Zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize[1–3]

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ABSTRACT

Background: Poor bioavailability of zinc from high-phytate diets is an important contributory factor to zinc deficiency in low-income populations.

Objective: The objective of this study was to determine the effect of low-phytate maize consumption on zinc absorption.

Design: The participants were apparently healthy children from the Central Highlands of Guatemala. Sixty children (20 per group) were randomly assigned to be fed only the low-phytate maize or 1 of 2 control maizes, the isohybrid wild-type maize or a local maize, for a 10-wk period. During the final week, the fractional absorption of zinc for all meals was measured during 1 d with the use of zinc stable isotopes and a dual isotope ratio technique based on urine enrichment data.

Results: Mean ($\pm$SD) phytate intakes for the low-phytate, wild-type, and local maize groups were 1536 ± 563, 2056 ± 517, and 2253 ± 687 mg/d, respectively. Corresponding zinc intakes were 8.6 ± 2.5, 8.1 ± 2.0, and 9.7 ± 2.6 mg/d, and the dietary phytate:zinc molar ratios were 18 ± 5, 26 ± 6, and 23 ± 5. Corresponding fractional absorptions of zinc were 0.32 ± 0.07, 0.28 ± 0.07, and 0.29 ± 0.06. The respective values for total absorbed zinc were 2.72 ± 0.88, 2.30 ± 0.96, and 2.78 ± 1.04 mg/d. No significant differences in either the fractional absorption of zinc or total absorbed zinc were seen between the maize groups.

Conclusion: Under the conditions of the present study, zinc absorption was not increased by the long-term use of low-phytate maize in children whose major dietary staple is maize.   *Am J Clin Nutr* 2006;83:59–64.

KEY WORDS   Zinc bioavailability, stable isotopes, maize, phytic acid, Guatemalan children

INTRODUCTION

The efficiency of zinc absorption is measured from current and recent intakes of zinc and by extrinsic factors that are ingested with zinc in the diet. It has been known since the work of O'Dell (1), and confirmed subsequently (2–5), that phytic acid (inositol hexaphosphate), a normal constituent of unrefined grains and legumes, interferes with intestinal zinc uptake. Experimental metabolic research has confirmed the adverse effects of exogenous phytate on human zinc absorption (6–8).

Zinc absorption from high-phytate diets was increased in animals by dephytinization during processing (9, 10), natural fermentation (11, 12), or addition of exogenous phytase enzymes

(13, 14). Human studies have generally confirmed a beneficial comparative effect on fractional zinc absorption after reducing the phytate content of foods or meals (15–23). In 2 longitudinal studies conducted in Swedish infants, however, the reduction of phytate in a weaning food had no effect on functional measures of trace element nutriture (24, 25).

Partially refined maize is the staple food in much of sub-Saharan Africa and in Mesoamerica, where maize has been the major dietary food staple of the Mayan people of Guatemala for over a millennium (26). In Malawi, where unrefined maize porridge is the food staple, field metabolic studies showed that phytase treatment of dough increased zinc uptake in hospitalized patients with tuberculosis but not in uninfected outpatients at a pediatric hospital (27). The maize dough used in Guatemala has a high phytic acid content (28). Maize inbreds and hybrids with low phytate content have been developed, and this maize enhanced mineral retention and growth in broiler chickens in feeding experiments (29). In single day metabolic stable isotope tracer studies conducted in healthy adult volunteers in Colorado, zinc absorption was significantly higher from polenta (23) or corn tortillas (22) prepared from low-phytate maize than from corresponding meals prepared from the wild-type isohybrid maize.

Given the worldwide prevalence of zinc deficiency and its adverse consequences on health and human development, there has been growing interest in the development of practical strategies to reduce the phytate content of staple grains. The objective of the present study was to determine whether the long-term substitution of low-phytate maize would enhance zinc absorption in a population that is dependent on maize as the major food staple. We present here the findings from a community-level

feeding intervention and metabolic study in schoolchildren from the Central Highlands of Guatemala.

SUBJECTS AND METHODS

Study design

In a cross-sectional, free-living study, school-aged village children were randomly assigned to be fed a low-phytate maize (*lpa1-1* ≈60% reduction), its wild-type isohybrid, or a locally grown maize for a 10-wk period. At the end of this period, the fractional absorption of zinc (FAZ, the overall mean for all meals consumed during one test day) was measured. The meals were extrinsically labeled with a stable zinc isotope and FAZ was measured by a dual isotope tracer ratio technique on the basis of the urine enrichment ratio of this extrinsic label to that of a second stable isotope of zinc that was administered intravenously (30, 31). The total dietary zinc intakes (TDZ) for the test day were measured in duplicate diets, and the total quantity of zinc absorbed on that day (total absorbed zinc, TAZ) was calculated from these 2 measurements.

Study site

The present study was conducted in the village of Buena Vista, of the municipality of San Pedro Sacatepequez, which is located in the Central Highlands 25 km west of Guatemala City, Guatemala. Ethnically, this population is of Kaqchikel Mayan origin.

Selection criteria

Families were approached for participation on the basis of the age of their apparently healthy children (6–11 y), their willingness to allow their children to participate in the study, and the willingness of the entire family to consume the study maize. The health status of the potential participants was assessed by a research physician to ascertain that the candidates were free of any major illness that would prevent them from participating.

Subjects

Sixty children (29 boys and 31 girls) within the target age group ($\bar{x}$ age ± SD: 8.9 ± 1.3 y) participated in the present study. The intravenous isotope administration was incomplete in one participant, and hence we were unable to calculate the FAZ and TAZ data for this participant.

Sample size estimates were based on measures of FAZ and calculated with PASS software (PASS User's Guide, 2000; NCSS, Kaysville, UT). A two-tailed type I error of 0.05 and a power of 0.90 were used. We assumed that the variability in FAZ between the participants in each intervention group would be limited because of the similarity in dietary intakes (ie, minimal dietary diversity and principally maize-based) in the families in this community. Furthermore, it was anticipated that the quantitative changes in variables of zinc status that resulted from the long-term (months) intervention with *lpa1-1* maize would have limited variance within each treatment group. Therefore, an estimated SD of 0.04 and an anticipated mean increase of 0.05 in FAZ for the use of the *lpa1-1* maize were used for the sample size calculations. These assumptions and estimates resulted in a sample size of 20 participants per intervention group.

The present study was approved by the Human Subjects Committee of the Center for Studies of Sensory Impairments, Aging,

and Metabolism and by the Colorado Multiple Institutional Review Board. The consent form was written in Spanish and explained both in Spanish and Kaqchikel with the support of resident health workers from Buena Vista. The study was initially described in information group sessions to anyone in the community who was potentially interested in having their family participate. Fathers and mothers were frequently present at these meetings. After adequate time for consideration, the interested families returned for additional discussion and, if they elected to participate, to give their written informed consent.

Maize alleles

The test maize was *lpa1-1*, a hybrid that is homozygous for the low-phytate gene with ≈60% reduction in phytate (32, 33). Both this maize and its wild-type isohybrid were provided for the study by Pioneer Hi-Bred Inc (Dupont, Johnston, IA) who grew this maize under a cooperative research and development agreement with the Agricultural Research Station of the US Department of Agriculture. These 2 maize varieties were grown in Iowa and shipped by airfreight to Guatemala in two shipments in successive years to provide a total of ≈25 tons. One batch of local yellow maize was purchased in San Pedro Sacatepequez in sufficient quantity to supply the families of the second control group. All maize was stored locally in the village of Buena Vista under dry and vermin-free conditions until distributed. The storage and distribution of the maize was supervised closely by a village health worker, in whose home the maize was maintained.

Maize distribution and consumption

The 60 families were randomly assigned to receive either the *lpa1-1* test maize or 1 of 2 control varieties. Randomization was stratified to include approximately the same number of families in each maize group during each of the 3 study periods. The necessity for the 3 study periods was determined by the manner in which the study funds were disbursed. The first of these 10-wk periods commenced in March 2000 and the third concluded in September 2001. The type of maize was not disclosed to the participants; however, the participants probably distinguished the local control maize. The investigators in Colorado were blinded to the treatment group assignments.

All varieties of maize were provided in sufficient quantities to meet the entire maize needs of each family for the 10-wk period. These needs were initially determined by food-frequency questionnaires; minor adjustments were made as necessary as the study proceeded. The research nutritionist and the primary care physician weighed and distributed the maize at weekly intervals. All meals were consumed in the home setting except on the day of isotope administration.

Isotope preparation

Accurately weighed quantities of zinc oxide preparations enriched with either ^{67}Zn (90.9% purity) or ^{70}Zn (95.56% purity) (Trace Sciences International, Richmond Hill, Ontario) were dissolved in 0.5 mol H_2SO_4/L to prepare a stock solution. For the preparation of orally administered doses of ^{70}Zn, the stock solutions were diluted with triply deionized water (Milli-Q; Millipore, Billerica, MA) and titrated to a pH of 5.0 with metal-free ammonium hydroxide. For the intravenously administered ^{67}Zn, the stock solution was adjusted to a pH of 6.0 with ammonium hydroxide and diluted with sterile isotonic sodium chloride to a

concentration of 1.5 mmol zinc/L. The oral and intravenous solutions were filtered through a 0.2 μm filter. The zinc concentrations of these solutions were measured by atomic absorption spectrophotometry with mass correction factors applied (34). Accurately weighed quantities were stored in plastic tubes for the oral doses or sealed sterile vials for the intravenous doses. The intravenous doses were tested for pyrogens and sterility before use.

Isotope administration

For a 1-d period, each test meal was extrinsically labeled with a stable zinc isotope tracer (^{70}Zn). The meals for that day were cooked at home as usual, but they were consumed under supervision in the courtyard of the senior health worker's home. Typically, zinc stable isotopes were administered to 5 children on the same day. The total quantity was distributed among all meals and snacks in proportion to the zinc content of the meals. For this purpose, the tracer was divided into 50-μg aliquots in 5 mL water. The children started to drink from the polypropylene test tubes in which the tracer was stored at the midpoint of the meal and continued through the remainder of the meal. Each tube was rinsed twice with Milli-Q water (Millipore) and these rinses were also ingested. The same protocol was followed for each of the meals and snacks over day 1 of the study period. The total quantity of isotope administered was ≈0.250–0.350 mg zinc.

An accurately weighed quantity (≈0.800 mg in 8 mL solution) of a second tracer (^{67}Zn) was administered intravenously during the afternoon on day 1. Administration was performed over a 5-min interval with a 10 mL syringe and a 3-way stopcock via a scalp vein needle inserted into a superficial forearm vein. The syringe was flushed twice with normal saline through the 3-way-stopcock.

Sample collections

Each food item of each test meal (and snack) was weighed before consumption and plate waste for each individual item was also weighed. Representative samples of all foods and recipes consumed during the study days were collected for subsequent analytic measurement of zinc content and calculation of dietary zinc intake.

Timed spot urine samples (20–50 mL) were collected twice daily (am and pm) from days 3 to 7. Immediately before the intravenous isotope infusion, a 10-mL blood sample was withdrawn via a butterfly infusion set that was placed in the arm. The samples were used for assays of plasma zinc concentrations, erythrocyte sedimentation rates, hemoglobin concentrations, and hematocrit. The samples were kept frozen at −20 °C until they were transported to the University of Colorado Health Sciences Center for additional processing and analyses.

Laboratory analyses

Dietary samples were wet- and dry-digested before reconstitution in 0.1 N hydrochloric acid for total zinc analyses. Urine samples were digested with both dry and wet digestion methods before a chelation procedure was performed to purify the zinc for isotope analyses (22). The total zinc in the digested samples was measured by flame atomic absorption spectrophotometry, and the isotope ratios (^{67}Zn:^{64}Zn and ^{70}Zn:^{64}Zn) were measured with inductively coupled plasma mass spectrometry (22). The isotope ratios were converted to percentage enrichment (defined to be all zinc in the sample from an isotopically enriched source divided by the total amount of zinc in the sample) by an algorithm that accounted for the isotope abundances and the average atomic mass of both the natural and the isotopically enriched zinc that was contained in the samples (Miller, unpublished observation). In general, enrichment levels in the urine were 15-fold (^{70}Zn) and 50-fold (^{67}Zn) above the detection limit of the analytic method.

Plasma zinc analyses were performed by atomic absorption spectrophotometry (35). Hemoglobin, hematocrit, and erythrocyte sedimentation rates (modified Westergren) were measured by standard laboratory assays in Guatemala City. Anion exchange HPLC was used to directly measure the phytate content of the dietary samples and maize kernels (36).

Data processing and statistical analyses

FAZ was measured from urine isotopic enrichments with the use of a dual isotope tracer ratio method (30, 31). TAZ was calculated for each participant by multiplying FAZ by TDZ for the test day.

Dietary phytate intakes were first calculated from the weighed intake records during day 1 of the metabolic study with the use of the WorldFood System nutritional analysis program (World-Food System 2; University of California, Berkeley, CA). Phytate intake from maize products was isolated from total phytate intake and subsequently adjusted for the actual phytate content (obtained by laboratory analysis) of the assigned maize group. This value was then summed with the nonmaize phytate intake to calculate the total phytate intake adjusted for maize assignment.

Data were analyzed with GRAPHPAD PRISM version 4.00 for Windows (GraphPad Software, San Diego, CA) and SPLUS (Insightful Corp, Seattle, WA). Results are presented as means ± SDs. Means from height, weight, and age data were compared between sexes with two-tailed unpaired t tests. Because no significant differences in these baseline measurements were found between sexes, the boys and girls were combined for subsequent analyses. Means for maize nutrient composition, dietary intakes, FAZ, and TAZ were compared with one-way analysis of variance and Tukey's multiple comparisons tests. Between-group comparisons of TAZ were also carried out by analysis of covariance with zinc intake as a covariate. The α level was set at 0.05. The correlation between plasma zinc concentrations and erythrocyte sedimentation rates was calculated with a two-tailed Pearson's correlation (r; $\alpha = 0.05$).

RESULTS

The anthropometric characteristics of the study children are summarized in **Table 1**. No statistical differences in age, weight, height, and z scores were observed between the boys and girls. The same pattern was seen when the participants were stratified by maize treatment groups, which suggested that the children were comparable at the beginning of the intervention. The participants were of short stature, when compared with the World Health Organization reference data (37). However, most of them had an adequate weight-for-height, which indicated the absence of wasting or acute malnutrition.

Selected nutrient compositions of unprocessed grain from the 3 maize varieties are presented in **Table 2**. Significant differences in phytate, zinc, and calcium contents and the phytate:zinc molar ratios were seen between the 3 types of study maize. The local maize had significantly higher zinc and calcium contents

TABLE 1
Summary of age and anthropometric measurements by maize group[1]

	lpa 1-1 maize group	Wild-type maize group	Local maize group
Age (y)	7.9 ± 1.2	8.3 ± 1.3	8.6 ± 1.4
Height (kg)	117 ± 5.9	116.8 ± 7.2	119.3 ± 7.5
Height-for-age *z* score	−1.66 ± 0.72	−1.94 ± 0.89	−2.01 ± 0.88
Weight (kg)	22.1 ± 2.6	22.2 ± 3.6	22.1 ± 3.0
Weight-for-age *z* score	−0.87 ± 0.73	−1.10 ± 0.75	−1.24 ± 0.64
Weight-for-height *z* score	0.48 ± 0.92	0.45 ± 0.52	0.28 ± 0.90

[1] All values are $\bar{x}$ ± SD. $n = 20$ per group. No significant differences ($P > 0.05$) in age, height, weight, and z scores were observed between groups (ANOVA with Tukey's multiple comparisons test).

TABLE 3
Dietary intakes of zinc and phytate and the phytate:zinc molar ratio, fractional absorption of zinc, and total absorbed zinc by maize group[1]

Intakes	*lpa 1-1* maize group	Wild-type maize group	Local maize group
Total phytate (mg/d)	1536 ± 563[a]	2056 ± 517[b]	2253 ± 687[b]
Zinc (mg/d)	8.6 ± 2.5[a]	8.1 ± 2.0[a]	9.7 ± 2.6[a]
Phytate:zinc molar ratio	18 ± 5[a]	26 ± 6[b]	23 ± 5[b]
Fractional absorption of zinc	0.32 ± 0.07[a]	0.28 ± 0.07[a]	0.29 ± 0.06[a]
Total absorbed zinc (mg/d)	2.72 ± 0.88[a]	2.30 ± 0.96[a]	2.78 ± 1.04[a]

[1] All values are $\bar{x}$ ± SD. $n = 20$ per group. Means within a row with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Tukey's multiple comparisons test).

than did both the wild-type and *lpa1-1* maize. However, the calcium content of the maize kernels was negligible compared with that of tortillas made from these types of maize after nixtamalization.

Approximately 50% of the daily caloric intake in the participants was attributable to maize intake. Food items that were prepared from maize contributed 65% of the total dietary phytate in the diets of the wild-type and local maize groups and 48% to the diets of the *lpa1-1* group. The mean dietary intakes of zinc and phytate and the phytate:zinc molar ratios on day 1 are presented in **Table 3** stratified by treatment groups. The mean FAZ and TAZ (TDZ × FAZ) are also presented in this table. The mean zinc intake was not significantly different across the groups, but total dietary phytate intake was significantly lower in the *lpa1-1* group than in the other groups, with a reduction of ≈25–30%. Although the mean fractional absorption of zinc was slightly higher in the *lpa1-1* group than in the other groups, no significant differences in FAZ ($P = 0.15$) or in TAZ were observed between the groups. The results did not significantly change ($P = 0.27$) when the between-group comparisons were performed by analysis of covariance, with daily zinc intake as a covariate and the quantity of zinc absorbed as the outcome. The relation between TAZ and TDZ for each group is shown in **Figure 1**.

The data showed a lot of variation in phytate within the groups but little variation between the groups. Because of the high intragroup variation in phytate, a regression of FAZ on phytate was performed, which resulted a nonsignificant ($P = 0.33$) negative relation. This negative relation was marginally significant ($P = $
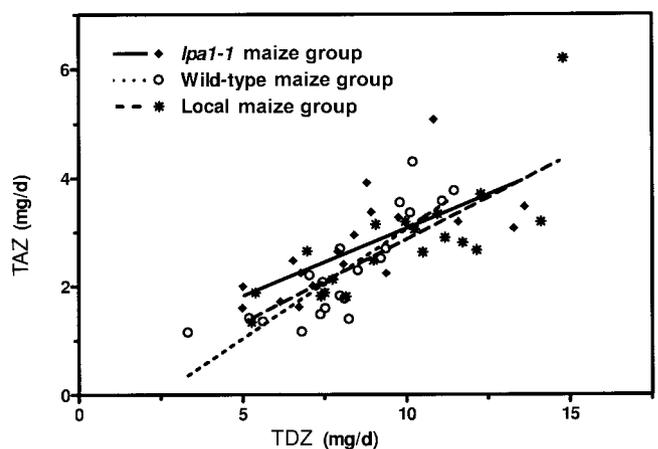
0.0548) after the omission of one outlier who had both high FAZ and high phytate intake.

An additional post hoc analysis was performed, because it was noted in Figure 1 that the low-phytate group tended to have higher TAZs when TDZs were low. When only TDZs below the median value of 8.47 mg Zn/d were included in the analysis, FAZ differed between the 3 maize groups ($P = 0.0190$); FAZ in the low-phytate group was greater than in the wild-type group ($P = 0.0053$; data not shown). Also TAZ adjusted for TDZ did differ between the 3 maize groups ($P = 0.0308$); again, TAZ in the low-phytate group was higher than in the wild-type group ($P = 0.0090$; data not shown).

The mean (±SD) plasma zinc concentration for the participants in the present study was 60.1 ± 11.8 μg/dL, with no significant differences between treatment groups or between boys and girls. A significant negative correlation was found between plasma zinc concentrations and erythrocyte sedimentation rates ($r = -0.2823$, $P = 0.04$).

DISCUSSION

Under the conditions of the present study, a long-term reduction in the high phytate content of the habitual diet did not enhance zinc absorption. Low power is a very possible explanation

TABLE 2
Selected nutrient composition of unprocessed grain of the 3 maize varieties[1]

Composition (per dry weight)	*lpa 1-1* maize	Wild-type maize	Local maize
Total phytate (mg/100 g)	268 ± 18[a,2]	840 ± 42[b]	763 ± 23[b]
Zinc (mg/100 g)	1.80 ± 0.04[a]	1.77 ± 0.07[a]	2.74 ± 0.01[b]
Phytate:zinc molar ratio	15	47	28
Calcium (mg/100 g)	0.59 ± 0.01[a]	0.49 ± 0.05[b]	0.84 ± 0.02[c]
Energy (kcal/100 g)	420 ± 60[a]	430 ± 40[a]	430 ± 30[a]
Protein (g/100 g)[3]	7.44[a]	8.03[a]	8.26[a]

[1] $n = 10$ for each sample, unless otherwise noted. Means within a row with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Tukey's multiple comparisons test).
[2] $\bar{x}$ ± SD (all such values).
[3] $n = 1$ for each type of maize.

FIGURE 1. Total absorbed zinc (TAZ) compared with total dietary zinc (TDZ) intakes for the 3 maize groups. Lines represent linear regressions for each maize group. No significant differences were observed between the slopes of the 3 lines ($P = 0.24$).

for the negative findings, because most effects were in the expected direction. Neither the large intragroup nor the small intergroup differences in phytate intake were adequately anticipated. One theoretical explanation is that the efficiency of zinc absorption in the control groups was not diminished by dietary phytate. However, although fractional absorption was higher than that predicted from the reference data, it was substantially lower than that predicted if the diet had contained minimal phytate (22, 38). Moreover, the results of secondary post hoc analyses suggested that there was a negative relation between the efficiency of zinc absorption and phytate intake.

The results of the present study were in contrast to the results of short-term (single day) studies with the same low-phytate maize and its wild-type isohybrid (22, 23). Several factors are likely to have contributed to this difference. First, the phytate reduction was less than that predicted. In the short-term studies, maize tortillas were the only source of dietary phytate, whereas the control maize in the present study accounted for only 65% of total phytate in the test meals. Therefore, the percentage reduction in total phytate that was achieved by substitution of the *lpa1-1* maize was less in the present field study than in the studies conducted in Colorado. Furthermore, for unexplained reasons, the phytate reduction in the maize tortillas (42%) that were consumed in the present study was less than anticipated from both our previous experience (22) and that of others (39). Second, the efficiency of zinc absorption for the control subjects was greater than predicted. As discussed earlier, the mean FAZs for the meals that were consumed by the local and wild-type maize control groups were higher than the FAZs predicted for the calculated dietary phytate:zinc molar ratio. These differences could not be attributed to differences in maize or analytic techniques, because *lpa1-1* maize and its wild-type isohybrid were used in the studies conducted in both countries and the analyses were performed in the same laboratories. These FAZs were also higher than those found in school-aged children in rural Malawi who consumed a maize-based diet that had a similar phytate:zinc molar ratio (40). Finally, the zinc intake of the children who participated in the present study was higher than was anticipated from the results of initial screening, which was undertaken before making the decision to locate the study in this community. This screening confirmed that the habitual diet consisted primarily of maize and some legumes, with little consumption of animal products. During the metabolic study period, the meals continued to be provided by the family but were eaten in a communal setting where it was feasible for the research team to administer the zinc stable isotope solutions, weigh the intake of individual foods, and collect dietary samples for analysis. In hindsight, this communal setting almost certainly influenced the participating families' choice of test meals. Furthermore, participation in the study provided additional wealth to the families because they didn't need to purchase or use their own maize for the family's consumption during this time. This could have affected the selection of meals for a limited period of time during the metabolic studies. In addition, the zinc content of wheat bread, a fairly common breakfast item, and of wheat pasta, which is also consumed in this community, was higher than expected. Additional investigation showed that all samples of wheat flour that could be purchased in the local markets were zinc-fortified. We could not determine the original source of this flour but, because there is no national Guatemalan fortification policy, this flour was presumably imported. The change in the school snacks that were provided to the children also had a variable and initially unsuspected effect on the study children's zinc intake. The snack that was provided during 1 of our 3 study periods was a highly zinc-fortified cookie. The use of standardized test meals prepared by the investigating team would have diminished the risk of unusually high zinc intake from the test meals, but was beyond the scope of the present study. Moreover, mimicking habitual dietary intakes would have remained a challenge.

Zinc deficiency is a documented cause of poor growth and diarrhea in the Central Highlands of Guatemala (41–43). Despite the relatively high zinc intake during the study period, which was only partially attributable to the study itself, our prestudy dietary survey indicated that zinc intake for the study children was not always favorable. This conclusion is supported by the mean plasma zinc data which, even after correction for the effect of high erythrocyte sedimentation rates, was ≈ 10 μg/dL less than the 50th percentile for this age (44). In contrast to current and recent intake, human zinc status does not have a readily detectable effect on the efficiency of zinc absorption (45), and possible changes in zinc status over the 10-wk study period are not thought to have contributed to the results of the present study. In conclusion, the present study did not show that the low-phytate maize altered the efficiency of zinc absorption in this population.

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