

Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids¹⁻³

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ABSTRACT

Background: Identification of allelic variants in a single gene that determine the phytate content of maize kernels and the subsequent breeding of low-phytate maize have facilitated studies designed to determine quantitatively the effects of maize phytate on the bioavailability of minerals in maize.

Objective: The objective was to determine the relation between the fractional absorption of zinc (FAZ) and the phytate content and phytate:zinc molar ratios of maize tortillas prepared from hybrids with different phytate contents.

Design: Six healthy adults were fed, as the only food for 2 d, maize tortillas prepared from 1 of 2 low-phytate mutants: *lpa1-1* (*lpa1-1-LP*) or Nutridense Low Phytate (ND-LP), which have phytate reductions of $\approx 60\%$ and $\approx 80\%$, respectively, compared with their respective wild-type isohybrids. Four additional subjects were fed tortillas prepared from the corresponding wild-type isohybrids (*lpa1-1-WT* and ND-WT) according to the same study design. Meals were extrinsically labeled with zinc stable isotopes, and FAZ was determined with a dual-isotope-tracer ratio technique. Overall FAZ values were examined in relation to dietary phytate and phytate:zinc molar ratios by using a mixed nonlinear regression model.

Results: The mean (\pm SD) FAZ values from tortillas prepared from ND-LP, *lpa1-1-LP*, *lpa1-1-WT*, and ND-WT were 0.38 ± 0.07 , 0.28 ± 0.04 , 0.15 ± 0.07 , and 0.13 ± 0.05 , respectively. A negative relation ($P < 0.001$) was found between FAZ and both dietary phytate and the phytate:zinc molar ratio. The effect of dietary zinc (8–14 mg Zn/d) under these experimental conditions was not significant.

Conclusions: FAZ from maize tortillas is positively related to the extent of phytate reduction achieved with low-phytate hybrids. *Am J Clin Nutr* 2004;79:1053–9.

KEY WORDS Maize, low-phytate maize hybrids, tortillas, zinc absorption, phytate:zinc molar ratio

INTRODUCTION

The inhibitory effect of phytate on mammalian zinc absorption has been recognized for 40 y (1, 2), and there is considerable experimental (3–9) and epidemiologic (10–14) evidence that dietary phytate has a negative effect on the bioavailability of dietary zinc in humans. Although substantial quantitative dose-response data on the inhibitory effect of dietary phytate are available from single test meal studies (3, 4, 6, 7), longer-term studies are required to estimate dietary zinc requirements in populations that are dependent on cereal grains or legumes as principal food

staples. Progress in plant genetics has led to the identification and successful breeding of grains and legumes that are homozygous for allelic variants at a single gene that alters the phytate content of the grain or legume (15–17). Maize is an example of a cereal grain that has a very high phytate content (11, 12, 18) and for which low-phytate alleles have been identified. Use of these allelic variants provides a novel means of facilitating measurements of the long-term effects of dietary phytate reduction on zinc bioavailability in subjects whose habitual diets are high in phytate.

The objective of this study was to determine the relation between the fractional absorption of zinc (FAZ) and the phytate content and phytate:zinc molar ratios of maize tortillas prepared from maize hybrids containing widely varying quantities of phytate and providing the only food for an entire day.

SUBJECTS AND METHODS

Subjects

Ten apparently healthy adult volunteers (4 women and 6 men) participated in this study, 6 in subgroup A and 4 in subgroup B. Sample size was calculated on the basis of FAZ data from a previous study (18). A sample size of 4 subjects provided 80% power to detect a minimum difference of 0.10 in FAZ ($\alpha = 0.05$). The average age was 26 y (range: 21–37 y). Detailed dietary records were not obtained, but each of the participants reported that their habitual diets were typically North American. None of the subjects were vegetarians and none consumed diets in which grains or legumes were the major food staples. This study was approved by the Colorado Multiple Institutional Review Board. All subjects provided written informed consent.

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Study design

Healthy adult volunteers consumed test meals consisting of maize tortilla only for an entire 2-d study period. Because only 2 zinc stable-isotope tracers were available to label test meals and there were 4 maize hybrids to study, the subjects were divided into 2 subgroups (A and B), each of which was fed 2 maize hybrids. According to a crossover design, one of these hybrids was fed for an entire day and the other was fed on the following day (the order alternated between subjects for each study). The maize hybrids studied in subgroup A were *lpa1-1-LP* and Nutridense Low Phytate (ND-LP; ExSeed Genetics, a Division of BASF Plant Sciences, Research Triangle Park, NC), which have phytate reductions of $\approx 60\%$ and 80% , respectively, compared with their corresponding wild-type hybrids. The maize hybrids studied in subgroup B were the isohybrid wild types for these 2 low-phytate hybrids (*lpa1-1-WT* and ND-WT).

All test meals (ie, all meals for 2 d) were extrinsically labeled with 1 of 2 enriched zinc stable isotopes (the other being administered on the second day). A third zinc stable isotope tracer was administered intravenously during the evening of day 1. FAZ was measured by a dual isotope tracer ratio (DITR) technique (19, 20). The relations between FAZ and dietary phytate and phytate:zinc molar ratios were modeled by using regression analysis.

Maize

The *lpa1-1-LP* maize is the prototype low-phytate hybrid ($\approx 60\%$ phytate reduction) produced by V Raboy (US Department of Agriculture/Agricultural Research Service) and Pioneer Hi-Bred Inc (Dupont, Johnston, IA) under a Cooperative Research and Development Agreement. The isohybrid wild type with a normal phytate content and matched to *lpa1-1-WT* was grown in the same location. ND-LP is another similar low-phytate hybrid that has a greater phytate reduction ($\approx 80\%$). This hybrid and its corresponding wild type with a normal phytate content (Nutridense, ND-WT) were bred by ExSeed Genetics.

Maize test meals

The test meals, which provided the only source of food over a 2-d period, consisted entirely of these maize hybrids provided as tortillas. For the preparation of the tortillas, ≈ 10 L water was added to 450 g maize kernels and brought to a boil. Five grams (1 tsp) of powdered limestone was added and stirred. The maize was left to simmer for 4 h, after which it was drained and spread out on a towel and left to dry for 3 h. The nixtamalized maize was then ground in a food processor, rolled into 4-cm diameter balls, dipped in corn oil, and flattened. The tortillas were cooked on a greased skillet for 1 min. The number of tortillas for an entire day were equally distributed for consumption between breakfast, lunch, and dinner. All test meals and plate waste were weighed before and after consumption of the meal.

Isotope preparation

Accurately weighed quantities of preparations of zinc oxide enriched with ^{67}Zn , ^{68}Zn , or ^{70}Zn (Trace Sciences International, Richmond Hill, Ontario) were dissolved in 0.5 mol $\text{H}_2\text{SO}_4/\text{L}$ to prepare a stock solution. For preparation of orally administered doses of ^{67}Zn and ^{70}Zn , the stock solutions were diluted with triply deionized water and titrated to pH 5.0 with metal-free ammonium hydroxide. For the intravenously administered ^{68}Zn ,

the pH of the stock solution was adjusted to 6.0 with ammonium hydroxide, and the stock solution was diluted with sterile isotonic sodium chloride to a zinc concentration of 1.5 mmol/L. 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 (21). Accurately weighed quantities were stored in plastic tubes (oral doses) or sealed sterile vials (intravenous doses). The intravenous doses were tested for pyrogens and sterility immediately before use.

Isotope administration

Two preparations of zinc stable isotopes (^{67}Zn and ^{70}Zn) were administered orally as extrinsic tracers. Administration of accurately weighed quantities commenced after consumption of approximately one-half of each test meal and continued in small quantities during the remainder of the meal. The tube containing the tracer preparations was rinsed with milli-Q (Millipore, Billerica, MA) water twice, and these rinses were also ingested. This is the standard procedure for administering extrinsic zinc stable-isotope labels with meals in our program. The validity of this method of adding an extrinsic tracer was confirmed (22; KM Hambidge, unpublished observations, 2003) by studies in which absorption determined by this method was compared with simultaneous measurements of the absorption of intrinsic zinc (23) in a range of different composite meals. The same protocol was followed for each of the meals over the 2-d study period. One of these isotope preparations (^{70}Zn) was administered equally between all meals on day 1 and the other (^{67}Zn) on day 2. The total quantity of isotope administered was ≈ 0.50 mg Zn/d.

An accurately weighed quantity (≈ 2 mg) of a third tracer (^{68}Zn) was administered intravenously into a superficial vein in the forearm over a 5–10-min interval with a 10-mL syringe and 3-way stopcock and scalp vein needle during the evening of day 1. The syringe was flushed twice with normal saline using the 3-way stopcock. Subsequent simulation studies that used our model of zinc metabolism (24) indicated that this particular timing of the intravenous dose resulted in an error in FAZ calculation of $+0.03$ for the second-day data. Accordingly, corresponding corrections were made in the final data.

Sample collections and analyses

Timed 20–50 mL-urine samples were collected twice daily in acid-washed Nalgene bottles (Nalge Nunc International, Rochester, NY) from days 4 to 9 after administration of the first tracer. Urine was transferred to Pyrex beakers (Corning Inc, Life Sciences, Acton, MA), dried at 100 °C, and then ashed twice for 24 h in a muffle furnace at 450 °C each time. Between the 2 ashings, a few drops of concentrated nitric acid were added to the sample and then the sample was dried on a hot plate. After digestion, each sample was dissolved in 2.5 mL ammonium acetate buffer (pH 5.6). Zinc in the dissolved samples was extracted into hexane after the addition of pyridine (Fischer Scientific, Pittsburgh). The organic chemicals left after evaporation were digested by adding concentrated nitric acid and 30% (by vol) hydrogen peroxide and then heated at 100 °C on a heating block (Fischer Scientific). The purified zinc was then reconstituted in 4 mL 2% (by vol) nitric acid (Optima, distilled at subboiling temperature; Fischer Scientific). All glass tubes used in the extraction were acid washed in aqua regia and rinsed in milli-Q triply deionized water before use (25).

TABLE 1Selected composition of the 2 low-phytate hybrids and their isohybrid wild types in unprocessed maize grain¹

Composition (per dry wt)	<i>lpa1-1</i> -LP (<i>n</i> = 6)	ND-LP (<i>n</i> = 6)	<i>lpa1-1</i> -WT (<i>n</i> = 4)	ND-WT (<i>n</i> = 4)
Total phytate (mg/g)	3.7 ± 0.3 ^{a,2}	2.6 ± 0.3 ^b	8.7 ± 0.0 ^c	8.4 ± 0.3 ^c
Zinc (mg/100 g)	1.99 ± 0.04 ^a	3.03 ± 0.03 ^b	2.13 ± 0.03 ^c	2.25 ± 0.05 ^d
Phytate:zinc molar ratio	18:1	8:1	40:1	36:1
Iron (mg/100 g)	2.00 ± 0.03 ^a	1.83 ± 0.03 ^a	1.81 ± 0.02 ^a	1.95 ± 0.02 ^a
Calcium (mg/100 g)	0.11 ± 0.00 ^a	0.13 ± 0.02 ^a	0.15 ± 0.01 ^a	0.13 ± 0.02 ^a
Energy (kcal/g)	4.0 ± 0.2 ^a	4.1 ± 0.0 ^a	4.1 ± 0.0 ^a	4.1 ± 0.0 ^a
Protein (g/100 g)	11.1 ± 0.3 ^a	10.1 ± 0.5 ^{a,b}	9.8 ± 0.5 ^{a,b}	8.9 ± 0.3 ^b

¹ *lpa1-1*-LP, low-phytate (≈60% reduction) hybrid maize; ND-LP, Nutridense Low Phytate (≈80% phytate reduction); *lpa1-1*-WT and ND-WT, the corresponding wild-type isohybrids. Means within a row with different superscript letters are significantly different, *P* < 0.05 (one-way post hoc ANOVA).

² $\bar{x} \pm SD$ (all such values).

Eight milliliters of a 50- μ g/L zinc solution in 2% (by vol) nitric acid was prepared from each sample. The solution was introduced into an inductively coupled plasma mass spectrometer (PlasmaQuad 3; VG Elemental, Cheshire, United Kingdom) for measurement of zinc stable-isotope ratios (⁶⁷Zn/⁶⁶Zn, ⁶⁸Zn/⁶⁶Zn, ⁷⁰Zn/⁶⁶Zn). The samples were introduced through an autosampler (ASX-500, model 510; CETAC, Omaha) by a peri-pump (Perimax 12; CPETEC, Erding, Germany). Instrument parameters were as follows: argon gas flow, 13 L/min; intermediate gas flow, 1.40 L/min; nebulizer gas flow, 0.82 L/min; forward power, 1350 W; temperature of pneumatic nebulizer, 4 °C; and sample flow rate, 1 mL/in. Data acquisition parameters were set as follows: one point per peak, 1800 sweeps, 10 replicates, 50 ns dead time, and dwell times of 3 min for ⁶⁶Zn, 4 min for ⁶⁷Zn, 3 min for ⁶⁸Zn, and 5 min for ⁷⁰Zn. A natural zinc standard was run every 6 samples and 2% HNO₃ every 12 samples. Counts per second of nitric acid were subtracted from counts per second of all samples. The precision of this method with the use of natural abundance standards is <0.3% relative SD for ⁶⁷Zn/⁶⁶Zn and ⁶⁸Zn/⁶⁶Zn and <0.6% relative SD for ⁷⁰Zn/⁶⁶Zn.

Test meal analyses

Samples of each individual batch of tortillas were collected and homogenized. Weighed aliquots were digested and the zinc content was determined by atomic absorption spectrophotometry. Anion-exchange HPLC was used to directly measure phytate and related inositol phosphates (26). FAZ values were determined with a DITR technique based on urine tracer enrichment ratios (19, 20).

Statistical analyses

Data were analyzed by using SAS software (SAS Institute Inc, Cary, NC). The results are presented as means ± SDs; for regression equations, results are presented as estimates ± SEs. Mean (±SD) compositions of the unprocessed and prepared maizes were compared for the 4 maize types by using one-way analysis of variance (ANOVA). Intakes of tortillas, zinc, and phytate and total FAZ were compared for the 4 maize types by using one-way ANOVA with adjustment for the repeated measurement of subjects. The relation between FAZ and dietary phytate intake and between FAZ and phytate:zinc molar ratios were modeled by using a nonlinear regression, assuming the exponential form

$$\text{FAZ} = A + B \exp(C \times X) \quad (1)$$

where *X* is the phytate intake or phytate:zinc molar ratio, and *A*, *B*, and *C* are variables to be estimated. Repeated measurements on subjects were accounted for by using a random subject effect in a nonlinear mixed model as implemented in SAS PROC NLMIXED (27). Analyses (including ANOVAs above) were also performed in which the period of measurement (first or second) was adjusted for, but in no cases was this factor significant so it was omitted from all reported models and results. Dietary zinc was also considered as a covariate to assess its effect on FAZ and to adjust for this effect on estimations of the relations between FAZ and phytate intake and between FAZ and phytate:zinc molar ratios.

RESULTS

The mean (±SD) concentrations of phytic acid, selected minerals and protein, and phytate:zinc molar ratios of the original maize grains and of the tortillas prepared from the 2 low-phytate hybrids and their corresponding wild types are given in Tables 1 and 2, respectively. The mean (±SD) intakes of tortillas, zinc, and phytate are given in Table 3. HPLC analyses of the grains and test meals used in this study showed that inositol hexaphosphate (phytic acid) represented ≈95% of the total soluble inositol phosphates found in all samples. The remaining 5% of total soluble inositol phosphates consists of a complex mixture of inositol pentaphosphates and tetraphosphates, which represent ≈3% and 2% of total inositol phosphates, respectively.

Also included in Table 3 are the mean FAZ values and the total amounts of absorbed zinc per day (TAZ) for each maize hybrid. For all subjects in subgroup A, the mean FAZ was significantly higher (*P* = 0.003) from the tortillas prepared with ND-LP maize than from the tortillas prepared with *lpa1-1*-LP maize. The mean FAZ values from the tortillas prepared with the 2 wild-type hybrids were not significantly different.

Assuming a relation between FAZ and phytate intake of the form $\text{FAZ} = A + B \exp(C \text{ phytate})$, the estimated coefficients were as follows: *A* = 0.122 ± 0.038 (*P* = 0.0045), *B* = 0.634 ± 0.182 (*P* = 0.0025), and *C* = -0.0010 ± 0.0004 (*P* = 0.0176), as shown in Figure 1. The coefficient *A* represents FAZ when the phytate intake is very high. After adjustment for dietary zinc, the magnitude of the effect of phytate intake on FAZ was essentially unchanged and dietary zinc was not significant (*P* = 0.73) over the range of ingested zinc for this study.

Assuming the same form for the relation between FAZ and phytate:zinc molar ratios, the estimated coefficients were as fol-

TABLE 2Selected composition of the 2 low-phytate hybrids and their isohybrid wild types in prepared tortillas¹

Composition	<i>lpa1-1-LP</i> (n = 6)	ND-LP (n = 6)	<i>lpa1-1-WT</i> (n = 4)	ND-WT (n = 4)
Total phytate (mg/g)				
Wet wt	2.1 ± 0.2 ^{a,2}	1.3 ± 0.0 ^b	4.3 ± 0.1 ^c	3.5 ± 0.4 ^d
Dry wt	3.8 ± 0.1 ^a	2.2 ± 0.1 ^b	9.6 ± 0.1 ^c	7.4 ± 1.1 ^d
Zinc (mg/100 g)				
Wet wt	1.31 ± 0.06 ^a	2.00 ± 0.07 ^b	1.17 ± 0.24 ^a	1.24 ± 0.20 ^a
Dry wt	2.34 ± 0.06 ^a	3.28 ± 0.17 ^a	2.52 ± 0.64 ^a	2.66 ± 0.49 ^a
Phytate:zinc molar ratio	17:1	7:1	37:1	28:1
Iron (mg/100 g)				
Wet wt	1.23 ± 0.03 ^a	1.19 ± 0.08 ^a	0.79 ± 0.04 ^b	1.17 ± 0.07 ^a
Dry wt	2.53 ± 1.57 ^a	2.35 ± 0.20 ^a	1.62 ± 0.48 ^b	2.05 ± 1.31 ^c
Calcium (mg/100 g)				
Wet wt	138 ± 98 ^a	129 ± 11 ^{a,b}	146 ± 9 ^a	113 ± 4 ^{b,d}
Dry wt	285 ± 23 ^a	254 ± 18 ^{a,c}	300 ± 7 ^a	197 ± 6 ^{b,c}
Energy (kcal/g)				
Wet wt	2.2 ± 0.8 ^a	2.2 ± 0.1 ^a	2.1 ± 0.5 ^a	2.3 ± 0.1 ^a
Dry wt	4.4 ± 0.2 ^a	4.4 ± 0.0 ^a	4.3 ± 0.1 ^a	4.6 ± 0.1 ^a
Protein (g/100 g)				
Wet wt	6.1 ± 0.1 ^a	5.1 ± 0.0 ^b	4.8 ± 0.1 ^{b,c}	4.6 ± 0.0 ^c
Dry wt	12.2 ± 0.2 ^a	10.1 ± 0.1 ^b	9.5 ± 0.2 ^{c,d}	9.2 ± 0.1 ^d

¹ *lpa1-1-LP*, low-phytate (≈60% reduction) hybrid maize; ND-LP, Nutridense Low Phytate (≈80% phytate reduction); *lpa1-1-WT* and ND-WT, the corresponding wild-type isohybrids. Means within a row with different superscript letters are significantly different, $P < 0.05$ (one-way post hoc ANOVA).

² $\bar{x} \pm SD$ (all such values).

lows: $A = 0.033 \pm 0.140$ ($P = 0.82$), $B = 0.490 \pm 0.093$ ($P < 0.0001$), and $C = -0.046 \pm 0.032$ ($P = 0.16$), as shown in **Figure 2**. The coefficient A represents FAZ when the phytate:zinc molar ratio is very high. The estimated FAZ was 0.523 ± 0.078 when the phytate:zinc molar ratio was zero. After adjustment for dietary zinc, the magnitude of the effect of the phytate:zinc molar ratio on FAZ was essentially unchanged and dietary zinc was not significant ($P = 0.59$).

DISCUSSION

This study extends the data derived from a previous study with a similar design, which was, however, limited to measurements of FAZ from *lpa1-1-LP* and its isohybrid *lpa1-1-WT*. The data in the current study for these 2 hybrids was virtually identical to that for the corresponding hybrids in the previous study (18).

Phytic acid represents ≈95% of total inositol phosphate in both wild-type maize seed and the seed of low phytic acid 1 maize types, which would include *lpa1-1-LP* and ND-LP; the remainder of inositol phosphates consists primarily of inositol penta- and tetraphosphates, as was previously reported (15, 28). The

difference in phytate:zinc molar ratios between the maize kernel and the corresponding tortillas is attributable to the nixtamalization and cooking (29). It is noted that this difference is only evident in the wild types, especially ND-WT.

The zinc content of meals is a major determinant of FAZ. However, plots of FAZ versus daily zinc intake are best fit by first-order exponential decay analysis. This is supported, for example, by mean data from the 10 groups of young males whose data were used by the Food and Nutrition Board of the Institute of Medicine for the purpose of determining the dietary reference intakes for zinc (30). The range of mean zinc intakes was ≈1–16.5 mg/d. The changes in FAZ were quite small over the range of dietary zinc intakes in this current study (8–12 mg/d). Therefore, it was not unexpected that adjustments for dietary zinc did not change the magnitude of the effect of the phytate:zinc molar ratio on FAZ.

ANOVA showed a significant difference in the zinc concentration of maize kernels between the different maize hybrids used in this study, with less pronounced differences in the tortillas prepared from these maizes. The notable difference was the sub-

TABLE 3Intakes of tortillas, phytate, and zinc and fractional zinc absorption from the test meals¹

	<i>lpa1-1-LP</i> (n = 6)	ND-LP (n = 6)	<i>lpa1-1-WT</i> (n = 4)	ND-WT (n = 4)
Tortillas (g wet wt/d)	866 ± 52 ^{a,2}	901 ± 72 ^a	807 ± 77 ^a	782 ± 100 ^a
Zinc (mg/d) ³	8.3 ± 0.6 ^a	12.5 ± 1.0 ^b	10.0 ± 2.4 ^{a,c}	10.1 ± 1.2 ^{a,c}
Phytate (mg/d)	1365 ± 71 ^a	851 ± 68 ^b	3457 ± 363 ^c	2699 ± 325 ^d
Fractional absorption of zinc	0.285 ± 0.042 ^a	0.383 ± 0.066 ^a	0.151 ± 0.071 ^b	0.135 ± 0.050 ^b
Total absorbed zinc (mg/d)	2.4 ± 0.5 ^a	4.9 ± 1.0 ^b	1.5 ± 0.7 ^a	1.4 ± 0.6 ^a

¹ *lpa1-1-LP*, low-phytate (≈60% reduction) hybrid maize; ND-LP, Nutridense Low Phytate (≈80% phytate reduction); *lpa1-1-WT* and ND-WT, the corresponding wild-type isohybrids. Means within a row with different superscript letters are significantly different, $P < 0.05$ (one-way post hoc ANOVA with repeated measures).

² $\bar{x} \pm SD$ (all such values).

³ Includes oral dose of zinc isotope.

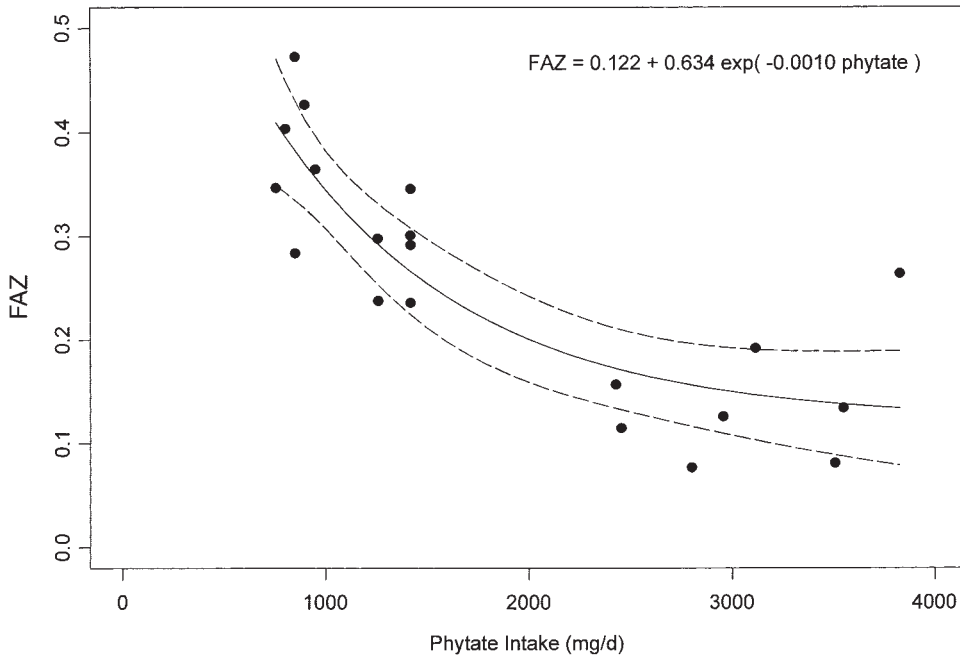


FIGURE 1. Relation between the fractional absorption of zinc (FAZ) from maize tortillas and the phytate intake from these tortillas. Zinc intakes ranged from 8 to 14 mg/d. The solid line represents the mean relation as estimated by nonlinear repeated-measures regression; the dashed lines represent 95% pointwise CI bands for the estimated mean FAZ.

stantially higher zinc concentration in the ND-LP maize. In contrast, the zinc content of ND-LP and ND-WT from plants grown adjacent to each other in a nursery in Idaho was shown to be identical. The ND-LP and ND-WT maizes used in the current study were grown in the same area of Indiana but not in the same farm or nursery. Although samples of fertilizer and soil from this

area were not analyzed, it is apparent that the differences in zinc content in this study were attributable to environmental rather than to genetic factors. We previously found similar differences in the zinc content of the same hybrids grown in different farms or nurseries with concentrations ranging from 1.3 to 3.2 mg Zn/100 g dry maize kernel. Although differences in the zinc

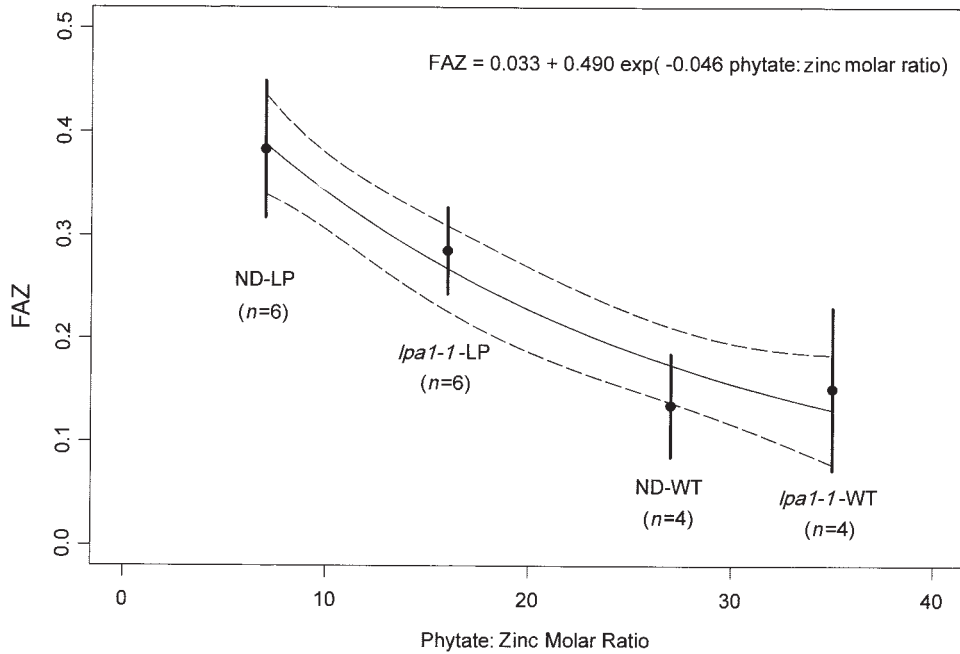


FIGURE 2. Relation between the mean fractional absorption of zinc (FAZ) from maize tortillas and the phytate:zinc molar ratio of these tortillas. The vertical bars represent the SEs, the solid line represents the mean relation as estimated by nonlinear repeated-measures regression, and the dashed lines represent the 95% pointwise CI bands for the estimated mean FAZ. *lpa1-1*-LP, low-phytate ($\approx 60\%$ phytate reduction) hybrid maize; ND-LP, Nutridense Low Phytate ($\approx 80\%$ phytate reduction; ExSeed Genetics, a Division of BASF Plant Sciences, Research Triangle Park, NC); *lpa1-1*-WT and ND-WT, the corresponding wild-type isohybrids.


content of the maize used were not the focus of this investigation, they emphasize the potential effect of agricultural environment and practices on the zinc content and the phytate:zinc molar ratios of maize.

The phytate effect was substantial and provided further evidence of the necessity of taking this effect into consideration when estimating dietary zinc requirements, especially for populations that depend on cereal grains or legumes for their principal dietary staples. However, FAZ was substantially higher at all dietary phytate intakes than has been reported by others for cereal-based test meals (3). FAZs for phytate:zinc molar ratios >5:1 are, however, substantially higher than those predicted by the World Health Organization (WHO) in a document published in 1996 (31) for diets with corresponding phytate:zinc molar ratios. Although the possibility of errors in techniques or other factors need to be considered when addressing these differences between current data and the data used by the WHO, one difference in study design that could be pertinent is the administration of labeled test meals for an entire day in the current study. This is in contrast with the data derived from single test meal studies used by the WHO committee. For zinc, there has been no reported comparison of results derived from single test meals with those obtained after the administration of labeled test meals for an entire day. Theoretically, however, it would not be expected that data derived from a single test meal in the postabsorptive state would be identical with data derived from meals consumed over an entire day. For iron, it has been reported that the effect of inhibitors of absorption is magnified by single test meal studies (32).

The test meals for this study were maize tortillas prepared by nixtamalization and, therefore, were high in calcium. This high calcium content may enhance the inhibitory effect of phytate on zinc bioavailability (3) and cannot explain the differences in effect discussed in the previous paragraph, which are in the opposite direction. The negative relations between FAZ and both dietary phytate and phytate:zinc ratios were observed down to the lowest level of dietary phytate and phytate:zinc molar ratio, ie, 7:1. This finding is consistent with the conclusion that there is no threshold for the inhibitory effect of dietary phytate on zinc bioavailability (33).

In these studies we compared FAZ from foods prepared with grains produced by normal maize hybrids (normal grain phytate contents) with those produced by the same maize hybrids, which differed only in that they were homozygous for allelic variants at a single gene that alters the grain phytate content (near-isohybrids). The differences in FAZ were attributed to the allelic variants at the single genes that determine seed phytate content. Although the current study and others (18, 29) showed the value of these low-phytate grains as a human mineral nutrition research tool, their value as a sustainable strategy for improving the bioavailability of zinc and other essential minerals from human plant-based diets remains to be shown. Reductions in the phytate content of cereal grain may need to be accompanied by a reduction in phytate in other plant foods, especially in legumes, to achieve the desired reduction in total diet phytate. Although it is not possible to remove all dietary phytate by this strategy, retention of some phytate in the diet may be prudent on a long-term basis until more is known about optimal dietary phytate contents.

The results of this study of zinc absorption from low-phytate maize and their wild-type controls, administered in test meals over an entire day, confirm the negative relation between FAZ and dietary phytate over a range of dietary phytate:zinc molar

ratios from 7:1 to 35:1. FAZs are, however, substantially higher than those predicted by the WHO for these dietary phytate levels and the zinc intake (8–13 mg/d) of the subjects in the current study. Currently, the phytate content of the habitual diet is regarded as a major determinant of dietary zinc requirements in most developing countries. Additional data on the quantitative effect of phytate on zinc bioavailability is necessary to develop reliable algorithms that account for the phytate content of the diet. Low-phytate grains attributable to homozygosity for allelic variants of a single gene provide an invaluable tool for investigating the long-term effects of dietary phytate reduction on human mineral bioavailability and homeostasis. 

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KMH, NFK, VR, JWF, GKG, JLW, LVM, and LS participated in the study design and data interpretation. JWH was responsible for the human studies. JWH, LS, and JAD were responsible for the laboratory analyses. GKG and JLW analyzed the data. KMH drafted the manuscript. None of the authors had any financial interest in the seed companies that donated the maize required for this study.

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