Zinc Supplementation Improves the Growth of Stunted Rural Guatemalan Infants[1–3]

Juan A. Rivera,*,[4] Marie T. Ruel,† Maria Claudia Santizo,** Bo Lönnerdal‡ and Kenneth H. Brown‡

*National Institute of Public Health, Av. Universidad 655, C.P. 62508, Cuernavaca, Morelos, Mexico; †International Food Policy Research Institute, Washington, DC 20036-3006; **Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala; and ‡Program in International Nutrition and Department of Nutrition, University of California, Davis, CA 95616

ABSTRACT The impact of zinc supplementation on the growth and body composition of Guatemalan infants was assessed in a community-based, double-blind intervention trial. Infants aged 6–9 mo were assigned randomly to receive 4 mL of a beverage containing 10 mg of zinc as zinc sulfate ($n = 45$) or a placebo ($n = 44$) daily (7 d/wk) for an average of 6.9 mo. The children's weight, length, mid-upper arm and head circumferences, and triceps skinfolds were measured at baseline and at 1–2 mo intervals until the end of supplementation. Midarm muscle area (MMA) was derived from the mid-upper arm circumference and triceps skinfolds measurements. Maternal anthropometry and family socioeconomic and demographic characteristics also were obtained. Zinc supplementation was associated with an overall increase of 0.61 cm^2 in MMA ($P = 0.02$). Children who received zinc supplements had a mean length increment that was 0.75 cm greater than those who did not ($P = 0.12$). However, there was a significant interaction between treatment group and initial length-for-age status ($P = 0.04$), such that supplemented children who were stunted at baseline (length-for-age Z score less than -2) gained 1.40 cm more than stunted children who received the placebo. We conclude that zinc supplementation of these rural Guatemalan infants during 6.9 mo increased accretion of fat-free mass and enhanced the linear growth of those who were stunted at baseline. Further research is required to determine whether zinc supplementation during longer periods of time may achieve larger and more generalized effects on physical growth. J. Nutr. 128: 556–562, 1998.

KEY WORDS: • *zinc* • *growth* • *Guatemalan infants* • *micronutrients* • *supplementation trial*

Growth retardation continues to be highly prevalent among children in low-income countries. About 43% of children <5 y of age in these settings (230 million) are stunted (de Onis et al. 1993) as defined by a height-for-age less than −2 SD with respect to the WHO/NCHS/CDC reference population (World Health Organization 1979). In Guatemala, the national prevalence of stunting is 58% (Institute of Nutrition of Central America and Panama 1992), among the highest in the world (de Onis et al. 1993). Infections (Rivera and Martorell 1988) and inadequate food intakes (Habicht et al. 1995) are well-established causes of stunting; however, the possible role of individual micronutrient deficiencies, particularly of zinc, in the etiology of growth retardation has gained attention recently.

A number of experimental trials testing the effects of zinc supplements or zinc-fortified foods on the growth of children has been conducted in the last 20 y. However, results of these trials are inconsistent. Positive effects of zinc supplementation on growth have been documented in infants from low-income families living in the U.S. (Walravens and Hambidge 1976) and France (Walravens et al. 1992); in preschool children from Ecuador (Dirren et al. 1994) and from poor families in the U.S. (Walravens et al. 1983 and 1989); in school-aged boys with low height for age in Canada (Gibson et al. 1989) and Chile (Castillo-Durán et al. 1994) and school-aged boys and girls from Iran (Ronaghy et al. 1974); in children recovering from severe malnutrition in Bangladesh (Khanum et al. 1988) and in Chile (Schlesinger et al. 1992); and in Bangladeshi children supplemented during diarrhea (Behrens and Tomkins 1990). However, other studies of preschool children with suspected zinc deficiency living in The Gambia (Bates et al. 1993) and Mexico (Rosado et al. 1997), of preschool and school-aged children from the U.S. (Hambidge et al. 1979) and of school-aged children in Guatemala (Cavan et al. 1993) did not find effects on growth. Although little is known about the zinc status of children in rural Guatemala, it is clear that the rural diet in Guatemala, based primarily on corn tortillas, impairs zinc absorption (Solomons et al. 1979) and that animal

---

[1] Preliminary results were presented at the Experimental Biology Meeting in 1995. [Rivera, J., Brown, K. H., Santizo, M. C., Ruel, M. & Lönnerdal, B. (1995) Effects of zinc supplementation on the growth of young Guatemalan Children. FASEB J. 9: A164 (abs. 956).]

[2] Supported by the University of California, Davis-Institute of Nutrition of Central America and Panama Institutional Linkage Project, the U.S. Agency for International Development (Cooperative Agreement No. DAN-5063-A-00-1115-00). Additional funding was obtained from the Thrasher Research Fund (principal investigator: Margaret Bentley) and from INCAP.

[3] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[4] To whom reprint requests should be addressed.

0022-3166/98 $3.00 © 1998 American Society for Nutritional Sciences.
Manuscript received 11 April 1997. Initial review completed 29 May 1997. Revision accepted 26 November 1997.

products, the principal sources of zinc, are not commonly consumed (Fitzgerald et al. 1993). Qualitative dietary information in the study population indicated that apart from breast milk (consumed by all study children), only five foods were consumed by a large proportion (>20%) of infants. These foods are tortilla (consumed by 72%), wheat bread (72%), coffee with sugar (43%), pan dulce—a biscuit-like sweet bread containing wheat, oil or shortening and sugar—(41%) and rice (28%). None of these foods is a good source of zinc; moreover, tortilla, the most frequently consumed food after breast milk, which accounts for 26% of the total energy intake of these infants, is high in phytate and calcium, both of which inhibit the absorption of zinc. Therefore, it is likely that zinc may be a limiting factor for the growth of rural Guatemalan children.

Diagnosis of zinc deficiency is limited by the lack of a specific and sensitive laboratory indicator of status (Gibson 1994). In addition, drawing blood samples from infants to determine plasma zinc concentration is not acceptable to most families in the study population. Therefore response to zinc supplementation remains the most valid approach to testing for zinc deficiency.

To test the hypothesis that zinc may be a limiting factor for the growth of rural Guatemalan children, a randomized double-blind study of 89 infants 6–9 mo of age was conducted. This age range was selected because it is the period when infants are becoming progressively more stunted with respect to the international reference population, and it is the time when complementary foods that may interfere with zinc absorption are introduced into the children's diets.

MATERIALS AND METHODS

Sample and design. The study was conducted in Santa Maria de Jesus, a rural Indian community in central Guatemala. The village is located 55 km from Guatemala City at an altitude of 2050 m above sea level. The population is estimated at 16,000 inhabitants, most of whom are Mayan Indians of the Cackchiquel linguistic group. More than 80% of the male heads of household are engaged in subsistence agriculture, water supply is scarce, the rates of diarrheal diseases are high (Cruz et al. 1992), and >60% of women are nonliterate.

The research team completed a census of the study village to identify children from 6 to 9 mo of age. A total of 108 children whose parents agreed to participate in the study were enrolled. The study protocol was explained in detail to each family, and oral consent in the presence of a witness was obtained. The protocol was approved by the Committees on the Use of Human Subjects in Research of the Institute of Nutrition of Central America and Panama (INCAP) and the University of California, Davis.

The study was a randomized, double-blind, placebo-controlled supplementation trial. Children received a daily dose 7 d/wk of 4 mL of a beverage that either did or did not contain 10 mg of zinc as zinc sulfate. The two beverages were indistinguishable and both contained sugar, citric acid, and artificial flavors.

The supplement was distributed daily for an average of 6.9 mo (range 4–8 mo) to the study participants. Originally we had planned to recruit ~33 children per month during a 3-mo period to achieve a target sample of ~100 children who were to be supplemented during 6 mo. However, we could not meet the monthly target and actually employed 5 mo for recruitment. Funding restrictions did not allow achieving 6 mo of supplementation to the groups of children who were recruited during the final 2 mo. Therefore four children were supplemented for 4 mo and six were supplemented during 5 mo. The rest of the children were supplemented for 6 (n = 33), 7 (n = 28) or 8 (n = 18) months. The field workers visited the houses early in the morning at a time when children had usually not consumed foods other than breast milk. If the child was asleep, the field worker returned to the house later during the day to ensure that the child consumed the supplement in her presence. The supplement distributors were instructed to provide the supplement between meals; however, this was not always possible. Distributors administered the supplement themselves or observed when mothers gave the beverage. Average compliance was 95%, and there were no differences between study groups.

Data collection. Baseline data were collected on child anthropometry and maternal weight and height; child's intake of breast milk and complementary foods; and child's appetite and physical activity patterns.

After the baseline measurements, child anthropometry was measured approximately at 1.5 and 3 mo and monthly thereafter until the end of supplementation. Measurements included: weight (to the nearest 5 g) using a beam scale, length (to the nearest mm) using a locally made wooden measuring board, mid-upper arm and head circumferences (to the nearest mm) using a flexible steel tape, and triceps skinfolds (to the nearest 0.1 mm) using a Holtain caliper (Holtain, Crosswell, Crymmych, Dyfed, Wales). Midarm muscle area (MMA)[5] was derived from mid-upper arm circumference and triceps skinfolds measurements (Frisancho 1981). Field workers were trained and standardized to take all measurements using standard techniques (Habicht 1974, Lohman et al. 1988). Technical errors of measurement (TEM) at the end of the standardization period were within values reported for carefully conducted studies such as the Fels Longitudinal Study (Lohman et al. 1988). For example, the intermeasurer TEM for the different field workers ranged between 1.2 and 2.7 mm for length and between 2.5 and 3.3 mm for mid-upper arm circumferences. Length and weight data were transformed to Z scores with the WHO/NCHS/CDC reference data (World Health Organization 1979).

Morbidity information was collected daily at the time of distribution of the supplement. A check list was used to record symptoms of illnesses observed during the last 24 h. Results of the morbidity observations are presented elsewhere (Ruel et al. 1997).

Data also were collected on children's dietary intake (direct observation and weighing), breast milk consumption (test-weighing), and appetite and physical activity, using 12-h observations in the homes at baseline and after 3 and 7 mo of follow-up. The activity data have been reported separately (Bentley et al. 1997)

Family socioeconomic and demographic characteristics were collected at the end of the study. Information was obtained on household composition, maternal age and parity, family possessions, quality of housing, hygienic facilities and parents' education.

Sample size. We hypothesized that zinc is a limiting factor for the growth of young rural Guatemalan children; therefore we expected that zinc supplementation would have a positive effect on the growth of study children. We also postulated differential effects by gender and initial degree of stunting.

Sample size calculations were based on an estimated impact on length of 1 cm, based on studies by Walravens and Hambidge (1976) and Xue-Cun et al. (1985). Using a two-tail test with an alpha level of 0.05 and a power of 90%, a sample of 42 children per group was calculated. Allowing for attrition, a total of 108 children were recruited. Nineteen children dropped out of the study due to parent's reluctance to participate, maternal work, migration or difficulties in complying with project requirements. Nine children who withdrew were from the placebo group and 10 from the zinc-supplemented group. Therefore the total number of children who concluded the study was 89; most children who failed to complete the study dropped out before supplementation began. Baseline characteristics of dropouts (anthropometry, feeding practices and maternal characteristics) did not differ from those of children who completed the study (t test results, data not shown).

Data analysis

Socioeconomic status score. A socioeconomic status score was derived by principal components analysis as one factor (standardized variable, mean = 0; SD = 1). Only variables with factor loadings >0.5 were maintained in the model. These included: house and floor material, number of rooms, sanitary facilities and level of poverty of the family. The latter variable was based on a community-specific

---

[5] Abbreviations used: LAZ, length-for-age Z score; MMA, midarm muscle area; TEM, technical errors of measurement.

ranking of families from poorer (score = 1) to wealthier (score = 4) by field workers who were residents of the community. Field workers were trained and standardized by an anthropologist in ranking families, based on predetermined, locally appropriate markers of wealth.

Statistical analyses. Characteristics of study children, of their mothers and of their families at baseline were compared between treatment groups by means of Student's *t* test for continuous variables and chi-square test for categorical variables, to identify potential confounding variables.

Ordinary least squares (OLS) regression analysis was used to test the treatment main effect on length, weight, mid-upper arm circumference, head circumference, triceps skinfolds and arm muscle area, adjusting for anthropometric measurements and indices at baseline, for other covariates and for potential confounding variables. Regression analyses were also used to test two-way interactions between treatment and gender, treatment and mother's schooling, treatment and socioeconomic score and treatment and the baseline value of the outcome variable for each model (i.e., initial length, weight or MMA) on their effects on the outcome (i.e., final length, weight or MMA). The models were used to compute least squares means final lengths for the categories resulting from combining initial stunting category and treatment groups.

The socioeconomic score was not included in the final models because it did not reach statistical significance. In the models that had final length as the outcome variable, least squares mean final lengths were computed according to a categorical variable indicating whether children were at or above -2 length-for-age *Z* score or below that cut off point. *Z* scores rather than absolute length values were used because the former already are adjusted for age and gender. The cut-off point (-2 *Z*) was selected because it is widely used in public health as an indication of stunting and because it corresponded to about the mean baseline value in the group studied.

Probability values <0.05 were considered statistically significant for main effects and <0.10 for interactions.

Analyses were performed using PC-SAS Statistical Program, version 6.04 (SAS Institute, Cary, NC).

RESULTS

Table 1 presents characteristics of the mothers and families of study children at baseline and the dietary energy intake, days exposed to supplementation and gender distribution of the children by treatment groups. The groups were very similar in most variables; however, in the zinc-supplemented group, there was a larger proportion of boys and mothers had more schooling relative to the placebo group ($P < 0.05$). Therefore the regression models used to assess the treatment effect included gender and maternal schooling because they were potentially confounding variables. No other variables were statistically significantly different between groups.

Age and anthropometric variables and indices of children in each experimental group at the baseline and final evaluations and the increments between baseline and final values are presented in **Table 2**. The increments in midarm muscle area, which increased from baseline to final evaluations by 1.2 cm^2 in the supplemented group and 0.6 cm^2 in the placebo group, tended to differ ($P < 0.1$). None of the other anthropometric variables and indices at the baseline and final evaluations nor the increments were different between treatment groups.

Table 3 presents results from OLS regression analysis for the effects of supplementation on length and on MMA. The 7.5 cm adjusted difference in final length between the zinc and the placebo group was not statistically significant. However, the interaction between treatment group and initial length for age was significant ($P = 0.04$), indicating that the difference in final length between treatment groups was different for the two categories of initial length for age. The adjusted difference in final MMA between the zinc and the placebo group was 0.61 cm^2, which was significant.

TABLE 1

Comparison of mother's anthropometric measurements and schooling, family socioeconomic status and children's dietary energy intake, days of supplementation and gender in the zinc-supplemented and placebo groups[1]

Variables	Placebo (*n* = 44)	Zinc supplemented (*n* = 45)
Continuous variables		
Mother's stature, *cm*	144.7 ± 5.0	144.6 ± 5.0
Mother's weight, *kg*	50.4 ± 6.7	53.2 ± 8.1
Mother's schooling, *y*	0.95 ± 1.74	1.80 ± 2.30*
Family socioeconomic status, factor score[2]	0.07 ± 0.95	−0.06 ± 1.05
Children's energy intake[3], *kJ · kg body* $wt^{-1} \cdot d^{-1}$	360 ± 130	326 ± 79
Supplementation, *d*	190 ± 31	188 ± 31
Categorical variables		
Percent boys	45	69*
Percent mothers without schooling	73	51*

[1] Values are means ± SD. * $P < 0.05$. Differences between means (*t* test) and differences between frequency distributions (chi-square test).

[2] Derived by principal components analysis. Variables in the model included: house and floor material, number of rooms, sanitary facilities, and a community-specific poverty ranking of families by field workers who were residents of the community.

[3] Total energy intake, includes food and breast milk.

Results from the other models tested are not presented because none of the adjusted differences between treatment groups nor the two-way interactions tested were statistically significant for weight, mid-upper arm circumference, triceps skinfolds or head circumference.

The magnitude of the differences between treatment groups by initial length for age categories are presented in **Figure 1**. For children with length-for-age *Z* score less than -2 SD of the reference population at baseline, those receiving the supplement had an adjusted final length 1.4 cm greater than children receiving the placebo ($P < 0.05$). In contrast, for children with length-for-age *Z* score at or more than -2 SD, the difference (0.35 cm in favor of the placebo group) was not significant.

Characteristics of mothers, families and children by stunting category are presented in **Table 4**. As expected, the families of stunted children had lower socioeconomic status and their mothers were shorter ($P < 0.05$). All other variables did not differ between groups.

Table 5 presents the differences in attained length between treatment groups at the end of various measurement intervals among the subgroup of infants who were stunted at baseline. The attained lengths and their differences are adjusted for length and age at the beginning of the measurement interval and for the interval duration. Information is presented only for the first six intervals. Information about the two last measurement intervals (195–210 and 210–240 d) is not presented due to the small number of cases (≤ 13 per treatment group). Because measurement intervals differed in duration, straight differences across intervals are not comparable; therefore, the last column in the table presents monthly differences, which are comparable across intervals. None of the differences was significant although that corresponding to the first interval (0–45 d) tended to be significant ($P < 0.10$).

DISCUSSION

Daily administration of 10 mg elemental zinc to 6- to 9-mo-old infants for an average of almost 7 mo positively affected

TABLE 2

Comparison of age and anthropometric variables and indices of children in the zinc-supplemented and placebo groups at the baseline and final evaluations[1]

	Placebo (n = 44)			Zinc supplemented (n = 45)		
Variables	Baseline	Final	Difference[2]	Baseline	Final	Difference[2]
Age, *mo*	7.51 ± 0.96	14.39 ± 1.75	—	7.73 ± 1.17	14.56 ± 1.71	—
Length, *cm*	63.5 ± 3.0	69.2 ± 3.7	5.7 ± 2.0	63.9 ± 2.9	70.0 ± 3.0	6.1 ± 2.0
Weight, *kg*	7.16 ± 1.01	8.12 ± 1.05	0.97 ± 0.51	7.22 ± 0.89	8.25 ± 0.95	1.03 ± 0.54
Head circumference, *cm*	42.7 ± 1.6	44.7 ± 1.5	1.9 ± 0.8	43.0 ± 1.6	45.0 ± 2.2	2.0 ± 2.0
Mid-upper arm circumference, *cm*	14.1 ± 1.3	14.1 ± 1.1	0.0 ± 1.0	14.0 ± 1.0	14.2 ± 1.1	0.2 ± 0.8
Triceps skinfolds, *mm*	9.3 ± 1.6	8.4 ± 1.7	−0.9 ± 1.8	9.2 ± 1.8	7.8 ± 1.7	−1.3 ± 2.0
Midarm muscle area, cm^2	10.0 ± 2.1	10.6 ± 1.6	0.6 ± 1.9	9.9 ± 1.8	11.1 ± 1.8	1.2 ± 1.7
Length-for-age, *Z* score[3]	−2.11 ± 1.02	−3.00 ± 1.16	−0.89 ± 0.74	−2.20 ± 0.80	−2.94 ± 0.84	−0.74 ± 0.68
Weight-for-age, *Z* score	−1.11 ± 1.09	−2.07 ± 0.90	−0.96 ± 0.56	−1.24 ± 0.86	−2.11 ± 0.82	−0.87 ± 0.55
Weight-for-length, *Z* score	0.75 ± 1.07	−0.09 ± 0.80	−0.84 ± 0.88	0.63 ± 0.89	−0.28 ± 0.76	−0.91 ± 0.78

[1] Values are means ± SD.
[2] Difference between baseline and final values (final-baseline).
[3] *Z* scores calculated using the WHO/NCHS/CDC reference data (WHO 1979).

length increments of infants who were initially stunted. The 1.4-cm increase in length gain of these zinc supplemented–stunted infants is considered biologically important because it is equivalent to slightly more than one-half of a standard deviation of expected linear growth for children of this age or 15% of the expected growth in length from 7 to 14 mo. The effect also is considered large given the duration of supplementation when compared with other well-documented interventions. For example, well-controlled supplementary feeding trials have identified effects on the order of 2.5 cm during a 3-y period (Habicht et al. 1995), although most of the effect occurred during the first 2 y of life (Schroeder et al. 1995).

Finding effects only in the most stunted children is biologically plausible. Severe stunting was associated with lower socioeconomic status (Table 4), which may be associated with poor diets and high infection rates, thus increasing risk of zinc deficiency. Other studies have found effects on children selected on the basis of their short stature or with evidence of zinc deficiency (Castillo-Durán et al. 1994, Gibson et al. 1989, Ninh et al. 1996, Walravens et al. 1983).

We further investigated the nature of the response to zinc supplementation in the subsample of stunted infants. We found that the differences in height increment between groups were not significant. The response was greater in the first 45 d of supplementation (6.1 mm/mo) than in the rest of the measurement intervals (range −1.9–4.4 mm/mo). However, there was not a clear decreasing trend after 45 d of supplementation. The lack of statistical significance is not surprising, because the number of children in each treatment group varied between 15 and 28 for the different age categories. These numbers are insufficient to test differences of the magnitude expected in the time intervals under study.

Finding restricted effects only in the most stunted children was unexpected given that the baseline mean length for age Z score was less than −2, which indicates that most children had some degree of linear growth deficit. This is particularly

TABLE 3

Multiple regression models of treatment, baseline length-for-age Z score and their interaction on final length and midarm muscle area adjusting for covariates and potential confounding variables

Dependent variable	Length, *cm*				Midarm muscle area, cm^2	
	Main effects model		Interactive model		Main effects model	
Independent variables	β	*P*	β	*P*	β	*P*
Intercept	57.63	0.0001	58.18	0.0001	9.70	0.0001
Treatment[1]	0.75	0.1189	−0.35	0.6129	0.61	0.0209
LAZ category[2]	−3.93	0.0001	−4.87	0.0001	−1.58	0.0001
Age, *mo*	0.98	0.0001	0.98	0.0001	—	—
Gender[3]	−1.05	0.0288	−1.21	0.0117	−1.03	0.0002
Weight-for-length, *Z* score	0.98	0.0002	0.99	0.0001	0.74	0.0001
Mother's schooling[4]	−1.12	0.0234	−1.08	0.0245	—	—
Treatment · LAZ[2]	—	—	1.91	0.0367	—	—
Baseline MMA[5]	—	—	—	—	0.17	0.0002
Adjusted *R*-square	0.64		0.66		0.54	

[1] Zinc = 1, placebo = 0.
[2] LAZ, length-for-age *Z* score. Categories less than −2 = 1; more than or equal to −2 = 0.
[3] Girls = 1, boys = 0.
[4] No schooling = 1, some schooling = 0.
[5] Midarm muscle area (cm^2).

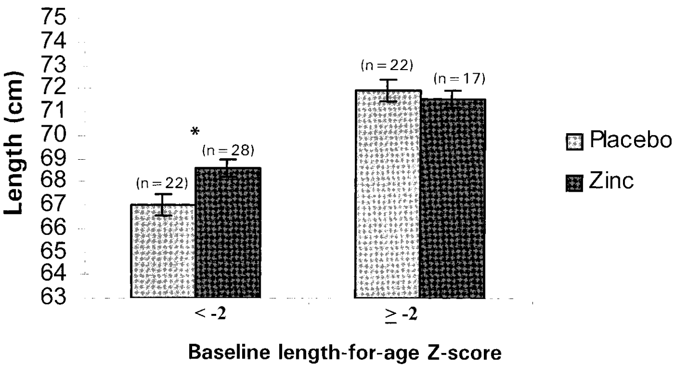

FIGURE 1 Adjusted final length (cm) by length-for-age *Z*-score categories at baseline and by treatment. Adjusted final lengths are least squares means ± SEM computed using ordinary least squares regression analysis. Age, gender, weight-for-length *Z* score and mother's schooling were adjusted for in the model. *The interaction between length-for-age *Z*-score categories and treatment was significant ($P < 0.05$).

important given the young age of the study children at admission. We had anticipated a larger overall main effect (~1 cm), based on results from previous studies (Dirren et al. 1994, Walravens and Hambidge 1976, Walravens et al. 1983 and 1992), on the evidence that the rural diet in Guatemala impairs zinc absorption (Solomons et al. 1979) and on the fact that supplementation took place at ages when stunting is most active in this population. Nevertheless, the difference of 0.75 cm observed between experimental groups is considered biologically important; because it amounts to more than one-quarter of a standard deviation in length for the age ranges of the study children. However, the study lacked the power to test this difference. Potential confounding variables are an unlikely explanation of the small magnitude of the main effect found, given the experimental and double-blinded design of the study and that we controlled for potential confounding factors in the analysis. The amount of zinc provided is also an unlikely explanation. Although we do not have quantitative information about the zinc content of the children's diet, qualitative dietary information presented in the introduction suggests low zinc intake. Therefore we provided a sufficient amount of zinc (10 mg) to cover the U.S. recommended intakes (National Research Council 1989) for the age range of study (6–16 mo): 5 mg for infants and 10 mg for children ≥1 y. Previous studies that documented effects on linear growth provided similar or lower amounts of zinc than our study (Dirren et al. 1994, Walravens and Hambidge 1976, Walravens et al. 1983 and 1992).

We cannot rule out the possibility that uptake of supplemental zinc was inhibited by foods that have high content of phytate and other inhibitors of zinc absorption, rendering the amount of zinc in the supplement insufficient to achieve the recommend intake. Although the supplement distributors were instructed to provide the supplement between meals to avoid supplementing during the postabsorptive state, in practice this was not always feasible. As mentioned earlier, tortillas, a corn-based staple food with high content of zinc absorption inhibitors (phytate and calcium), was consumed by 72% of the children. The average amount consumed per day was 33 g and accounted for 36% of the total energy intake. It is possible that children sometimes may have consumed tortillas immediately before or after the supplement administration some times in the course of supplementation. However, even at a net (apparent) absorption as low as 5%, the amount of daily supplement provided would be enough to meet the zinc required for growth (Hambidge 1997). Therefore, occasional consumption of inhibitors is an unlikely explanation of the lack of statistical significance of the main effect on growth in our study. We avoided providing large amounts of zinc to prevent potential interference with the absorption of other micronutrients.

Other investigators who have documented effects within 6 mo of supplementation studied younger infants (Friel et al. 1993, Walravens and Hambidge 1976, Walravens et al. 1992). Studies that supplemented for >6 mo showed more pronounced effects after 6 mo of supplementation (Dirren et al. 1994). It is therefore possible that greater effects would have been observed after a longer period of supplementation. Another possible explanation for the small effect is the existence of other limiting factors not corrected by our intervention.

No effects were observed on weight, mid-upper arm circumference and triceps skinfolds. The only main treatment effect found was in MMA, a gross indicator of fat-free mass. The greater increment in MMA associated with zinc supplementation resulted from a combination of a larger reduction in the triceps skinfold and a larger increment in mid-upper arm circumference in the supplemented group. Older infants generally become leaner with age. Therefore reduction in triceps skinfold is not uncommon during the ages of the infants in our study, particularly in populations at risk of malnutrition. For example, Peruvian infants with growth patterns similar to children in our study experienced reductions in triceps and subscapular skinfolds between 6 and 18 mo of age (Trowbridge et al. 1987). The larger decrease in skinfolds and increase in mid-upper arm circumference in supplemented children may be interpreted as greater accretion of lean tissue, which has been observed in children recovering from severe malnutrition who were supplemented with zinc (Golden and Golden 1981b). Zinc supplementation in these children was associated with a reduction in the energy cost of tissue deposition due to greater lean tissue synthesis and less adipose tissue accretion.

TABLE 4

Comparison of mother's anthropometric measurements and schooling, family socioeconomic status and children's dietary energy intake, days of supplementation and gender in stunted and not stunted children[1,2]

Variables	Stunted (n = 50)	Not stunted (n = 39)
Continuous variables		
Mother's stature, *cm*	143.6 ± 5.1	146.1 ± 4.5*
Mother's weight, *kg*	51.1 ± 7.4	52.8 ± 7.8
Mother's schooling, *y*	1.14 ± 1.88	1.69 ± 2.28
Family socioeconomic status, factor score	−0.22 ± 0.86	0.28 ± 1.11*
Children's energy intake,[3] $kJ \cdot kg\ body\ wt^{-1} \cdot d^{-1}$	251 ± 126	331 ± 84
Days exposed to supplementation	182 ± 27	176 ± 37
Categorical variables		
Percent boys	56	59
Percent mothers without schooling	63	58

[1] Values are means ± SD or percent. * $P < 0.05$. Differences between means (*t* test) and differences between frequency distributions (chi-square test).

[2] Stunted (length-for-age *Z* score less than −2). Not stunted (length-for-age *Z* score more than or equal to −2).

[3] Total energy intake, includes food and breast milk.

TABLE 5

Attained length (mm) by treatment at the end of various measurement intervals and differences between treatments in infants who were stunted at baseline[1]

Measurement intervals, d	Adjusted attained length, *mm*[2]				Length difference, *mm*	
	n	Zinc	*n*	Placebo	Absolute	Per month
0–45	25	638.2 ± 3.1	22	629.1 ± 3.3	9.1	6.1
45–90	25	645.7 ± 3.2	21	641.9 ± 3.5	3.8	2.5
90–120	28	655.1 ± 3.3	21	651.2 ± 3.9	3.9	3.9
120–150	26	662.8 ± 4.3	21	660.0 ± 4.9	2.8	2.8
150–180	25	664.8 ± 3.1	19	666.7 ± 3.6	−1.9	−1.9
180–195	23	669.9 ± 3.3	15	667.7 ± 4.5	2.2	4.4

[1] Values are means ± SD.

[2] Adjusted for length and age at the beginning of the measurement interval and for the interval duration.

In addition, zinc supplementation of children recovering from severe malnutrition has been associated with greater net absorption of nitrogen and higher rate of protein turnover (Golden and Golden 1981a, 1981b and 1992). Our results suggest that in addition to its effect on linear growth, zinc supplementation may have had a positive effect on lean mass not restricted to the most stunted children.

Possible mechanisms explaining the effects of zinc supplementation on growth are decreased morbidity, increased efficiency in the use of dietary protein and energy or increased appetite and food intake. There is evidence in the literature supporting the first two possible mechanisms. We (Ruel et al. 1997) and others (Ninh et al. 1996, Rosado et al. 1997, Sazawal et al. 1995) have found an impact of zinc supplementation on the incidence of diarrhea. However, in previous analyses, we found that the reduction in diarrheal incidence was not restricted to stunted children, suggesting that the mechanisms by which zinc supplementation affects growth and morbidity may be different. Changes in the efficiency of use of nutrients were not measured in our study. However, as mentioned earlier, there is evidence of increased use of energy and protein resulting in reduced energy cost of tissue deposition (Golden and Golden 1981a and 1981b). Finally, we did not find evidence of increased total energy intake (Santizo et al. 1995) as a result of zinc supplementation. Similar negative findings were reported in zinc-supplemented children recovering from severe malnutrition (Golden and Golden 1992).

We found a positive effect of zinc supplementation for 6.9 mo on the linear growth of stunted rural Guatemalan children aged 6–9 mo. Also, an overall group effect was documented on the MMA, suggesting effects of zinc supplementation on accretion of fat-free mass. The results indicate that interventions aimed at improving the zinc status of young Guatemalan children may have important effects on growth, particularly if the interventions are focused on the most stunted children. Further research is required to explore if supplementation during longer periods of time may achieve larger and more generalized effects. In addition, the possibility that nutrients other than, or in addition to, zinc may be limiting the growth of children should be evaluated. A possible application of our findings to public health programs is the use of stunting for the selection of beneficiaries of interventions involving supplementation or food fortification with zinc.

ACKNOWLEDGEMENTS

We thank Humberto Mendez and his team for their assistance with data processing, Dora Ines Mazariegos for preparation of the supplement, and Margarita Garcia for supervision of the field work.

LITERATURE CITED

Bates, C. J., Evans, P. H., Dardenne, M., Prentice, A., Lunn, P. G., Northrop-Clewes, C. A., Hoare, S., Cole, T. J., Horan, S. J., Longman, S. C., Stirling, D. & Aggett, P. J. (1993) A trial of zinc supplementation in young rural Gambian children. Br. J. Nutr. 69: 243–255.

Behrens, R. H. & Tomkins, A. M. (1990) Zinc supplementation during diarrhoea, a fortification against malnutrition. Lancet 336: 442–443.

Bentley, M. E., Caulfiled, L. E, Ram, M., Santizo, M. C, Hurtado, E., Rivera, J. A., Ruel, M. T. & Brown K. H. (1997) Zinc supplementation affects the activity patterns of rural Guatemalan infants. J. Nutr. 127: 1333–1338.

Castillo-Durán, C., Garciía, H., Venegas, P., Torrealba, I., Panteon, E., Concha, N. & Pérez, P. (1994) Zinc supplementation increases growth velocity of male children and adolescents with short stature. Acta Paediatr. 83: 833–837.

Cavan, K. R., Gibson, R. S., Grazioso, C. F., Isalgue, A. M., Ruz, M. & Solomons, N. W. (1993) Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. Am. J. Clin. Nutr. 57: 344–352.

Cruz, J. R., Bartlet, A. V., Mendez, H. & Sibrian, R. (1992) Epidemiology of persistent diarrhea among Guatemalan rural children. Acta Paediatr. 381 (suppl.): 22–26.

de Onis, M., Monteiro, C., Akré, J. & Clugston, G. (1993) The worldwide magnitude of protein-energy malnutrition: an overview from the WHO global database on child growth. WHO Bull. 71: 703–712.

Dirren, H., Barclay, D., Ramos, J. G., Lozano, R., Montalvo, M. M., Dávila, N. & Mora, J. O. (1994) Zinc supplementation and child growth in Ecuador. In: Nutrient Regulation during Pregnancy, Lactation, and Infant Growth (Allen, L., King, J. & Lönnerdal, B., eds.) Plenum Press, New York. pp. 215–222.

Fitzgerald, S. L., Gibson, R. S., Quan Serrano, J., Portocarrero, L., Vasquez, A., Zepeda, E., Lopez-Palacios, C. Y., Thompson, L. U., Stephen, A. M. & Solomons, N. (1993) Trace element intakes and dietary phytate/Zn and Ca × phytate/Zn millimolar ratios of periurban Guatemalan women during the third trimester of pregnancy. Am. J. Clin. Nutr. 57: 195–201.

Friel, J. K., Andrews, W. L., Matthew, J. D., Long, D. R., Cornel, A. M., Cos, M., McKim, E. & Zerbe, G. O. (1993) Zinc supplementation in very-low–birth-weight infants. J. Pediatr. Gastroenterol. 17: 97–104.

Frisancho, A. R. (1981) New norms for upper limb fat muscle area for assessment of nutritional status. Am. J. Clin. Nutr. 34: 2540–2545.

Gibson, R. S. (1994) Zinc nutrition in developing countries. Nutr. Res. Rev. 7: 151–173.

Gibson, R. S., Smit Vanderkooy, P. D., MacDonald, A. C., Goldman, A., Ryan, B. A. & Berry, M. (1989) A growth-limiting, mild zinc deficiency syndrome in some Southern Ontario boys with low height percentiles. Am. J. Clin. Nutr. 49: 1266–1273.

Golden, M.H.N. & Golden, B. E. (1981a) Plasma zinc, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein based diet. Am. J. Clin. Nutr. 34: 892–899.

Golden, M.H.N. & Golden, B. E. (1981b) Effect of zinc supplementation on the dietary intake, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition. Am. J. Clin. Nutr. 34: 900–908.

Golden, M.H.N. & Golden, B. E. (1992) Effect of zinc on lean tissue synthesis during recovery from malnutrition. Eur. J. Clin. Nutr. 46: 697–706.

Habicht, J.-P. (1974) Estandarización de métodos epidemiológicos cuantitativos sobre el terreno. PAHO Bull. 76: 375–384 (in Spanish).

Habicht, J.-P., Martorell, R. & Rivera, J. A. (1995) Nutritional impact of supplementation in the INCAP longitudinal study: analytic strategies and inferences. J. Nutr. 125: 1042S–1050S.

Hambidge, K. M. (1997) Zinc deficiency in young children. Am. J. Clin. Nutr. 65: 160–161.

Hambidge, K. M., Chavez, M. N., Brown, R. M. & Walravens, P. A. (1979) Zinc nutritional status of young middle-income children and effects of consuming zinc-fortified breakfast cereals. Am. J. Clin. Nutr. 32: 2532–2539.

Instituto de Nutrición de Centroamérica y Panamá (1992). Situación alimentaria-nutricional y de salud en centroamérica. INCAP/OPS Publication No. ME/003. Guatemala, Guatemala, C.A.

Khanum, S., Alam, A. N., Anwar, I., Akbar Ali, M. & Mujibur Rahaman, M. (1988) Effect of zinc supplementation on the dietary intake and weight gain of Bangladeshi children recovering from protein-energy malnutrition. Eur. J. Clin. Nutr. 42: 709–714.

Lohman, T. G., Roche, A. F. & Martorell, R., eds. (1988) Anthropometric Standardization Reference Manual. Human Kinetics Books, Champaign, Illinois.

National Research Council (1989) Recommended Dietary Allowances, 10th ed. National Academy Press, Washington, DC.

Ninh, N. X., Thissen, J. P., Collette, L., Gerard, G., Khoi, H. H. & Ketelslegers, J. M. (1996) Zinc supplementation increases growth and circulating insulin-like growth factor I (IGF-I) in growth-retarded Vietnamese children. Am. J. Clin. Nutr. 63: 514–519.

Rivera, J. & Martorell, R. (1988) Nutrition, infection and growth. I. Effects of infection on growth. Clin. Nutr. 7: 156–162.

Ronaghy, H. A., Reinhold, J. G., Mahloudji, M., Ghavami, P., Spivey Fox, M. R. & Halsted, J. A. (1974) Zinc supplementation of malnourished schoolboys in Iran: increased growth and other effects. Am. J. Clin. Nutr. 27: 112–121.

Rosado, J. L., Lopez, P., Muñoz, E., Allen, L. A. & Martinez, H. (1997) The effects of zinc and iron supplementation on the growth, body composition and morbidity of Mexican preschoolers from a population with a high incidence of growth stunting. Am. J. Clin. Nutr. 65: 13–19.

Ruel, M. T., Rivera, J. A., Santizo, M. C., Lönnerdal, B. & Brown K. H. (1997) Impact of zinc supplementation on morbidity from diarrhea and respiratory infections among rural Guatemalan children. Pediatrics 99: 808–813.

Santizo, M. C., Rivera, J. A., Ruel, M. T., Brown, K. H., Hurtado, E., Bentley, M. E. & Caulfield, L. E. (1995) The impact of zinc supplementation on nutrient intake from breastmilk and diet among rural Guatemalan children. FASEB J. 9: A164 (abs).

Sazawal, S., Black, R. E., Bhan, M. K., Bhandari, N., Sinha, A. & Jalla, S. (1995) Effect of zinc supplementation during acute diarrhea on duration and severity of the episode. A community based, double-blind controlled trial. N. Engl. J. Med. 333: 839–844.

Schlesinger, L., Arévalo, M., Arredondo, S., Diíaz, M., Lönnerdal, B. & Stekel, A. (1992) Effect of zinc fortified formula on immunocompetence and growth of malnourished infants. Am. J. Clin. Nutr. 56: 491–498.

Schroeder, D., Martorell, R., Rivera, J. A., Ruel, M. T. & Habicht, J.-P. (1995) Age differences in the impact of nutritional supplementation on growth. J. Nutr. 125: 1051S–1059S.

Solomons, N. W., Jacob, R. A., Pineda, O. & Viteri, F. (1979) Studies on the bioavailability of zinc in man. Effects of the Guatemalan rural diet and of the iron-fortifying agent, NaFeEDTA. J. Nutr. 109: 1519–1528.

Trowbridge, F. L., Marks, J., Lopez de Romana, G., Madrid, S., Boutton, T. W. & Klein, P. D. (1987) Body composition of Peruvian children with short stature and high weight-for-height. II. Implications for the interpretation for weight-for-height as an indicator of nutritional status. Am. J. Clin. Nutr. 46: 411–418.

Walravens, P. A., Chakar, A., Mokni, R., Denise, J. & Lemonnier, D. (1992) Zinc supplements in breastfed infants. Lancet 340: 683–685.

Walravens, P. A. & Hambidge, K. M. (1976) Growth of infants fed a zinc supplemented formula. Am. J. Clin. Nutr. 29: 1114–1121.

Walravens, P. A., Hambidge, K. M. & Koepfer, D. M. (1989) Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double blind, controlled study. Pediatrics 83: 532–538.

Walravens, P. A., Krebs, N. F. & Hambidge, K. M. (1983) Linear growth of low income preschool children receiving a zinc supplement. Am. J. Clin. Nutr. 38: 195–201.

World Health Organization. (1979) Measurement of Nutritional Impact. WHO, Geneva, Switzerland.

Xue-Cun, C., Tai-An, Y., Jin-Sheng, H., Qui-Yan, M., Zhi-Min, H. & Li-Xiang, L. (1985) Low levels of zinc in hair and blood, pica, anorexia, and poor growth in Chinese preschool children. Am. J. Clin. Nutr. 42: 694–700.