen JJ, Gudmand-Hoyer E. Fructans of chicory: intestinal transport and fermentation of different chain lengths and relation to fructose and sorbitol malabsorption. Am J Clin Nutr 1998;68: 357–64.
27. Dai D, Walker WA. Protective nutrients and bacterial colonization in the immature human gut. Adv Pediatr 1999;46:353–82.