

# Effect of zinc supplementation on the morbidity, immune function, and growth of low-birth-weight, full-term infants in northeast Brazil<sup>1-3</sup>

Pedro IC Lira, Ann Ashworth, and Saul S Morris

**ABSTRACT** In Brazil, the highest incidence of low birth weight (LBW) occurs in the northeast, and diarrhea and respiratory infections are the main causes of infant mortality and morbidity. We hypothesized that LBW infants may be zinc deficient, and that this might be adversely affecting their immune function, morbidity, and postnatal growth. We therefore examined the effect of zinc supplementation on these outcomes during the first 6 mo of life. LBW full-term infants (mean birth weight 2337 g) were given daily for 8 wk either 5 mg Zn ( $n = 71$ ), 1 mg Zn ( $n = 68$ ), or a placebo ( $n = 66$ ). Morbidity was determined prospectively through daily home visits (except on Sunday) during weeks 0–8, then twice weekly in weeks 9–26. Anthropometric measurements were made at 0, 4, 8, 17, and 26 wk. Immune function was assessed at 8 wk by the phytohemagglutinin skin test. Supplementation (5 mg Zn) was associated with a 28% reduction in diarrhea prevalence over the 6-mo period [after adjustment for confounders ( $P = 0.043$ )], and a 33% reduction in the prevalence of cough (NS, adjusted prevalence  $P = 0.073$ ). All infants had a positive immune response at 8 wk. Although supplementation had no significant effect on weight and length gains from 0 to 26 wk, infants given 5 mg Zn gained more weight than infants given placebo during weeks 17–26 ( $P = 0.024$ , analysis of variance). There was no effect on any outcome with 1 mg Zn. We conclude that 5 mg Zn/d is of benefit to LBW, full-term infants who only have a modest weight deficit. *Am J Clin Nutr* 1998(suppl);68:418S–24S.

**KEY WORDS** Zinc supplementation, low birth weight, diarrhea, morbidity, respiratory infection, immune function, growth

## INTRODUCTION

Low birth weight (LBW, < 2500 g) is a major public health problem in technologically less-developed countries. Approximately 90% of all LBW infants worldwide are born in these countries and most are born at term, in contrast with the situation in industrialized countries in which most LBW infants are preterm (1). Even though they may be of similar birth weight, infants who are born too small differ in many important ways from those born too soon. For example, full-term, LBW infants have more severe and longer lasting impairments in immunocompetence than preterm infants, and they do not grow as well postnatally (2). Full-term, LBW infants are also disadvantaged

by having smaller livers than preterm infants of comparable birth weight, the magnitude of this difference being 35–40% (3). Full-term, LBW infants can thus be expected to have a smaller reserve capacity for micronutrients stored in the liver than preterm infants. Zinc, which has an important immunologic and growth-promoting role, is among these micronutrients. We surmised that LBW, full-term infants in poor communities may be deficient in zinc because they have less stored zinc in reserve and limited access to dietary sources, and that this deficiency may impair their immune function, increase their morbidity, and limit their postnatal growth. Unfortunately, it is difficult to assess zinc status reliably, and any detrimental effect of zinc deficiency can only be established by zinc supplementation trials.

Because Chandra (4) found significant improvement in immune function even in preterm infants supplemented with zinc, we hypothesized that full-term, LBW infants might also benefit. In the population studied, LBW, full-term infants have higher prevalences of diarrhea and vomiting than infants of normal birth weight (5). The main purpose of our study was therefore to determine whether zinc supplementation might be an effective public health intervention to reduce morbidity in LBW, full-term infants. Other outcomes of interest were immune function and growth.

## SUBJECTS AND METHODS

### Study design

A community-based, randomized, double-blind trial was developed in which 5 mg Zn or a placebo were to be given daily during the first 8 wk of life to LBW infants born at term. Mor-

<sup>1</sup> From the Centre for Human Nutrition and the Maternal and Child Epidemiology Unit, the Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London; and the Department of Nutrition, Centre for Health Sciences, Federal University of Pernambuco, Recife-Pe, Brazil.

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<sup>3</sup> Address reprint requests to A Ashworth, Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom. E-mail: a.hill@lshtm.ac.uk.

idity, immune function, and growth from birth to 26 wk of age were the main outcomes of interest. A mistake in the manufacture of the zinc solution resulted in the initial cohort of 68 infants in the active treatment group being given 1 mg Zn instead of 5 mg Zn. When this was discovered, the design was modified to include a second cohort of nonrandomly assigned, LBW, full-term infants who would all receive 5 mg Zn. Inclusion and exclusion criteria remained the same and all fieldworkers and the infants' families were unaware of the change, and they remained blinded.

### Study population

The study was conducted in 5 small towns in the interior of the state of Pernambuco, northeast Brazil. A total of 205 LBW infants were recruited over 20 mo, from January 1993 to August 1994, from the maternity wards of 1 state hospital (in Palmares), 2 private hospitals (in Palmares and Ribeirão), and 3 smaller government health centers (in Catende, Água Preta, and Joaquim Nabuco). In this area, >90% of deliveries occur in these facilities, with an LBW incidence of 9.2%.

Eligible subjects were singleton newborns weighing 1500–2499 g who had a gestational age of 37–42 wk, had no congenital anomalies, were from families who earned <4 minimum salaries monthly (1 minimum salary being ≈US\$70), and intended to reside in the area for the next 6 mo or more. Infants were excluded if they showed evidence of fetal distress or needed intensive care at delivery.

After the initial screening (for family income and intended residence), all potentially eligible newborns were weighed, measured, and examined by 1 of the 2 study pediatricians. Infants were weighed without clothes with regularly calibrated baby scales (digital baby scale, model 15/2B, Filizola, São Paulo, Brazil; beam baby scale, model 725, Soehnle-Waagen, Murrhardt, Germany) with 15 kg × 10 g capacity. Most of the weights (67%) were taken within 12 h of birth, and only 2 were weighed after 24 h. Length, from crown to heel, was measured with an infantometer (Harpندن model; Holtain Ltd, Crymych, United Kingdom) with an accuracy of 0.1 cm. Gestational age was assessed according to the method of Capurro et al (6). If the infant was eligible, the mother was fully informed about all study procedures and data confidentiality, and her consent sought. After enrollment, mothers were interviewed about their demographic and socioeconomic conditions, reproductive history, and prenatal care. Maternal weight and height were measured. Finally, the first dose of the supplement or placebo was administered in the maternity center in the presence of the mother to show how the fieldworkers would administer it at home.

### Ethics

All sick infants were referred immediately to a local health service according to a standard protocol, and the study team did not interfere with the treatment prescribed by the health personnel. Mothers knew that they could withdraw their child from the study at any time and were present for the skin test. Ethical permission was obtained from the Ethics Committees of the London School of Hygiene and Tropical Medicine, the Universidade Federal de Pernambuco, and the Fundação Nacional de Saúde.

### Zinc supplement and placebo

The supplement was zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) masked in sorbitol and flavor. The placebo (sorbitol and flavor) was similar

in taste and appearance. The fieldworker gave 0.5 mL of the placebo or supplement at home in the presence of the mother or guardian daily, except on Sundays, from birth to 8 wk. A disposable 1-mL syringe (with needle removed) was used and then discarded to avoid contamination. If an infant was hospitalized, the fieldworker went to the hospital to give the dose. The placebo and supplements were prepared in bulk and then placed in 50-mL bottles, one for each infant. The bottles were kept by the fieldworkers to prevent tampering, and a photograph of the infant's mother was affixed to the bottle to prevent misallocation.

### Morbidity

After leaving the maternity center, all infants were visited at home by trained nonmedical interviewers every day except on Sundays up to age 8 wk, and twice weekly thereafter up to age 26 wk. The morbidity questionnaire included daily information on the presence or absence of 5 maternally perceived symptoms: diarrhea, cough, vomiting, fever, and *cansaço*, the local term for rapid breathing (mothers were asked about *cansaço* only when cough was reported). The events occurring on Sundays were recorded the next day. All questions were prompted, with the exception of the question relating to diarrhea, which was only coded as present when the mother spontaneously mentioned it in response to an opening general question about the child's health since the previous visit. This reflected the investigators' desire not to include mild gastrointestinal disorders in this category. After the morbidity symptoms had been reported, respondents were asked to describe the child's appetite and to recall the number of stools passed in the previous 24 h, the number that were liquid or semiliquid, and the presence or absence of blood in the stools. If the child was reported to have a cough, the respiratory rate was measured with a respiratory rate timer (UNICEF prototype, New York). From 9 to 26 wk, the same information was recalled twice weekly except for the number of stools, which were asked about only for the preceding 24 h.

### Breast-feeding

Interviewers also recorded the number of breast-feedings and nonbreast-feedings (excluding water, tea, and juice) given to the infant in the previous 24-h period. This information was collected daily from birth to 8 wk and twice weekly thereafter.

### Anthropometry

Weight and length were measured at home at 4, 8, 17, and 26 wk of age by a team of 3 specially trained fieldworkers. Standard techniques were used (7), and 94% of the measurements were made within 3 d of the scheduled date. Infants were weighed without clothes with use of a portable scale (models MP10 and MP25; CMS Ltd, London) with a capacity of 10 kg or 25 kg × 10 g. Length was measured with an infantometer (Harpندن model; Holtain Ltd), with a precision of 0.1 cm. Infants were considered wasted at birth if their ponderal index ( $\text{g} \times 100/\text{cm}^3$ ) was <2.5. They were considered stunted at birth if their length was less than  $-2$   $z$  scores of the sex-specific reference median published by the National Center for Health Statistics (8).

### Immune function

The response to purified phytohemagglutinin (PHA) antigen (Wellcome Reagents Ltd, London) was tested at 8 wk of age. PHA (2  $\mu\text{g}$ ) was injected in a volume of 0.1 mL sterile saline into the flexor surface of the right forearm. The reac-

tion was read 24–30 h later. Indurations of  $\geq 5$  mm were considered positive (9).

### Other zinc sources

To check comparability of the groups regarding other sources of zinc, a questionnaire was administered at 4, 8, 17, and 26 wk of age to record any zinc-containing skin ointments, creams, or tonics given by parents, and any consumption of specific zinc-rich foods.

### Quality control

Gestational age was assessed independently for 20% of the infants, with good interobserver reliability ( $\kappa = 0.86$ ), and 10% of home visits were repeated to check the reliability of morbidity interviews and length measurements. The zinc contents of the supplement and placebo solutions were verified by the Pernambuco State Institute for Technology, Brazil, and the Middlesex Hospital, United Kingdom. A 10% sample of individual bottles was also checked against the randomization code. No errors were detected.

### Statistical methods

Questionnaires were precoded and checked daily for consistency, accuracy, and completeness. Double data entry was performed by different data clerks using DBASE-III+ (Ashton Tate, Torrance, CA). Data entry was verified by using crosschecks and logical checks. Differences among the 3 groups were assessed by using a standard chi-square test for categorical variables, or a chi-square test for linear trend for ordered categorical variables. One-way analysis of variance (ANOVA) was used for continuous variables (10).

Morbidity burdens were assessed by calculating, for each individual, the percentage of time ill with each symptom (longitudinal prevalence) between birth and 6 mo of age (11). The non-parametric Kruskal-Wallis test was used to compare mean differences in longitudinal prevalence among the groups (12). For multiple linear regression analysis (13), the longitudinal prevalence was transformed to a logarithmic scale (after 1 was added to eliminate zeros) because the untransformed longitudinal prevalence had a highly skewed distribution. The regression coefficients from this analysis were subsequently exponentiated (antilog transformation). This has the advantage of enabling differences between groups to be expressed on a ratio scale and of enabling these differences to be controlled for the confounding effects of socioeconomic variables associated with the disease outcome. The effect of the intervention on medical consultations (ever versus never), hospitalizations (ever versus never), and mortality was assessed by using Cox proportional hazards regression analysis (14). The Cox model was fitted using the stepwise method to identify potential confounders (namely, other variables associated with the outcome with  $P < 0.2$ ). The treatment group dummy variables were then forced to enter into the equation, and statistical significance was assessed with the Wald test (14).

Gains in weight and length were analyzed using two-way ANOVA with sex and treatment group as factors. Gains were calculated for the separate periods (0–4, 4–8, 8–17, and 17–26 wk) and for the whole period (0–26 wk). Differences in the average induration of the PHA reaction were assessed by using one-way ANOVA (10).

Analyses were conducted with the STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES/PC+ (version 5.0.1; SPSS Inc, Chicago) and EGRET (SERC, Seattle).

**TABLE 1**

Loss to follow-up at 26 wk for morbidity and growth outcomes according to treatment group

	Placebo ( <i>n</i> = 66)	1 mg Zn ( <i>n</i> = 68)	5 mg Zn ( <i>n</i> = 71)	Total ( <i>n</i> = 205)
	<i>n</i> (%)			
Losses				
Morbidity	14 (21.2)	12 (17.6)	19 (26.8)	45 (22.0)
Growth <sup>1</sup>	12 (18.2)	10 (14.7)	17 (23.9)	39 (19.0)
Reasons				
Moving from area	7 (10.6)	9 (13.2)	16 (22.6)	32 (15.6)
Death	7 (10.6)	3 (4.4)	3 (4.2)	13 (6.4)

<sup>1</sup>There were fewer losses for growth than for morbidity because 6 infants who moved after 8 wk were traced for the final anthropometric measurements.

### RESULTS

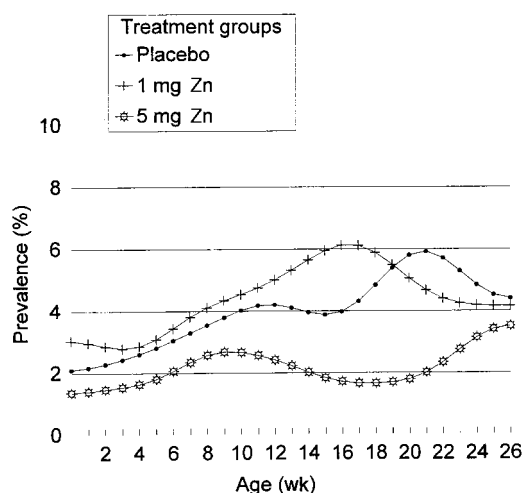
During the enrollment period (between January 1993 and January 1994), 134 LBW infants were recruited and given either 1 mg Zn (*n* = 68) or a placebo (*n* = 66). Between February 1994 and August 1994, 71 more LBW infants were recruited and given 5 mg Zn. Losses during the 26-wk follow-up were 22%, with 6% dying and 16% moving away (**Table 1**). The mean duration of participation averaged 45.7 d for all 3 groups. The zinc supplement was well tolerated and there was less vomiting during the supplementation period in the group with 5 mg Zn than in the other groups. The mean duration of morbidity follow-up was 158 d.

The mean ( $\pm$  SD) birth weight was  $2337 \pm 152$  g. Only 9 infants weighed  $< 2000$  g. Sixty percent of the infants were wasted only, 18% were stunted only, 17% were both wasted and stunted, and 5% met neither of these criteria. Overall, 56% of the infants were female, the mean gestational age was 38.9 wk, and 10% of infants were born by cesarean delivery.

### Comparability of the groups

Because infants were randomly allocated to receive either a placebo or 1 mg Zn, between-group differences in characteristics that might influence growth, morbidity, and immune function could only have arisen by chance. However the group receiving 5 mg Zn was not randomly allocated. Formal statistical tests were therefore used to compare the 3 groups with respect to the characteristics of the infants, their mothers, and their family environments. Of the 47 variables examined, 5 were significantly different and 3 others showed nonsignificant but nonetheless important between-group differences. The group given 5 mg Zn was longer at birth, with a mean length of 46.5 cm compared with 46.1 cm in the group given 1 mg Zn and 45.8 cm in the placebo group ( $P = 0.019$ , ANOVA), and fewer were less than  $-2$  SD in length, although there was no statistical difference among the groups ( $P = 0.246$ , chi-square). The mothers of the infants given 5 mg Zn were also significantly taller (mean: 154.9 cm) than the mothers of the infants given 1 mg Zn and placebo (153.3 and 152.2 cm, respectively;  $P = 0.027$ , ANOVA). The placebo group included a higher proportion of female infants (65%;  $P = 0.15$ , chi-square), they lived in houses with poorer wall structure, and fewer had electricity compared with the other groups ( $P = 0.008$  and  $P = 0.036$ , respectively, chi-square).

Few infants regularly received zinc-containing ointments, tonics, or zinc-fortified infant formulas. The most common complementary foods were powdered milks and maize or rice gruel. Breast-feeding prevalence was comparable in the 3 groups at 4,



**FIGURE 1.** Point prevalence of diarrhea (mothers' definition) in the 3 treatment groups (3-wk moving averages derived from daily prevalences).

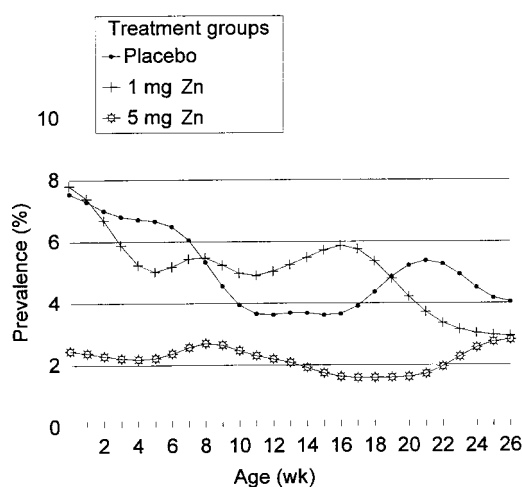
8, 17, and 26 wk. Only 27% of all infants were still breast-fed at 17 wk and this did not vary by treatment group.

### Diarrhea

The point prevalence of diarrhea from 0 to 26 wk for each treatment group, as reported by mothers, is shown in **Figure 1**. The prevalence of diarrhea using a definition of  $\geq 3$  liquid or semiliquid stools in the past 24 h is shown in **Figure 2**. The group receiving 5 mg Zn had less diarrhea than the other 2 groups by both definitions (Kruskal-Wallis ANOVA:  $P = 0.029$  for reported diarrhea and  $P < 0.001$  when defined by stool consistency and frequency). After adjustment for socioeconomic variables with multiple linear regression with stepwise entry of confounders (**Table 2**), the longitudinal prevalence of reported diarrhea on average was 28% less with 5 mg Zn than with the placebo, and 48% less when diarrhea was defined as  $\geq 3$  liquid or semiliquid stools in 24 h. Both bloody stools and persistent diarrhea were too rare to permit comparison between groups.

### Cough

The point prevalence of cough for each week from birth to 26 wk is compared in **Figure 3**. All 3 groups experienced increas-



**FIGURE 2.** Point prevalence of diarrhea (defined as  $\geq 3$  liquid or semiliquid stools in preceding 24 h) among the treatment groups (3-wk moving averages derived from daily prevalences).

ing morbidity from cough during the study period, but the group given 5 mg Zn experienced the lowest prevalence throughout ( $P = 0.008$ , Kruskal-Wallis). The results of the multivariate analysis are presented in **Table 2**. The unadjusted longitudinal prevalence ratio was significantly lower in the group receiving 5 mg Zn than in the placebo group ( $P = 0.047$ ), but this ceased to be significant after adjustment for socioeconomic confounding ( $P = 0.073$ ). No significant differences were found for more severe respiratory symptoms, namely cough with fever, cough with raised respiratory rate, and cough with cansaço.

### Vomiting, fever, and poor appetite

No statistically significant differences were found for vomiting, fever, and poor appetite, although the group given 5 mg Zn had slightly lower prevalences for each of these 3 symptoms.

### Mortality, hospitalizations, and consultations

Thirteen infants died, 7 in the placebo and 3 each in the groups given 1 or 5 mg Zn (**Table 3**). Diarrhea and respiratory infections were the major causes of death, and 11 of the 13 deaths were postneonatal. There were no significant differences among the groups in the rates of hospitalizations and medical consultations

**TABLE 2**

Effect of zinc supplementation and placebo on the longitudinal prevalence of diarrhea and cough (0–26 wk), before and after adjustment for socioeconomic status and housing conditions (water supply): multiple linear regression analysis

	Unadjusted longitudinal prevalence ratio, (95% CI)	Adjusted longitudinal prevalence ratio, (95% CI) <sup>1</sup>
Diarrhea (mothers' definition)		
Placebo	1.00	1.00
1 mg Zn	1.00 (0.72, 1.40)	1.05 (0.76, 1.46)
5 mg Zn	0.68 (0.49, 0.95) <sup>2</sup>	0.72 (0.52, 0.99) <sup>3</sup>
Diarrhea: ( $\geq 3$ liquid or semiliquid stools)		
Placebo	1.00	1.00
1 mg Zn	0.84 (0.64, 1.22)	0.89 (0.65, 1.23)
5 mg Zn	0.49 (0.40, 0.76) <sup>4</sup>	0.52 (0.38, 0.72) <sup>4</sup>
Cough		
Placebo	1.00	1.00
1 mg Zn	1.14 (0.72, 1.79)	1.21 (0.78, 1.88)
5 mg Zn	0.64 (0.41, 0.99) <sup>5</sup>	0.67 (0.44, 1.04)

<sup>1</sup> Controlled for water supply.

<sup>2-5</sup> Significantly different from placebo group: <sup>2</sup>  $P = 0.029$ , <sup>3</sup>  $P = 0.043$ , <sup>4</sup>  $P < 0.001$ , <sup>5</sup>  $P = 0.047$ .

**TABLE 3**

Medical consultations, hospitalizations, and mortality rate among the treatment groups before and after adjustment for socioeconomic status and housing conditions

Variable group	Subjects experiencing event <i>n</i> (%)	Unadjusted hazard rate ratio (95% CI)	Adjusted hazard rate ratio (95% CI)	<i>P</i>
Consultations with medical personnel				
Placebo ( <i>n</i> = 66)	31 (47.0)	1.00	1.00	
1 mg Zn ( <i>n</i> = 68)	35 (51.5)	1.09 (0.67, 1.76)	1.21 (0.73, 2.0)	0.462 <sup>1</sup>
5 mg Zn ( <i>n</i> = 71)	27 (38.0)	0.76 (0.45, 1.27)	0.77 (0.46, 1.29)	0.317 <sup>1</sup>
Hospitalizations				
Placebo ( <i>n</i> = 66)	13 (19.7)	1.00	NC <sup>2</sup>	
1 mg Zn ( <i>n</i> = 68)	20 (29.4)	1.73 (0.84, 3.54)	NC	
5 mg Zn ( <i>n</i> = 71)	12 (16.9)	0.97 (0.44, 2.16)	NC	
Mortality rate				
Placebo ( <i>n</i> = 66)	7 (10.6)	1.00	1.00	
1 mg Zn ( <i>n</i> = 68)	3 (4.4)	0.41 (0.11, 1.60)	0.5 (0.13, 2.01)	0.334 <sup>3</sup>
5 mg Zn ( <i>n</i> = 71)	3 (4.2)	0.40 (0.11, 1.57)	0.53 (0.13, 2.14)	0.370 <sup>3</sup>

<sup>1</sup> Controlled for refrigerator ownership and water supply.

<sup>2</sup> No confounders were identified.

<sup>3</sup> Controlled for electric lighting.

(Table 3). Of the 45 infants who were hospitalized, 15 (33%) were hospitalized more than once. Only 2 infants were hospitalized during the neonatal period. The mean ( $\pm$ SD) duration of hospitalization was shorter in the group given 5 mg Zn ( $5.0 \pm 2.7$  d) than in the placebo group ( $7.7 \pm 4.7$ ) and the group given 1 mg Zn ( $8.4 \pm 4.8$  d;  $P = 0.053$ ). Diarrhea and respiratory infections were the main causes of hospitalization.

### Immune function

The PHA skin test was performed to evaluate cell-mediated immunity at 8 wk of age immediately after zinc supplementation ended. The mean ( $\pm$ SD) induration was very similar among the groups [placebo (*n* = 23):  $7.3 \pm 1.3$  mm; 1 mg Zn (*n* = 25):  $7.7 \pm 1.3$  mm; and 5 mg Zn (*n* = 64):  $7.5 \pm 1.4$  mm], and all infants had a positive reaction of  $\geq 5$  mm induration.

### Growth

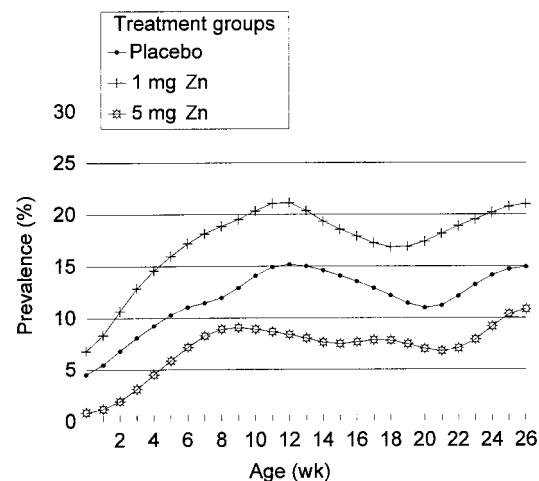
Mean ( $\pm$ SD) weight and length gains from birth to 6 mo in the 3 groups are compared in **Table 4**. There was no significant effect of zinc supplementation on weight gain from 0 to 26 wk. Because a zinc effect on growth was found only for males in some studies, the analyses were repeated for each sex separately. No significant differences from 0 to 26 wk were found for either sex. The greatest difference in weight gain among the groups was between 17 and 26 wk of age. During this period, the group given 5 mg Zn gained on average  $1057 \pm 402$  g compared with  $844 \pm 510$  g for the placebo group ( $P = 0.024$ , ANOVA). No significant differences in length gain were found from 17 to 26 wk.

### DISCUSSION

We hypothesized that 5 mg Zn/d for 8 wk would improve immune function and thereby reduce morbidity and improve growth. The decision to supplement for only 8 wk was based on the supposition that if, in the future, zinc supplements for LBW infants were to be offered through existing health service channels, compliance by caretakers may be of limited duration. Concern regarding external validity therefore prompted the choice of

8 wk. Even though the duration was short, and after adjustment for confounders, supplementation with 5 mg Zn was associated with a 28% difference in the prevalence of reported diarrhea over the first 6 mo of life. The magnitude of the reduction was comparable with the 33% excess in diarrhea prevalence observed in LBW full-term infants in this population (5). The benefits of therapeutic zinc for infants and children with diarrhea, particularly during acute episodes, is well documented (15–19). In our study with prophylactic zinc, when diarrhea did occur the episodes were mostly acute, only 5 infants having persistent diarrhea. Few infants in any group had diarrhea in the first 6 wk of life. The results thus support the findings of Behrens et al (20) that the beneficial effect of zinc on diarrhea extends beyond the actual period of supplementation.

When a clinical definition of diarrhea ( $\geq 3$  liquid or semiliquid stools in the past 24 h) was used, the difference in prevalence with 5 mg Zn was more marked than for maternal reporting (48% compared with 28%). However, we believe the former is an over-



**FIGURE 3.** Point prevalence of cough in the 3 treatment groups (3-wk moving averages derived from daily prevalences).

**TABLE 4**Average weight and length gains from birth to 26 wk of age according to treatment group<sup>1</sup>

	Weight gain	Length gain
	g	cm
Placebo		
Male (n = 19)	4296 ± 1045	17.88 ± 2.64
Female (n = 35)	3895 ± 1063	16.62 ± 2.09
Both sexes (n = 54)	4036 ± 1064	17.06 ± 2.35
1 mg Zn		
Male (n = 26)	4585 ± 1112	17.35 ± 2.91
Female (n = 32)	3977 ± 1022	16.11 ± 2.84
Both sexes (n = 58)	4229 ± 1097	16.67 ± 2.96
5 mg Zn		
Male (n = 24)	4665 ± 725	17.62 ± 1.43
Female (n = 30)	4060 ± 760	15.89 ± 1.60
Both sexes (n = 54)	4329 ± 798	16.67 ± 1.74

<sup>1</sup> $\bar{x} \pm SD$ . There were no significant differences by ANOVA.

estimate because in the early weeks of life, stools tend to be frequent and poorly formed, especially if the infant is breast-fed. This may lead to misclassification. This seems to be the case in Figure 2, where the prevalence of diarrhea in the placebo group and the group given 1 mg Zn in the early weeks appears much higher than the prevalence shown for these groups in Figure 1, based on mothers' report. Interestingly both definitions give similar prevalences for the group given 5 mg Zn, even in the early weeks. We conclude that 5 mg Zn alters stool consistency, possibly by improving intestinal absorption of water, sodium, and potassium (21). We therefore urge those planning zinc supplementation trials in very young infants to use reported diarrhea and not a clinical definition based on stool consistency.


Infants given 5 mg Zn had the lowest prevalence of cough throughout the 6-mo period and a significant difference was observed for the unadjusted prevalence. This effect, however, disappeared after adjustment for confounders. Interestingly, vitamin A supplementation appears to have similar differential effects on morbidity, having a positive effect on diarrhea and no effect on respiratory infections (22). Two studies have investigated the effect of zinc supplementation on respiratory infections in older children, but results have been conflicting. In Mexico, a significant beneficial effect was found in preschool children (23) but in Guatemala no effect was found (24). Both studies were placebo-controlled, randomized trials.

Weight gain, but not length gain, was higher in infants receiving 5 mg Zn than in the placebo group, but the difference was not significant except for weight gain during weeks 17–26. This effect,  $\approx 10$  wk after supplementation ended, may well have been a chance finding. Alternatively, initial zinc status in the placebo infants may have been marginally adequate while they were still breast-fed, but later on demand may have outstripped supply. Hepatic zinc metallothionein, which may act as a zinc reserve in young infants (25), usually declines during early postnatal life. Supplementation with 5 mg Zn in the first 8 wk may thus have conserved some of this reserve. The lack of effect of zinc on linear growth may have been because only 27% of infants in the group given 5 mg Zn were born stunted. No effect of zinc supplementation on appetite was detected. The growth results differ in some respects from the findings of Castillo-Duran et al in Chile (26), where full-term infants born small for gestational age showed significantly greater gains in length and weight when

supplemented with 3 mg Zn/d for 6 mo.

All infants showed a positive response to PHA at 8 wk. With hindsight, it might have been preferable to use a more sensitive test of immune function because PHA is a strong antigen and may have provoked a response even though there may have been impairments in some key components of the immune system.

As far as we know, this is the first zinc supplementation trial in full-term LBW infants with morbidity as an outcome. Because zinc is toxic, there is concern about the dosage appropriate for LBW infants. In our study, 1 mg Zn/d for 8 wk was ineffective. Infants who received 1 mg Zn were randomly assigned to this treatment group and the trial was placebo controlled and double blind. We are therefore confident in concluding that this dosage is inappropriate. Our 5-mg daily dose was associated with a reduction in diarrhea morbidity and was well tolerated, but the infants in this group were not randomly assigned. We therefore cannot state unequivocally that zinc was the causal factor. Nevertheless, we believe that this was so for the following reasons: 1) the group given 5 mg Zn had socioeconomic and demographic characteristics comparable with those of the other groups, 2) the macroenvironment was similar in 1993 and 1994, and 3) a cohort of control infants (birth weight: 3000–3499 g) recruited during 1994 had weight and length gains that were comparable with those of a cohort of infants of the same birth weight recruited from the same maternity centers in 1993. We are confident that the infants in all 3 groups were delivered at term. Intensive morbidity surveillance and data quality checks throughout the study gave us confidence as to the reliability of the data.

We conclude that zinc supplementation is likely to be advantageous for LBW infants in northeast Brazil, as well as in poor communities in other countries. Outstanding questions include whether the morbidity effect might be even greater if the supplement were to be given for longer, were timed to coincide with the age at peak diarrhea prevalence, or both. The magnitude of the effect under operational conditions also needs elucidating. 

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