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Neither a Zinc Supplement nor Phytate-Reduced Maize nor Their Combination Enhance Growth of 6- to 12-Month-Old Guatemalan Infants[1–4]

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Abstract

After age 6 mo, the combination of breast-feeding and unfortified plant-based complementary feeding provides inadequate zinc (Zn). Additionally, high phytate intakes compromise the bioavailability of zinc. Our principal objective in this randomized controlled, doubly masked trial was to determine the effect of substituting low-phytate maize, a daily 5-mg zinc supplement, or both, in infants between ages 6–12 mo on impaired linear growth velocity, a common feature of zinc deficiency. In the Western Highlands of Guatemala, 412 infants were randomized to receive low-phytate or control maize. Within each maize group, infants were further randomized to receive a zinc supplement or placebo. Length, weight, and head circumference were measured at 6, 9, and 12 mo of age. There were no significant differences between the 2 maize groups or between the Zn supplement and placebo groups and no treatment interaction was observed for length-for-age (LAZ), weight-for-length (WLZ) or head circumference Z-scores. Overall mean (± SD) Z-scores at 6 mo for combined treatment groups were: LAZ, −2.1 ± 1.1; WLZ, 0.7 ± 1.0; and head circumference Z-score, −0.7.0 ± 1.0. At 12 mo, these had declined further to: LAZ, −2.5 ± 1.1; WLZ, −0.0 ± 0.9; and head circumference Z-score, −0.9 ± 1.1; 83.3% were stunted and 2% were wasted. Low linear growth in older Guatemalan infants was not improved with either low-phytate maize or a daily 5-mg zinc supplement. Low contribution of maize to the complementary food of the infants negated any potential advantage of feeding low-phytate maize. J. Nutr. 140: 1041–1048, 2010.

Introduction

Zinc deficiency has been shown recently to be among the top 10 preventable causes of mortality in children under 5 y of age (1,2). In term breast-fed infants, the period of greatest risk for zinc depletion is after ~6 mo of age and continues through later infancy and early childhood (3). The principal reasons for this enhanced risk are: the sharp decline in levels of zinc in breast milk as lactation advances, a decline which is generally not affected by maternal zinc status (4); the relatively low intake of zinc from commonly consumed complementary foods (3,5); and the high intake of dietary phytate, especially from the cereal grains and legumes that typically provide the major staple for complementary feeding globally. Although quantitative data on the inhibitory effect of phytate on zinc absorption in early life are lacking, data from adults that identify phytate as the major dietary factor that inhibits zinc absorption (6) predict that high phytate intakes together with low total dietary zinc result in a high risk of zinc deficiency in any age group.

Recently, selective plant breeding has led to the availability of maize that is low in phytic acid (7). Pilot studies have demonstrated that zinc absorption from low-phytate maize is higher than from their near isohybrid wild-type controls, and the increase in absorption is inversely related to the phytate content of the maize (8). The primary goal of this study was to determine whether improved physical growth would be associated with the use of low-phytate maize in a group of older infants from a population with high stunting rates and for whom maize is a major food staple. Linear growth was selected as the primary outcome measure because impairment of physical, including linear growth, is an early and prominent feature of experimental zinc depletion in mammals (9). Furthermore, enhanced linear growth velocity has been documented in many (10), although not all (11), studies of zinc supplementation in young children

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[3] Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.
[4] The data reported in this paper were presented at Experimental Biology 2008 on April 6th in San Diego, CA.
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targeted because of low growth velocity. Currently, population stunting rates of >20% are widely used as an indirect indicator of populations at high risk for zinc deficiency (12). There is also literature linking zinc deficiency/zinc supplementation to the secondary outcome measures, including weight (10), an extensive literature linking zinc supplements to decreases in infectious disease morbidity (13,14) and mortality (2), and a growing but still much too limited literature on zinc and brain development and function (15–17).

A significant increase in linear growth velocity was hypothesized to result from phytate reduction in the diet of older infants whose complementary food was plant-based with high-phytate intake. Dietary phytate reduction was hypothesized to improve zinc absorption in this population. However, because of the low-zinc intake, improving bioavailability alone was not expected to increase the absorption of zinc sufficiently to meet estimated physiologic requirements (18). For this reason, a second nested, randomized controlled trial of 5 mg zinc/d or placebo was included. The second primary hypothesis was that, in the same population, infants assigned to the daily administration of a low-dose zinc supplement would also exhibit a greater linear growth increment than that of placebo-treated controls. The study also was designed to detect an interaction between the zinc supplement and the low-phytate maize to determine whether the effect of the zinc supplement differed between the 2 maize treatment groups.

Secondary hypotheses related to additional measures of physical growth, resistance to infectious disease morbidity, and to neurodevelopment were tested.

Experimental Methods

Study design

The project was designed as a masked, randomized controlled trial of feeding low-phytate vs. isohybrid control maize as a major food staple in the complementary feeding of infants aged 6–12 mo. Within each maize group, infants were randomized to receive either a 5-mg zinc supplement or placebo daily (**Supplemental Fig. 1**). The primary outcome measure was linear growth velocity during the intervention period. Major secondary outcomes were other growth measures, neurodevelopment, and prevalence and incidence of infectious disease morbidity.

Location and demographics

The study was undertaken in the rural town of San Juan Comalapa, in the province of Chimaltenango, in the Western Highlands of Guatemala, ~80 km northwest of Guatemala City by a paved road. Comalapa is at an altitude of 2100 m and its annual temperature range is 20–25 C°. Including the households surrounding associated rural communities, Comalapa has a population of ~38,000 inhabitants. Most are concentrated in the township, in which the study was based. Ethnicity was almost entirely 'post-Mayan' Kaq'chikel Native American (93%), with the remainder of mixed European ascendancy (Ladino). Maize is the dominant crop grown by this community and the region and is the major food staple for adults and children. This study was undertaken with the full support of the Comalapa Community Health Center, the civic leaders, locally based nongovernmental organization, and other stakeholders. For the duration of the study, the investigating team established a research base in the township of Comalapa where participants underwent initial screening and longitudinal assessments were performed. A team of locally recruited and trained community research workers were supervised in part by local staff, including a part-time physician, and partly by senior staff based in Guatemala City led by one of the authors (M.M.).

Participants

Sample size. The study design required a total of 420 infants, 105 infants in each supplement group within each maize group. We determined this value by assuming a 2-tailed type I error of $\alpha = 0.05$, a power of 80%, 15% drop-out of infants by 12 mo due to loss at follow-up, and a 6% increase in expected linear growth rate of (mean ± SD) 1.3 ± 0.2 cm/mo in the zinc supplement group compared with the placebo group, within each maize group. Therefore, there was sufficient power to make comparisons between the treatment and control/placebo for both the maize and zinc groups. The study was also powered to detect an interaction between the zinc supplement and the low-phytate maize to determine whether the effect of the zinc supplement differed between the 2 maize treatment groups.

A convenience sample of 872 infants was identified between 2004 and 2006 using birth data from the civil registry, the community health center, and a network of traditional birth attendants. Of these infants, 777 were eligible for inclusion and 412 were enrolled. Inclusion criteria included: living within 12 km of the township of Comalapa; apparently healthy (based on maternal history without any prenatal or natal concerns and no history of serious illness postnatally); <6 mo of age; and home-cooked maize as the major family food staple. Primary reasons for ineligibility included: did not agree to verbal screening ($n = 42$); did not eat tortillas in the home ($n = 31$); infant too old ($n = 12$); and family did not plan to stay in the geographical area ($n = 9$) for the next year. Some potential recruits had more than 1 ineligibility issue. Because many of the infants were born in the home, birth weights were not routinely available for this population.

Potential participating families were invited to attend small group information sessions about the study held on a regular basis in the community. If the family of a potential participant expressed a desire to enroll in the study after attending such a session, a senior research team member met with the mother and family of the candidate in the presence of their community health worker. The consent form was first presented to potential participants verbally with the aid of a pictorial consent form. Once eligibility and potential interest were established, the consent form (at a 5th grade literacy level in the appropriate language) was read to the caregiver of the candidate participant by the senior field team member. The consent forms included a statement in the authorization that all of the information contained in the consent form had also been included in the pictorial consent presentation of the purpose, nature, and risk of study participation. Both the mother of the potential participant and the community health worker signed the consent form to indicate consent.

This protocol was approved by the Inter-Institutional Ethics Committee of Guatemala, the Colorado Multiple Institutional Review Board, and the ethics board at Research Triangle Institute (RTI).[10]

Demographic data

Baseline evaluation data included demographic and household asset information, agricultural practices, habitual maize consumption, and health and obstetric history of mothers. House-

[10] Abbreviations used: ALRI, acute lower respiratory infection; BSID, Bayley Scales of Infant Development; LAZ, length-for-age Z-score; MDI, mental development index; PDI, psychomotor development index; RTI, Research Triangle Institute; SES, socioeconomic status; WLZ, weight-for-length Z-score.

hold asset information (i.e. electricity, materials used for household, access to running water) was collected to compute a socioeconomic status (SES) index. The index was calculated using a composite of the household asset variables and corresponding factor scores as used by the World Bank (19).

Interventions

Maize. The germplasm for the low-phytate maize and the near isohybrid control maize was provided by BASF. The maize was grown in adjacent plots in Indiana, where it was dried and stored in 40-pound bags until required for use in Guatemala. The maize was transported to the U.S. West Coast in sealed containers and then shipped by freighter to Guatemala. The cargo was finally placed on flatbed trucks at the port and hauled to Comalapa. There it was stored in a dedicated spacious, dry, vermin-proof store room. Quality control checks of maize prior to shipment and from randomly selected bags at the time of distribution in Guatemala was undertaken by one of the investigating team (V. R.) at the USDA facility in Aberdeen, Idaho, to verify correct delivery of the assigned maize. Apart from the members of the Data Management Center at RTI, V.R. was the only unmasked member of the investigating team. His quality control data and results of other phytate analyses were reported directly to RTI. The phytate content of the control maize was typical of the high-phytate content of this grain, averaging 710 mg/100 g. The phytate concentration of the test maize was ~80% reduced from the isohybrid control maize. The zinc concentration of both of these maize hybrids was relatively high, with an average of 2.6 mg/100 g, ~50% higher than is typical for maize either in Guatemala or in the US. The low-phytate and control maize were each assigned 3 colors with which the 18-kg sacks were clearly identified. Both types were yellow maize with a similar appearance. The study and control maize was readily accepted by all participating families, although some difference in color from the locally grown maize existed. Many families were especially appreciative of the superior cooking qualities of the study maize.

Families were provided with all the maize they needed for the entire household on a weekly basis, which was typically one 18-kg sack. The delivery and consumption of maize commenced immediately after the enrollment of the infant before 6 mo of age and ended when the infant attained 12 mo of age.

Zinc supplement or placebo tablets. Ten milligrams of zinc in scored dispersible tablets or identical zinc-free placebos in a 15-tablet blister pack were provided to the mother on a monthly basis. The mothers were trained to break the scored tablets and orally administer one-half a study tablet to the infant each morning and to ensure that the entire dosage dissolved and was swallowed by the infant. The tablets were provided by Nutriset. The zinc content of the test and placebo tablets was confirmed by laboratory analyses in Colorado.

Randomization

At the time of enrolment, families were assigned to receive maize labeled with 1 of 6 randomization colors. Permuted blocks were used in the generation of the randomization list. At age 6 mo, infants were further randomized to the zinc supplementation trial. The infants were randomized to the treatment or control group within the family's maize group assignment, also using permuted blocks.

Compliance monitoring

Percent compliance for the maize intervention was calculated by the total number of days the infant consumed the study maize divided by the total number of days of intervention in the study $\times$ 100. Percent supplement compliance was defined as the number of days that the infant consumed the study tablet divided by the total number of days of intervention in the study $\times$ 100. Compliance was defined as eating the study maize or taking the study tablet over 80% of the days in the study. Compliance monitoring of the intervention was carried out through home visits by the study personnel initially on a weekly basis and then every other week. The home visit included reinforcement to the mothers of the purpose and nature of the study, confirmation that the correct study maize had been delivered to the family, evaluation of the use of the study maize, and verification that nonstudy maize was not being used in the home.

Outcome measures

Dietary intakes. Once complementary foods had been introduced into the infant diet, 24-h dietary recall data were collected monthly for a randomized sample of two-thirds of the participants. These infants were then further randomized to collect a second 24-h recall to assess the variation in food and nutrient intakes. Mothers were interviewed by a thoroughly trained, master's level research nutritionist using a standardized questionnaire and with standardized sample volumes available at each interview. Dietary recalls were keyed into a computer database and analyzed using the WorldFood System. Database values for the phytate and zinc content of foods containing the study maize were adjusted using laboratory analyses of the maize and selected foods (e.g. tortillas). Dietary intakes of zinc and phytate from complementary foods were calculated using these adjusted values. Zinc intakes from breast milk were not measured.

Anthropometry. Measurements of recumbent length, weight, and head circumference were carried out at 6, 9, and 12 mo at the study headquarters. Staff with specialized training, led by a senior staff member who had extensive experience with accurate anthropometry, was responsible for these measurements. Anthropometric data were processed and converted into Z-scores using the WHO Infant Growth Standards (20). Wasting and stunting were defined as < -2 SD Z-scores below the median [weight-for-length (WLZ) and length-for-age (LAZ), respectively] when compared with these standards (20). Duplicate measurements of infant recumbent length were obtained following the WHO guidelines (21) using a calibrated Harpenden Infantometer (Holtain) and recorded to the nearest 0.1 cm. A 3rd measurement was obtained if the difference between the 2 duplicate measurements was >0.4 cm. Infant weight was obtained by using a digital seca 334 scale with the infant either naked or while wrapped in a standard blanket with a known weight, which was subtracted to obtain infant net weight. Weight was recorded to the nearest 2 g in duplicate measurements. A 3rd measurement was carried out if a difference > 10 g was observed. An Inser-Tape (Abbott Nutrition) graduated tape in millimeters was used to measure head circumference. Duplicate head circumference measurements were recorded to the nearest 0.1 cm. A 3rd measurement was carried out if a difference > 0.2 cm was observed with the first 2 measurements. In the case of a 3rd measurement for any of these growth parameters, the 2 closest values were averaged for the final data.

Infectious disease morbidity. Morbidity monitoring for diarrheal disease and acute lower respiratory infection (ALRI) was carried out by field staff interviewing the mothers during

weekly home visits. The criterion for diarrheal disease was 3 or more loose or watery stools in 24 h. The cardinal criteria for ALRI were the presence of tachypnea with or without chest retraction as diagnosed by a physician member of the team or associated local medical practitioners.

Bayley Scales of Infant Development. Two certified specially trained individuals carried out neurodevelopmental evaluations (motor and mental scales) in all infants at 12 mo of age. A dedicated evaluation room was available to facilitate accurate testing results. The Spanish version of the Bayley Scales of Infant Development (BSID II) has been previously validated by Instituto de Nutrición de Centro América y Panamá/Institute of Nutrition of Central America and Panama investigators and applied in other Guatemalan infant populations (22). The test was conducted in Spanish with translation into the local Kaq'chikel language as needed. Research staff members who were native speakers translated a few test items to less than 10 participants.

Biochemical measurements. A venous blood sample was collected from infants at 6 and/or 12 mo of age to measure biomarkers of zinc and iron status. Eighty-four infants were recruited as a convenience sample from the enrolled participants in the main study. A section of the consent form explained the optional blood draw and consent for this procedure was given or declined by all potential participants. Blood samples were centrifuged and plasma separated within 30 min of collection and stored frozen until shipment to University of Colorado. Plasma zinc concentrations were measured by atomic absorption spectrophotometry (23). Methods and results from other biochemical markers will be presented in a separate manuscript.

Data management

Interviewer-administered paper and pencil questionnaires were conducted with study participants in Spanish during home visits or at the project headquarters. Prior to computer entry, all data forms were reviewed by senior field personnel. All de-identified data were keyed into the data management system located at the project headquarters in Comalapa and subsequently transferred to RTI for storage, management, and statistical analyses.

Analyses were conducted by RTI using SAS software (SAS Institute). Statistical tests were 2-sided with significance at $P < 0.05$. Data are presented as means ± SD unless otherwise noted. In all analyses, numerical data were examined graphically for unusual features such as outliers or skewness. Baseline characteristics and sociodemographics of infants were compared between the control maize vs. low-phytate maize groups using 2-sample tests (t test). Similarly, 2-sample tests were used to compare characteristics of infants randomized at 6 mo to zinc supplement vs. placebo groups. The gender effect on growth outcomes was also tested for independence of treatment using 2-sample t tests.

For the primary outcome, 2 hypotheses were tested. The first was that low-phytate maize would increase infant linear growth velocity between 6 and 12 mo of age independent of the receipt of a zinc supplement. The second, conversely, was that a small zinc supplement (5 mg/d) would increase infant linear growth velocity independent of the type of maize eaten. For each hypothesis, a t test was used to compare linear growth velocity (mm/mo) between the treatment group and the respective control group (length was also reported as a standardized Z-score and these were also examined over time). The secondary growth outcomes, weight gain and increase in head circumference, were analyzed similarly. The primary analysis was carried out using the intention-to-treat approach.

The secondary outcome of diarrheal morbidity was expressed as prevalence (percent days ill). ALRI was expressed as incidence, which was calculated as number of episodes divided by number of days at risk, where days of illness after the first day are omitted from the risk set (24). Each of these morbidity outcomes was evaluated separately.

The BSID data were processed by standard methods: mental and motor raw scores were standardized by mental development index (MDI) and psychomotor development index (PDI), respectively. The mean score on the MDI or PDI is 100 with a SD of 15, where 50 is the minimum score and 150 is the maximum. A score of <70 (>2 SD below the mean) indicates significant delay. Infants who were thought to be so severely developmentally delayed that they were not able to be tested were assigned a score of 49 on each index. The medians for the indexed scores were tested with a non-arametric 2-sample median test.

Differences in maize and phytate intake between maize groups and between supplement groups were examined using t tests similar to the methods described above that were used to analyze the primary outcome.

Plasma zinc data were analyzed cross-sectionally for all available data and longitudinally for those infants who had results at both 6 and 12 mo of age.

Results

Participants. A total of 420 infants were enrolled in the study. The number of participants randomized to each intervention group (412) and the numbers who completed the longitudinal study (384) are given in the consort diagram (Supplemental Fig. 1). While the consort diagram shows that participants were assigned to 4 distinct treatment groups based on how they were randomized to the 2 interventions (control vs. low-phytate maize and then placebo vs. zinc supplement), all analyses (Tables 1–5) examined each intervention individually to maintain consistency with the hypotheses stated in the protocol. Therefore, for the investigation of the maize intervention effects, all participants receiving the low-phytate maize treatment were pooled into 1 group (n = 203) and all receiving the control maize were pooled into another (n = 209). Similarly, for the investigation of the zinc intervention effects, all infants receiving the zinc supplement (n = 204) and all receiving the placebo (n = 208) were placed into separate, distinct groups. The small attrition rate in participant retention was primarily due to relocation from the area or withdrawal of consent because of perceived study burden.

Demographic data. The intervention arms did not significantly differ at baseline in demographic characteristics (**Table 1**). In general, most infants were of low SES and were representative of the general status of the community.

Study compliance. Study compliance was high in both consumption of study maize (>97%) and zinc supplement/placebo (100%), with no significant difference between intervention groups. No adverse events (e.g. vomiting) were reported in association with the study interventions.

Dietary intake data. Mean phytate intake by infants in the maize intervention group was lower at 6, 9, and 12 mo ($P < 0.01$) than in the group receiving the control maize. Infants in the group receiving a 5-mg/d zinc supplement had a higher mean

TABLE 1 Baseline demographic data of control vs. low-phytate maize groups and for zinc supplement vs. placebo groups[1]

	Maize treatment		Zinc treatment	
Variable	Control	Low-phytate	Placebo	Zinc
n	207	203	207	203
Male, *%*	47.5	53.5	50.3	50.7
Mother's age at enrollment, *y*	29.0 ± 6.6	28.9 ± 6.0	29.6 ± 6.3	28.2 ± 6.3
Mother's education, *y*	3.5 ± 2.9	4.0 ± 3.2	3.5 ± 2.9	3.9 ± 3.1
Gravidity, *n*	4.5 ± 2.9	4.5 ± 2.8	4.7 ± 3.0	4.3 ± 2.6
Parity, *n*	3.9 ± 2.2	4.0 ± 2.1	4.2 ± 2.3	3.8 ± 2.0
Living in township, *% (vs. hamlet outside of township)*	65	62	64	63
Work for pay, *% yes*	60	61	65	56
Summary SES score[2]	0.32 ± 0.61	0.33 ± 0.66	0.30 ± 0.62	0.35 ± 0.65

[1] Values are mean ± SD unless otherwise noted.
[2] SES summary score algorithm based on source of drinking water; availability of electricity and electrical appliances, telephone, and transportation; type of toilet facilities and flooring; and number of family members per sleeping room (19).

total zinc intake than those receiving the placebo ($P < 0.0001$), although dietary zinc intake from complementary foods was not significantly different between the treatment groups at any time point (**Table 2**). Mean energy intakes from complementary foods (kJ/d) ranged from 569 to 687 at 6 mo, 1377 to 1427 at 9 mo, and 1436 to 1536 at 12 mo.

Growth data. Anthropometric characteristics (means ± SD) at 6, 9, and 12 mo are presented in **Table 3**. The mean length-for-age Z-score (LAZ) for combined groups at 6 mo was −2.09. For males, the mean LAZ was −2.52 ± 1.06 and for females −1.67 ± 0.95 ($P < 0.001$). Stunting rates (LAZ < −2 SD) at 6 mo also differed between males and females ($P < 0.001$): 64.7% of males and 33.5% of females were stunted. Wasting (WLZ < −2 Z-scores) rates for the combined groups were 1% at 6 mo and 2% at 12 mo. Head circumference was within the normal range for all participants. Length and weight at 6 mo of age did not differ across treatment groups. At 12 mo, LAZ scores were lower than at 6 mo of age ($P < 0.0001$). Linear growth velocity did not differ by treatment group between ages 6 and 12 mo (**Table 4**). Stunting rates at 12 mo increased to 88.8% in males and 77.6% in females, giving an overall value of 83.3%.

The interaction between the 2 interventions was also examined by comparing the change in LAZ from 6 to 12 mo among the 4 distinct participant groups determined by treatment combination (control maize/Zn placebo; control maize/Zn supplement; low-phytate maize/Zn placebo; low-phytate maize/Zn supplement). Growth velocity did not differ among these 4 groups (F-test *P*-value = 0.71), indicating no significant interaction between the 2 interventions for the primary outcome.

Infectious disease morbidity. There were no significant differences among treatment groups in prevalence of diarrhea or incidence of ALRI. Morbidity outcomes also did not differ between males and females. The overall mean for diarrhea prevalence was 6.0% and the incidence of ALRI was 0.29 episodes/y.

BSID. The median MDI and PDI scores were 78 and 58, respectively, and were the same for each of the 4 intervention groups. The median intervention group behavioral indices ranged from 116 to 119 and were higher in the zinc-supplemented group (119) than in the placebo group (116; $P = 0.02$).

Plasma zinc. Eighty-four infants had cross-sectional measurements at 6 and/or 12 mo of age. Fifty-three of these infants had longitudinal measurements at both ages. Changes in the plasma zinc concentration in the zinc-supplemented group tended to be greater than for the control group for both the cross-sectional ($P = 0.08$) and longitudinal ($P = 0.06$) data sets (**Table 5**).

TABLE 2 Maize, phytate, and zinc intakes from complementary foods estimated from 24-h recalls for Guatemalan infants at 6, 9, and 12 mo of age in control vs. low-phytate maize groups and in zinc supplement vs. placebo groups[1,2]

	6 mo				9 mo				12 mo			
	Maize treatment		Zinc treatment		Maize treatment		Zinc treatment		Maize treatment		Zinc treatment	
Variable	Control	Low-phytate	Placebo	Zinc	Control	Low-phytate	Placebo	Zinc	Control	Low-phytate	Placebo	Zinc
n	30	30	30	30	60	66	54	72	56	53	50	59
Maize, *g/d*	23.3 ± 23.6	25.0 ± 19.4	23.6 ± 22.6	24.7 ± 20.6	45.2 ± 51.7	41.9 ± 49.6	41.2 ± 47.8	45.2 ± 52.6	65.9 ± 67.3	65.2 ± 69.2	64.7 ± 64.4	66.3 ± 71.3
Phytate, *mg/d*	206 ± 191	76.3* ± 76.3	136 ± 128	141 ± 183	336 ± 234	222 ± 241	279 ± 188	276 ± 279	409 ± 261	246* ± 183	316 ± 245	342 ± 237
Phytate from maize, *mg/d*	80 ± 92	16* ± 9	49 ± 84	45 ± 57	145 ± 105	31* ± 21	94 ± 112	80 ± 79	194* ± 145	44* ± 31	108 ± 91	132 ± 152
Dietary Zn,[3] *mg/d*	1.1 ± 1.4	0.7 ± 0.7	0.9 ± 1.5	0.8 ± 0.7	1.7 ± 1.2	1.7 ± 1.1	1.6 ± 0.9	1.8 ± 1.3	1.8 ± 1.0	1.7 ± 1.0	1.8 ± 0.9	1.7 ± 1.1
Zn from maize, *mg/d*	0.2 ± 0.3	0.2 ± 0.1	0.2 ± 0.3	0.2 ± 0.2	0.5 ± 0.4	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.4	0.7 ± 0.6	0.6 ± 0.4	0.6 ± 0.3	0.7 ± 0.6

[1] Values are means ± SD. *Different from control, $P < 0.05$.
[2] At enrollment, families were first randomized to either low-phytate or control maize. Within each maize group, infants were randomized to receive either a 5-mg zinc supplement or placebo daily. Dietary recall data were assessed on a randomized sample of two-thirds of the infants once complementary foods were initiated.
[3] Dietary zinc does not include the amount of zinc in the supplement.

TABLE 3 Anthropometric measurements for Guatemalan infants at 6, 9, and 12 mo of age in control vs. low-phytate maize groups and in zinc supplement vs. placebo groups[1]

	6 mo				9 mo				12 mo			
	Maize treatment		Zinc treatment		Maize treatment		Zinc treatment		Maize treatment		Zinc treatment	
Variable	Control	Low-Phytate	Placebo	Zinc	Control	Low-Phytate	Placebo	Zinc	Control	Low-Phytate	Placebo	Zinc
n	207	203	207	203	203	194	202	195	195	189	196	188
Length, *cm*	62.0 ± 2.3	62.2 ± 2.2	62.1 ± 2.2	62.0 ± 2.3	65.6 ± 2.5	65.8 ± 2.3	65.7 ± 2.3	65.4 ± 2.4	68.5 ± 2.5	68.8 ± 2.6	68.7 ± 2.5	68.2 ± 2.6
LAZ[2]	−2.12 ± 1.14	−2.07 ± 1.05	−2.06 ± 1.10	−2.13 ± 1.09	−2.34 ± 1.18	−2.30 ± 1.04	−2.31 ± 1.12	−2.33 ± 1.11	−2.59 ± 1.14	−2.50 ± 1.10	−2.52 ± 1.13	−2.57 ± 1.11
Weight, *kg*	6.8 ± 0.8	6.9 ± 0.9	6.9 ± 0.9	6.8 ± 0.9	7.5 ± 0.9	7.7 ± 1.0	7.6 ± 0.9	7.6 ± 1.0	8.0 ± 0.9	8.1 ± 1.0	8.1 ± 1.0	8.0 ± 1.0
WAZ[3]	−0.97 ± 1.09	−0.93 ± 1.18	−0.89 ± 1.12	−1.01 ± 1.15	−1.16 ± 1.12	−1.08 ± 1.15	−1.09 ± 1.11	−1.15 ± 1.16	−1.4 ± 1.07	−1.30 ± 1.12	−1.30 ± 1.08	−1.40 ± 1.12
WLZ[4]	0.66 ± 0.92	0.65 ± 1.02	0.70 ± 1.00	0.61 ± 0.94	0.32 ± 0.94	0.35 ± 1.06	0.37 ± 1.02	0.29 ± 0.99	−0.01 ± 0.87	0.02 ± 0.97	0.10 ± 1.10	0.05 ± 0.91
Head circumference, *cm*	41.9 ± 1.2	42.0 ± 1.2	42.1 ± 1.2	41.8 ± 1.2	43.4 ± 1.3	43.5 ± 1.3	43.5 ± 1.2	43.4 ± 1.3	44.3 ± 1.4	44.4 ± 1.3	44.5 ± 1.3	44.3 ± 1.3
HCZ[5]	−0.68 ± 1.07	−0.65 ± 0.97	−0.55 ± 0.98	−0.77 ± 1.05	−0.79 ± 1.10	−0.72 ± 1.03	−0.69 ± 1.04	−0.83 ± 1.10	−0.86 ± 1.16	−0.84 ± 1.01	−0.78 ± 1.07	−0.92 ± 1.11

[1] Values are presented as means ± SD.
[2] LAZ, length-for-age Z-scores.
[3] WAZ, weight-for-age Z-scores.
[4] WLZ, weight-for-length Z-scores.
[5] HCZ, Head circumference Z-score.

Discussion

The absence of an effect of either treatment on linear growth, the designated primary outcome measure, was contrary to the principal hypothesis underlying this study. There are several potential reasons. First, the maize intake of these infants was quite low. Accordingly, the effect of the reduction in phytate intake achieved with substitution of the low-phytate maize was more modest than anticipated. More important is the low intake of zinc from complementary foods at an age when zinc intake from breast milk is very low (25). As a consequence, the substantial reduction in phytate:zinc molar ratios achieved with the low-phytate maize could do little to enhance the quantity of zinc absorbed. The energy intake from complementary foods was relatively low with respect to estimated needs (26) although weight relative to length was appropriate, suggesting that energy intake from breast milk was relatively high (26). It is regrettable that it was not possible to find support for an extended study until the participants were at least 2–3 y of age, when the intake of maize is likely to be sufficient for hypothesized benefits of the low-phytate grain to be apparent.

With respect to zinc supplementation intervention, the lack of any effect on linear growth was also contrary to the study hypotheses, despite confirmation of the very low-zinc intakes of the study population. Again, it is feasible that a positive effect may have been observed with a longer period of treatment. Though the rationale for this possibility is less evident than it is for the low-phytate intervention, there is precedent for effects of zinc supplementation not being observed until the second year of life (27). If ever a population and setting with extraordinarily high infant stunting rates and risk of zinc deficiency would justify exploration of a supplementation intervention effect, it would be in the Mayan highlands of Guatemala. These baseline growth data are very similar to those reported in this region nearly 40 y ago by Mata (28) and the participants were primarily Mayan in both studies. However, Martorell's group (29) also reported comparable mean (± SD) data in a cohort of non-indigenous Ladino children from Eastern Guatemala who participated in a longitudinal study in 1969–77. These latter data appear to mitigate genetic factors as an explanation in our mainly indigenous population and rather point to nutritional or other environmental factors. Although the zinc supplement was at the lower end of the typical range of zinc supplementation for young children (11), the absorption of zinc from this supplement alone should have been more than adequate to meet the physiologic requirement for zinc at this age (18). The relatively close monitoring and the ready acceptance of these dispersible tablets give no reason to doubt the approximate accuracy of the favorable compliance figures obtained. The negative results of the recent zinc mortality trial in Pemba (30), especially the absence of an increase in serum zinc, led to speculation about the bioavailability of zinc from the Nutriset product used for providing the zinc supplement. However, absorption of zinc from this product has been found to be closely comparable to that from aqueous zinc sulfate (31,32). The unusually high plasma zinc data for non-zinc–supplemented controls raises concerns about sample contamination with zinc despite rigorous and well-tested guidelines for sample collection and processing (33). Despite this concern, the trend for a higher mean for zinc-supplemented compared with non-zinc–supplemented controls

TABLE 4 Growth velocity from 6 to 12 mo in Guatemalan infants in control vs. low-phytate maize and in zinc supplement vs. placebo groups[1]

	Maize treatment		Zinc treatment		WHO Child Growth
Variable	Control	Low-phytate	Placebo	Zinc	Standards (20)
n	195	189	196	188	Median
Linear growth, *mm/mo*	11.5 ± 1.9	11.1 ± 1.8	11.1 ± 1.9	10.9 ± 1.9	13.6
Ponderal growth, *g/d*	6.5 ± 2.7	6.6 ± 2.4	6.6 ± 2.6	6.6 ± 2.6	9.7
Head circumference, *mm/mo*	4.2 ± 1.1	4.1 ± 0.8	4.1 ± 1.1	4.2 ± 0.9	4.5

[1] Values are means ± SD.

TABLE 5 Plasma zinc concentrations in 6- and 12-mo-old Guatemalan infants in control vs. low-phytate maize groups and in zinc supplement vs. placebo groups[1]

	6 mo		12 mo					
	Maize treatment		Zinc treatment		Maize treatment		Zinc treatment	
	Control	Low-phytate	Placebo	Zinc	Control	Low-phytate	Placebo	Zinc
Cross-sectional data								
n	41	43	45	39	35	40	40	35
Plasma Zn, *μmol/L*	17.1 ± 5.2	16.7 ± 6.3	16.8 ± 5.4	17.0 ± 6.2	16.7 ± 7.1	16.3 ± 5.6	15.3 ± 5.6	17.8* ± 6.8
Longitudinal data[2]								
n	27	26	29	24	27	26	29	24
Plasma Zn, *μmol/L*	16.7 ± 5.4	16.9 ± 6.7	16.2 ± 5.4	17.5 ± 6.7	17.5 ± 7.8	16.9 ± 6.0	15.6 ± 5.9	19.2* ± 7.6

[1] Values are means ± SD. *Different from control, $P < 0.05$.
[2] Longitudinal data set only includes data for those infants who had data at both 6 and 12 mo follow-up.

is also compatible with the conclusion that zinc was absorbed from this supplement by these infants.

The working primary hypothesis was based on observations dating back to positive growth effects of zinc supplementation in Colorado infants in the 1970s (34) and summarized in an extensive meta-analysis of intervention studies published through 2001 (10). The International Zinc Consultative Group (12,35) has postulated that high stunting rates in a setting of dependence on high-phytate cereal crops is a harbinger of endemic zinc deficiency; the Guatemalan highlands situation clearly fulfills these criteria. Indeed, a linear growth response to zinc supplementation has been reported previously in young children in the same ethnic and geographic population (36) and the possibility exists that longer term follow-up may yet reveal linear growth enhancement in the zinc-supplemented cohorts. However, this project is only one of several recent studies in which zinc supplements administered to young children with poor linear growth have failed to increase linear growth velocity; Ramakrishnan et al. (11) presented a meta-analysis of zinc interventions published since the original series in which the result was a null effect. The explanation for the contrast between the outcome of this recent meta-analysis (11) and that reported in 2002 (10) is not immediately apparent but combined with the results of this trial indicates that there is still much to learn about the relationship between intake of bioavailable zinc and physical growth in young humans. Moreover, the low mean LAZ and high percentage of stunting suggests that unidentified factors play a major role in curtailing linear growth in early postnatal life before zinc deficiency is considered likely (3). Low-grade chronic systemic inflammation or recurrent bouts of diarrhea may impair linear growth (37) and it is plausible that this effect could be mediated in part by effects of inflammation on zinc metabolism that are not reversed by a modest zinc supplement.

The secondary outcomes included morbidity and infant cognitive development. Careful and systematic monitoring for symptoms of common childhood illness in the cohorts failed to detect any effect of the treatments. The exceptionally low PDI scores likely reflect the extent to which these infants are immobilized on their mothers' backs. The MDI data are also low compared with other studies of participants close to 1 y of age (17). There is no obvious explanation for the results of this scale.

In conclusion, this set of zinc interventions was directed to a population of infants aged 6–12 mo with a typical diet of low bioavailable Zn and a very high prevalence of stunting. No effect on linear growth was observed with dietary phytate reduction, zinc supplement, or their combination. The absence of any effect of substituting low-phytate maize is probably explicable on the basis of the low contribution of maize to the complementary foods of these infants. The failure of the zinc supplement to enhance linear growth velocity is consistent with meta-analysis findings from the most recent field trials of this intervention. Finally, although noted in earlier and other studies, perhaps the most notable finding in this project is the still-unexplained severity of linear growth retardation with high prevalence of stunting by the age of 6 mo in this population.

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