Impact of Zinc Supplementation on Morbidity From Diarrhea and Respiratory Infections Among Rural Guatemalan Children

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ABSTRACT. *Objective.* A community-based, randomized, double-blind intervention trial was conducted to measure the impact of zinc supplementation on young Guatemalan children's morbidity from diarrhea and respiratory infections.

Methods. Children aged 6 to 9 months were randomly assigned to receive 4 mL of a beverage containing 10 mg of zinc (as zinc sulfate) daily (7 d/wk) for 7 months ($n = 45$) or a placebo ($n = 44$). Morbidity data were collected daily. Diagnoses of diarrhea, fever, and anorexia were based on mothers' definitions. Respiratory infections were defined as the presence of at least two of the following symptoms: runny nose, cough, wheezing, difficulty breathing, or fever.

Results. High rates of diarrhea and respiratory infections were reported. Children from the placebo group had a 20% episodic prevalence of diarrhea, with 8 episodes/100 d, and a 7% episodic prevalence of respiratory infections, with 3 episodes/100 d. The median incidence of diarrhea among children who received zinc supplementation was reduced by 22% (Wilcoxon rank test), with larger reductions among boys and among children with weight-for-length at baseline lower than the median of the sample (39% reductions in both subgroups). Zinc supplementation also produced a 67% reduction in the percentage of children who had one or more episodes of persistent diarrhea (χ^2 test). No significant effects were found on the episodic prevalence of diarrhea, the number of days per episode, or the episodic prevalence or incidence of respiratory infections.

Conclusions. The large impact of zinc supplementation on diarrhea incidence suggests that young, rural Guatemalan children may be zinc deficient and that zinc supplementation may be an effective intervention to improve their health and growth. *Pediatrics* 1997;99: 808–813; *diarrhea, Guatemala, experimental trial, micronutrients, respiratory infections, zinc.*

ABBREVIATIONS. LAZ, length-for-age z-score; WLZ, weight-for-length z-score.

Little is known about the zinc status of young children in rural Guatemala. There are, however, reasons to suspect that zinc may be a limiting factor for their growth and health. The staple diet of lime-treated maize and beans is high in phytates and calcium, which are known to inhibit zinc absorption[1,2]; and consumption of animal products, which are high in zinc, is minimal among poor families.[3]

High diarrheal rates among Guatemalan preschoolers[4] may further exacerbate marginal zinc status. Studies have shown that diarrhea may affect zinc status as a result of reduced dietary intake, impaired intestinal absorption, and increased intestinal losses.[5,6] On the other hand, zinc deficiency, which is associated with impaired immune function,[7–10] can also contribute to elevated rates of diarrhea. Moreover, diarrhea is consistently found in severe zinc deficiency and is quickly reversed by zinc supplementation,[11] and even mild to moderate zinc deficiency has been reported to cause diarrhea in humans.[12,13]

We conducted a community-based, randomized, double-blind, zinc-supplementation trial to test the hypothesis that Guatemalan children were zinc deficient to a degree that affected their morbidity, appetite, intake of breast milk and complementary foods, and physical growth and activity patterns.[14–16] This article describes the impact of zinc supplementation on the children's morbidity.

METHODS

Sample

The study was conducted in Santa Maria de Jesus, a poor rural village located 55 km from Guatemala City at an altitude of 2050 meters above sea level. The population is estimated at 16 000 inhabitants, the majority of whom are Mayan Indians. Santa Maria de Jesus has a severely limited water supply and high rates of diarrheal diseases among preschoolers.[4]

A census was done to identify children from 6 to 9 months of age. Because the main outcome of the study was linear growth, sample size calculations were based on an estimated impact on length of 1 cm (with a standard deviation of 2 cm), based on studies from Walravens and Hambidge and Xue-Cun et al.[17,18] Using a two-tail test with an α level of .05 and a power of 90%, a sample of 42 children per group was calculated. Allowing for drop-outs, a total of 108 children were recruited.

Nineteen children dropped out of the study attributable to migration, or inability to comply with the project requirements because of maternal work, or late parental refusal. Ten of these children were from the zinc-supplemented group and nine were from the placebo group. Baseline characteristics of children who dropped out of the study (maternal characteristics, child intake of breast milk and complementary foods, and anthropometry) were not statistically significantly different from those of the rest of the

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sample (t test results; not shown). The final sample size for the analyses presented here is 45 children in the zinc-supplemented group and 44 in the placebo group.

Study Design

The study was designed as a double-blind randomized community trial. Children were assigned to receive a 4 mL daily dose of a flavored liquid preparation with or without 10 mg of zinc included as zinc sulfate. The two beverages were indistinguishable, and both contained sucrose, citric acid, and artificial flavor.

Field workers distributed the supplement daily, 7 d/wk for an average of 7 months. Although the planned duration of supplementation was 6 months, some variation occurred, such that 20% of the sample received the supplement for 4 to 6 months, 62% for 6 to 8 months, and 18% for 8 to 9 months. There were no differences between the groups in the duration of treatment. Field workers observed the children's consumption of the supplement. When the child was asleep they returned to the home later to ensure that the child consumed the supplement in their presence. Average compliance was 95% and there were no differences between study groups.

Data Collection Procedures

Baseline data were collected on: family socioeconomic and demographic characteristics (family composition, maternal age and parity, family possessions, quality of housing, hygienic facilities, and parents' education); maternal and child anthropometry; child intake of breast milk and complementary foods; and child's appetite and physical activity patterns.

Morbidity information was collected daily, at the time of supplement distribution. A check list was used to record symptoms of illnesses observed during the previous 24 hours. The list included symptoms of respiratory infections (runny nose, cough, wheezing, difficulty breathing); gastrointestinal disorders (diarrhea based on mother's definition, number of stools/day, presence of fecal mucus or blood; vomiting); other infections; and selected complications of illness (apathy, anorexia, irritability, and fever).

Data were also collected on the children's diets (direct weighing) and breast milk intake (test-weighing),[19] using 12-hour observations in the homes at baseline and at 3 and 7 months follow-up. Total energy intake (kcal/g of body weight) and energy density of diet and breast milk (kcal/g consumed) were calculated by adding up the energy intake from complementary foods and from breast milk (73 kcal/dL)[20] during the 12-hour observation period. Feeding frequency and number of episodes of breastfeeding were also calculated over a period of 12-hours only.

Analytical Methodology

Definition of Morbidity Variables

Respiratory infections were defined as the presence of at least two of the following symptoms: runny nose, cough, wheezing, difficulty breathing, or fever. Diarrhea was based on the mother's definition because information on stool consistency was not available. Fever and anorexia were also based on the mother's definition. Persistent diarrhea was defined as episodes that lasted more than 14 days.

Episodic prevalence[21] was defined as the percentage of days ill, calculated as the number of days ill divided by the total number of days of observation. Incidence was defined as the number of new episodes divided by the number of days at risk, which was defined as the number of days of observation minus the number of days with diagnosis, allowing for two illness-free days between episodes.

Socioeconomic Status Score

A socioeconomic status score was derived by Principal Components Analysis as one factor. The variables included were: house and floor material, number of rooms, sanitary facilities, and level of poverty of the family.

Statistical Analyses

Univariate analyses were done on each morbidity variable to eliminate outliers (ie, values that did not belong to the distribution) using schematic plots (box plots). Values more extreme than three interquartile ranges (an interquartile range is the distance between the 25th and 75th sample percentiles) of the box were considered outliers and were eliminated from the analysis.[23] This represented a maximum of 3% of the observations for morbidity from diarrhea, 2% for morbidity from respiratory infections and fever, and 8% for the episodic prevalence of anorexia.

Statistical tests of comparison were done using t tests, χ^2 tests, and the Wilcoxon rank test where appropriate.

Ordinary least squares regression analysis was used to: 1) test the impact of zinc supplementation on morbidity outcomes, controlling for potential confounding factors (main effects model); and 2) test whether some children benefited more from the supplementation than others, as indicated by the presence of statistically significant two-way interaction terms between treatment and the characteristics studied (interactive effects models). The independent variables included in the morbidity models were: socioeconomic status, maternal schooling, child nutritional status at baseline, frequency of feeding of complementary foods and of breast milk, energy density of diet (food plus breast milk), and total energy intake.

Analyses were performed using the PC-SAS statistical program, version 6.04 (SAS Institute, Cary, NC). Probability values $<.05$ for main effects and $<.10$ for interaction terms were considered statistically significant.

The study protocol was approved by the Committees on the Use of Human Subjects in Research both at the Institute of Nutrition of Central America and Panama and the University of California, Davis. Verbal informed consent was obtained from all participants.

RESULTS

At baseline, there were no differences between treatment groups in any of the characteristics studied, except maternal schooling and gender ratio (Table 1). Among the zinc-supplemented group, there

TABLE 1. Characteristics of the Sample at Baseline

Characteristics	Placebo (n = 44)	Zinc-supplemented (n = 145)
Child	Mean (SD)	Mean (SD)
Age (mo)	7.51 (0.96)	7.73 (1.17)
Weight-for-age z-score*	−1.11 (1.09)	−1.24 (0.86)
LAZ	−2.11 (1.02)	−2.20 (0.80)
WLZ	0.75 (1.07)	0.63 (0.89)
Energy intake† (kcal/kg wt)	86 (31)	78 (19)
Energy density† (kcal/g)	1.25 (0.84)	1.00 (0.84)
Feeding frequency† (food) (12-h)	1.69 (1.31)	1.81 (1.42)
Breastfeeding frequency† (12-h)	5.88 (2.03)	6.19 (2.24)
Mother		
Stature (cm)	144.7 (5.0)	144.6 (5.0)
Weight (kg)	50.4 (6.7)	53.2 (8.1)
Schooling (y)	0.95 (1.74)	1.8 (2.30)‡
Socioeconomic status (factor score)	0.07 (0.95)	−0.06 (1.05)
	%	%
Mothers who can read (%)	27	58‡
Houses with electricity (%)	83	89
Houses with mud floor (%)	61	67
Houses with bamboo walls (%)	50	67
Houses with latrines (%)	84	71
Children partially breastfed (%)	100	100
Boys (%)	45	69‡

* Weight and length were measured to the nearest 5 g and 1 mm, respectively. Z-scores were calculated by comparing individual values to the World Health Organization/Centers for Disease Control and Prevention reference data.[22]

† All four variables related to child-feeding practices are based on 12-hour observations conducted at baseline. Both energy intake (kcal/kg of body weight) and energy density (kcal/g of food) refer to total energy (food plus breast milk). The energy content of breast milk (73 kcal/dL) was derived from a study conducted in another indigenous population of Guatemala.[20]

‡ $P < .05$. Differences between means and frequency distribution were tested by t-test and χ^2 test, respectively.

was a statistically significant greater percentage of mothers who could read (58% vs 27% in the placebo group), and their average number of years of schooling was approximately one year greater than mothers from the placebo group. A greater percentage of boys was also found among the zinc-supplemented group (69% vs 45% among the placebo group). All other socioeconomic characteristics, maternal and child anthropometry, and child feeding practices were similar between groups. As documented in other studies from Guatemala,[23,24] children were severely stunted at baseline (56% had length-for-age z-score (LAZ) < -2 SD), but wasting was nonexistent (no child had weight-for-length z-score (WLZ) < -2 SD). All children were partially breastfed throughout the study.

The incidence of diarrhea was very high among the placebo group (median, 8 episodes/100 child-days), but was significantly reduced by zinc supplementation to a median of 6 episodes/100 child-days (Table 2). Zinc supplementation also significantly reduced the percentage of children with persistent diarrhea by 67%: among the placebo group, 27% of children had one or more episode of persistent diarrhea compared to only 9% among the zinc-supplemented group ($P < .05$; χ^2 test). The incidence of respiratory infections and fever was not affected by zinc supplementation.

Children from the zinc-supplemented group tended to have a lower episodic prevalence of diarrhea, fever, and anorexia and a greater episodic prevalence of respiratory infections than those from the placebo group, although none of these differences was statistically significant (Table 3). The duration of illness did not differ between the groups: the average duration of diarrhea was 3.99 days (SD, 1.84) among the zinc-supplemented group and 4.09 days (SD, 2.69) among the placebo group.

TABLE 2. Morbidity Incidence (Episodes/100 Child-Days) by Treatment Group

Incidence of:	Placebo	Zinc	% Difference*
	Median [25%–75%]*	Median [25%–75%]	
Diarrhea	8.1 [5.8–10.2]	6.3 [4.2–8.9]	−22%†
Respiratory infections	2.8 [1.5–4.6]	3.2 [1.6–4.6]	+14%
Fever	2.0 [0.9–2.7]	1.9 [1.2–2.3]	−1%
Persistent diarrhea (% with ≥1 episode)	27%	9%	−67%‡

* 25% to 75% refers to the 25th and 75th percentile values. % Difference = (median placebo group − median zinc-supplemented/median placebo) × 100.

† $P < .05$; differences between groups tested by the Wilcoxon rank test.

‡ $P < .05$; difference between groups tested by the χ^2 test. Persistent diarrhea is defined as an episode that lasted longer than 14 days.

TABLE 3. Episodic Prevalence of Morbidity (% Days Ill) by Treatment Group

Episodic Prevalence of:	Placebo	Zinc	% Difference*
	Median [25%–75%]*	Median [25%–75%]	
Diarrhea	19.9 [12.7–28.5]	16.0 [11.2–24.1]	−20%
Respiratory infections	7.3 [3.9–12.4]	10.1 [4.4–14.4]	+38%
Fever	4.1 [1.9–6.8]	3.9 [2.4–6.2]	−5%
Anorexia	6.1 [2.7–7.8]	4.7 [3.0–6.8]	−23%

* 25% to 75% refers to the 25th and 75th percentile values. % Difference = (median placebo group − median zinc-supplemented/median placebo) × 100. Differences between groups were tested by the Wilcoxon rank test; $P < .05$ was considered statistically significant. None of the differences were statistically significant.

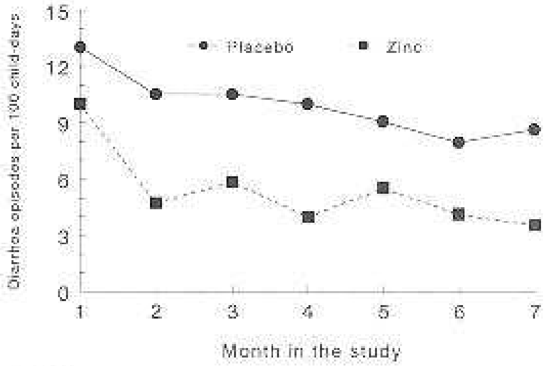

Fig 1. Median incidence of diarrhea by study group and month (boys).

Analyses done separately by gender revealed that, although a reduction in diarrhea incidence was found in both boys and girls, the difference was statistically significant only among boys. None of the other morbidity indicators showed any difference between genders. Figure 1 shows, for boys only, the difference in diarrhea incidence between treatment groups, by month of supplementation. Note that bias due to seasonality is not an issue in this longitudinal analysis because children started supplementation in four different cohorts at 1- to 2-month intervals between June and November, 1993. Thus, a particular month of supplementation corresponds to a different calendar month for children from different cohorts. Figure 1 shows that a rapid drop in diarrhea incidence occurred between the first and second months of supplementation and that zinc-supplemented boys maintained a consistently lower incidence of diarrhea throughout the intervention period. Because of the large variance in diarrhea incidence and the relatively small sample size for this analysis (placebo, 20; zinc-supplemented, 31), only differences at 3, 4, and 7 months were statistically significant ($P < .05$, Wilcoxon rank test). Note that there was also a slight reduction in diarrhea incidence among the placebo group over time. This may have been attributable to maturation (children getting older and less vulnerable) or to participation in the study (fieldworker effect).

The determinants of diarrhea incidence were analyzed by ordinary least squares regression (Table 4). The significant impact of zinc supplementation on reducing diarrheal morbidity remained after controlling for child WLZ and age at baseline, gender, family socioeconomic status, maternal schooling, number of days of supplementation, child feeding practices, and daily energy intake (Model A). Other models were run to test whether LAZ at baseline, and other family socioeconomic factors (presence of

TABLE 4. Determinants of Diarrhea Incidence (Episodes/100 Days): Results of Ordinary Least Squares Regression Analysis

Variable	Main Effects (Model A)		Interactive Model (Model B)	
	β	P	β	P
Intercept	21.22	(.00)	21.64	(.00)
Treatment (0 = placebo; 1 = zinc)	−2.88	(.02)	−8.81	(.00)
Initial WLZ	−0.28	(.69)	−2.87	(.04)
Gender (1 = M; 2 = F)	−1.45	(.28)	−4.38	(.00)
Age at baseline (mo)	−0.23	(.72)		
Socioeconomic status factor	0.56	(.37)		
Maternal schooling (yr)	−0.04	(.89)		
Days of supplementation	−0.06	(.00)	−0.03	(.01)
Total energy intake* (kcal/kg)	0.02	(.41)		
Energy density* (kcal/kg)	−0.79	(.32)		
Feeding frequency* (food)	0.28	(.46)		
Breastfeeding frequency*	0.39	(.26)		
Interaction: TRT × WLZ			1.81	(.03)
Interaction: TRT × gender			3.64	(.02)
Interaction: gender × WLZ			1.92	(.03)
R square (adj)	0.10		0.25	
Mean square error	5.27		3.46	
N	81		87	

Abbreviations: β = regression coefficient; F = female; M = male; P = P value of t-test of regression coefficient; TRT = treatment; WLZ = weight-for-length z-score.

* All four variables related to child feeding practices are based on 12-hour observations conducted at the end of the supplementation period. Both energy intake (kcal/kg of body weight) and energy density (kcal/g of food) refer to total energy (food plus breast milk). The energy content of breast milk (73 kcal/dL) was derived from a study conducted in another indigenous population of Guatemala.[25]

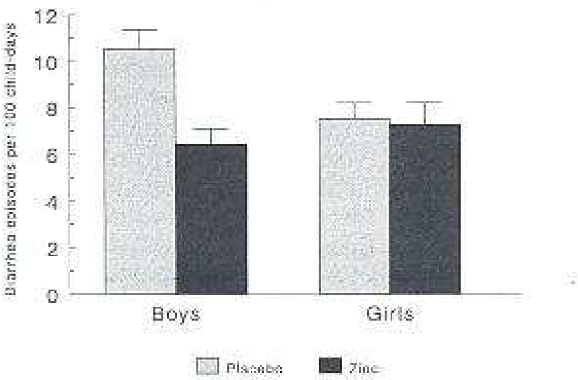

Fig 2. Interactive effect of treatment and gender on diarrhea incidence.

animals, hygienic facilities, and so forth) were associated with diarrheal morbidity. The results were consistent in showing a significant impact of zinc supplementation on the reduction of diarrheal incidence, but none of the child and family characteristics were associated with diarrhea. The number of days children received the supplement was significant in all models, indicating a dose-response effect. With more days of supplementation, a greater reduction in the incidence of diarrhea was obtained.

Analyses of interactive effect models showed the existence of two statistically significant two-way interactions with treatment group, ie, initial WLZ and gender (Table 4, Model B). Figures 2 and 3 present the adjusted means for diarrhea incidence by gender and treatment and by WLZ and treatment, respectively.

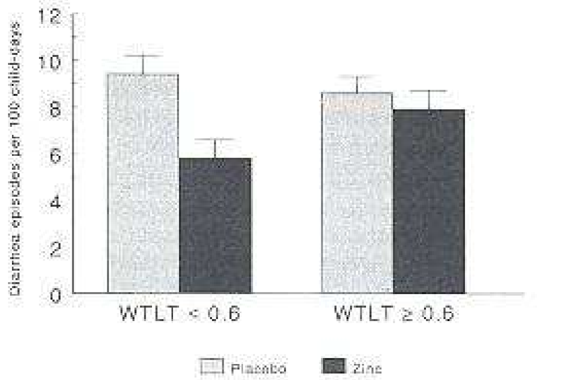

Fig 3. Interactive effect of treatment and weight-for-length z-score (WLZ) at baseline on diarrhea incidence.

For illustrative purposes, WLZ at the onset of supplementation was dichotomized using the median for this sample as the cut-off point to divide the sample into two groups: lighter (WLZ < .6) and heavier (WLZ ≥ .6). Males and children with lower WLZ at baseline benefited more from zinc supplementation than girls and children with greater initial WLZ. Zinc supplementation reduced diarrhea incidence by 39% among boys and among children with lower initial WLZ, whereas the reduction was only 3% among zinc-supplemented girls and 8% among heavier children. None of the other child and family socioeconomic variables tested was statistically significant in two-way interactions with treatment.

Regression models of the episodic prevalence of diarrhea and of the incidence or episodic prevalence of respiratory infections, fever, and anorexia were also developed. Results of multivariate analyses confirmed those of bivariate analyses, namely that zinc supplementation had no effect on these outcomes.

DISCUSSION

Zinc supplementation reduced the incidence of diarrhea by 22% among our sample of 6- to 9-month-old Guatemalan children. The magnitude of the effect was significantly larger among boys (39%) and among children with lower WLZ at the onset of supplementation (39%). A dose-response relationship was found indicating that a greater impact on diarrhea was obtained as the number of days of supplementation increased. Zinc supplementation also produced a 67% reduction in the percentage of children who had one or more episodes of persistent diarrhea ($P < .05$). The episodic prevalence of diarrhea (percent days ill) was also reduced by 20% among the zinc-supplemented group, but this difference was not statistically significant attributable to lack of statistical power (the power to detect a difference was only 47% for this analysis).[25] These results combined with the previously reported findings of a positive impact of zinc supplementation on growth among stunted children[14] suggest that young, rural, indigenous Guatemalan children may be zinc deficient.

Overall, children in our sample had very high diarrheal rates. There are various potential explanations for these findings. First, children were between

6 to 18 months of age, which is the age of greatest diarrheal rates reported among this population.[4] Secondly, morbidity surveillance was carried out daily. Less frequent surveillance is known to result in underestimates of diarrheal rates, attributable to underreporting of mild and short episodes.[26] Finally, our definition of a diarrheal episode allowed for two illness-free days between diarrhea-days. Failure to allow for illness-free days between episodes results in slightly lower incidence estimates attributable to a larger denominator.

Mothers' definitions of diarrhea were used in our study as opposed to the clinical definition of more than three unformed stools a day, because information on stool consistency was not available. Comparison of mothers' definitions with a definition based on the number of stools only (more than three stools a day), showed a correspondence of 88%, similar to findings documented by Cogswell and collaborators.[27] Moreover, the correspondence between the two definitions was exactly the same for the zinc and the placebo groups. We repeated all analyses using the definition of diarrhea based on number of stools per day and found similar results as when maternal definition was used, although with slightly reduced statistical power attributable to a larger variance. Because of the randomized design used in this study, and because the correspondence between the two definitions of diarrhea was the same for the two comparison groups, we have no reason to believe that the use of mothers' definitions of diarrhea could have biased our results.

The beneficial effect of zinc supplementation on the incidence of diarrhea has been reported recently in community-based studies in Mexico, India, and Vietnam.[28–30] Zinc supplementation has also been found to reduce the duration and severity of diarrhea in some settings.[31–33] No effect on duration of diarrhea was found in our study. A possible reason for this difference in findings may be that the other studies were targeted to children during diarrhea episodes, or to children suffering from kwashiorkor. Additionally, the doses of zinc were larger,[31,32] or were increased during diarrhea episodes.[33]

The effect of zinc supplementation on respiratory infections is not consistent. Three studies reported a reduction in the incidence of respiratory infections,[8,28,30] and two found no impact.[7,34] Our study showed an increase in both the incidence and the episodic prevalence of respiratory infections among the zinc-supplemented group, but neither was statistically significant. This result is of possible concern, however, and should be explored in other studies. It should also be noted that even though several studies have shown a positive effect of zinc supplementation on immune function,[35] this may not be the case for all components of the immune system. Schlesinger et al[36] have shown that monocyte function was impaired by zinc supplementation in malnourished children. It is therefore possible that infections affected by such immune components will not be reduced by zinc supplementation and may even be increased.

Gender differences in the response to zinc supplementation have been documented extensively, particularly in studies examining the effects on growth.[17,37,38] Relative to diarrheal morbidity, only the study in India found similar results to ours, ie, that the positive effect of zinc supplementation on the incidence of dysentery was restricted to male children.[29] The usual interpretation of the greater response of boys to zinc supplementation is that they may have greater zinc requirements than girls.[17,38] Among boys from our sample, this is likely to be the case because boys had greater incidence and episodic prevalence of diarrhea than girls, and thus may have had greater zinc requirements because of excessive losses during diarrhea. It is not clear, however, whether the greater requirements would be a consequence of diarrhea; or whether diarrhea was caused by greater deficit resulting from higher requirements. There are no indications from our preliminary analyses of diet and breast milk intake of differences in nutrient intake per kg of body weight between boys and girls. It is difficult to understand why girls, who also had high diarrheal morbidity, did not benefit from the supplementation.

The other determinant of benefit found in our study, WLZ $< .6$ at baseline, has not been identified previously in zinc-supplementation trials investigating effects on the incidence or prevalence of specific illnesses. It was, however, found to determine benefit in the duration and severity of diarrhea in the study by Sazawal et al,[33] who reported that the reduction in the number of days of watery diarrhea after zinc supplementation was greater among wasted children. It could be hypothesized that thinner children in our study had already experienced more diarrhea, and that thinness acted as a proxy for the risk of diarrhea rather than as an indicator of nutritional deficit. This does not seem to be the case, however, because there were no differences in the incidence of diarrhea between thinner and heavier children among the placebo group (Fig. 3). The lack of response to zinc supplementation among heavier children who seemed equally at risk of diarrhea is difficult to interpret.

Conclusions

Findings from our study and the other three recent community-based zinc supplementation trials showed reductions in the incidence of acute and persistent diarrhea ranging from 14 to 65%. This magnitude of effect is at least as large as the range of effects reported for other interventions that are currently promoted by diarrheal disease control programs.[39] For example, successful promotion of breastfeeding would be likely to result in 8 to 20% reductions of diarrhea in the first year of life,[40] whereas improved water supply and sanitation is expected to reduce diarrheal diseases by 27%.[41] Thus, if well targeted, zinc supplementation could be a powerful intervention to reduce morbidity from diarrheal diseases among children from developing countries.

More research is needed to continue to explore the determinants of benefit from zinc supplementation for targeting purposes. Future community-based trials should test with appropriate sample sizes the differential effect of zinc supplementation on subgroups of children who are most likely to benefit, so as to improve the impact and cost-effectiveness of interventions. Other studies should also test whether similar results can be replicated in populations with lower rates of diarrheal infections.

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