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## Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas<sup>1-3</sup>

Concepción Mendoza, Fernando E Viteri, Bo Lönnnerdal, Kevin A Young, Victor Raboy, and Kenneth H Brown

### ABSTRACT

**Background:** Genetically modified, low-phytic acid strains of maize were developed to enhance mineral absorption, but have not been tested previously in humans.

**Objectives:** We evaluated the mineral and phytic acid contents of a low-phytic acid "flint" maize (LPM, the *lpa-1-1* mutant) and its parent, wild-type strain (WTM) and measured iron absorption from tortillas prepared with each type of maize and from a reference dose of ferrous ascorbate.

**Design:** Proximate composition and mineral and phytic acid contents were measured by standard techniques. Iron absorption from tortillas was evaluated by using the extrinsic tag method and was measured as the incorporation of radiolabeled iron into the red blood cells of 14 nonanemic men 2 wk after intake.

**Results:** The phytic acid content of LPM was 3.48 mg/g,  $\approx 35\%$  of the phytic acid content of WTM; concentrations of macronutrients and most minerals were not significantly different between strains. Iron absorption results were adjusted to 40% absorption of ferrous ascorbate. Iron absorption was 49% greater from LPM (8.2% of intake) than from WTM (5.5% of intake) tortillas ( $P < 0.001$ , repeated-measures analysis of variance).

**Conclusion:** Consumption of genetically modified, low-phytic acid strains of maize may improve iron absorption in human populations that consume maize-based diets. *Am J Clin Nutr* 1998;68:1123-7.

**KEY WORDS** Iron, iron absorption, iron deficiency, phytic acid, corn, maize, tortilla, men

### INTRODUCTION

Nonheme iron from cereals and other plant sources is poorly absorbed because of the presence of inhibitors of iron absorption, such as phytic acid, tannins, and selected dietary fibers, which irreversibly bind iron in the intestinal lumen (1-3). One possible approach to improving iron absorption is to reduce the phytic acid content of foods by genetically modifying their capacity to synthesize phytic acid (4; V Raboy et al, unpublished observations, 1994). Recent experiments indicate that much of the phytic acid in maize and other cereals can be removed through genetic engineering without affecting the total phosphorus content of the grain or the health of the plant (V Raboy, K Young, P Gerbasi, unpublished observations, 1994). This offers great promise for human trace mineral nutrition,

especially in populations that are primarily dependent on plant-derived diets.

Before promoting the large-scale production of low-phytic acid grain for human consumption, it is necessary that we determine whether a reduction in phytic acid content affects other components of the grain and whether low-phytic acid mutants do indeed have the expected effect on mineral absorption from mixed diets consumed by humans. We therefore conducted several laboratory analyses of the nutrient content of low-phytic acid and unmodified strains of maize and completed a clinical study of the effect of substituting the low-phytic acid maize on the absorption of nonheme iron from maize tortillas. The studies were conducted with maize tortillas because this is the most common form in which maize is consumed by vulnerable populations in Mexico and Central America.

The specific null hypotheses tested were as follows: 1) Genetically modified low-phytic acid maize (LPM) and the parent (wild-type) unmodified maize (WTM) will have similar macronutrient and mineral profiles except for their phytic acid and inorganic phosphorus contents. 2) The content of phytic acid and minerals (other than calcium) in the respective unprocessed strains of maize will not be altered by traditional processing techniques for maize tortillas. 3) Iron absorption from tortillas prepared from LPM will be the same as that from tortillas prepared from WTM.

### SUBJECTS AND METHODS

#### Composition of maize and effects of processing

WTM (nonmutant "flint" maize) and LPM (the *lpa-1-1* mutant) were provided by the US Department of Agriculture,

<sup>1</sup>From the Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala; the Department of Nutrition and Program in International Nutrition, University of California, Davis; the Department of Nutritional Science, University of California, Berkeley; and the US Department of Agriculture Agricultural Research Service National Small Grains Germplasm Research Facility, Aberdeen, ID.

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<sup>3</sup>Reprints not available. Address correspondence to C Mendoza, Department of Nutrition, University of California, One Shields Avenue, Davis, CA 95616. E-mail: cmendoza@ucdavis.edu.

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Agricultural Research Service, Cereal Crops Research Center at Montana State University. Although flint maize is not typically consumed by Central American populations, it was used for these preliminary studies because sufficient quantities of other types of maize were not yet available.

Tortillas were made from each of the 2 maize strains by traditional methods of preparation (5). Briefly, maize was boiled in 0.5% Ca(OH)<sub>2</sub> and water (5:1 proportion, liquid:maize) for 60 min and cooled to room temperature. After it was rinsed with water, the maize was ground to prepare a wet dough. Tortillas were formed from rolled dough and cooked on a metal grill. Samples of the unprocessed maize, maize dough, and maize tortillas were analyzed for proximate components and phytic acid, iron, zinc, calcium, and magnesium concentrations by using standard methods of the Association of Official Analytical Chemists (6). Ion-pair HPLC procedures were used for determination of inositol tri-, tetra-, penta-, and hexaphosphates (7). Differences in chemical composition between processed and unprocessed maize strains were evaluated by analysis of variance (ANOVA).

### Iron absorption studies

#### Subjects

The research protocol was approved by the University of California–Davis and the University of California–Berkeley human subjects committees and radiation use committees. Written, informed consent was obtained from all subjects before the study. Fourteen men aged 19–35 y were enrolled in the study. The volunteers were nonanemic (hemoglobin > 130 g/L), were not iron deficient (serum ferritin > 12 µg/L), and did not habitually consume iron-containing nutrient supplements. One of the originally enrolled 14 subjects was excluded from the analysis because he failed to complete the study schedule. The initial characteristics of the remaining 13 subjects are shown in Table 1. In general, hemoglobin and hematocrit values remained unchanged throughout the study period. Likewise, there were no significant changes in geometric mean serum ferritin concentration with time ( $P = 0.35$ ).

#### Procedures

Iron absorption from tortillas was measured by using the extrinsic labeling technique (8). Three different sets of tortillas were prepared from 100% WTM, a mixture of 50% WTM and 50% LPM, and 100% LPM. A single reference dose of iron ascorbate (3 mg Fe as FeSO<sub>4</sub> + 30 mg ascorbic acid + 1 mL 0.1 mol HCl/L + 9 mL H<sub>2</sub>O, labeled with 55.5 kBq <sup>59</sup>Fe) was also given separately to assess each subject's capacity for iron absorption. The tortillas were labeled with a tracer dose of iron containing either 111 kBq <sup>55</sup>Fe or 55.5 kBq <sup>59</sup>Fe, which was pipetted directly onto the tortillas and evenly distributed. The tortillas were then incubated overnight in a refrigerator (4 °C) before being reheated in a microwave oven and served at breakfast the following morning. During the first 14-d study period, each of 2 types of tortillas was given at breakfast for 2 d within a 4-d period; blood was sampled 14 d later for analysis of incorporation of radioiron into red blood cells (9). During the second 14-d study period, the remaining type of tortilla and the reference dose of ferrous ascorbate were given for 2 d each and a final sample of blood was obtained 14 d later. The order of the different types of tortillas was systematically varied. On each study day the volunteers consumed only the tortillas (43 g wet weight

**TABLE 1**  
General characteristics of the study subjects<sup>1</sup>

Characteristic	
Age (y)	28.3 ± 7.3 (19.0–46.0)
Weight (kg)	76.6 ± 16.1 (56.6–115.3)
Height (m)	1.8 ± 0.1 (1.7–2.0)
Hemoglobin (g/L)	149.5 ± 8.2 (130.0–160.0)
Hematocrit	0.429 ± 0.025 (0.370–0.465)
Serum ferritin (µg/L)	67.0 ± 1.9 (47.0, 95.6) [20.0–187.0]

<sup>1</sup> $\bar{x} \pm SD$ ; range in parentheses.  $n = 13$ .

<sup>2</sup>95% CI in parentheses; range in brackets.

per subject) after an overnight fast and no additional food was permitted for ≥ 4 h.

The amount of isotope incorporated into red blood cells was measured in triplicate at the University of California–Berkeley as described by Viteri and Kohaut (9). The total amount of iron absorbed was calculated by assuming a blood volume of 71 mL/kg body wt and 85% incorporation of radioactive iron into hemoglobin (10). Percentage iron absorption from the tortillas was adjusted for each subject to a reference dose absorption of 40% (8). Within-subject differences in iron absorption from the respective tortillas were compared by repeated-measures ANOVA, followed by post hoc Tukey's tests. Data were analyzed with PC-SAS (release 6.04; SAS Institute Inc, Cary, NC).

## RESULTS

### Composition of maize and effects of processing

The proximate composition and concentrations of phytic acid and selected minerals in the unprocessed grains, doughs, and tortillas prepared from WTM and LPM are shown in Table 2. Results are presented per unit dry weight. The components of the 2 strains of unprocessed maize were not significantly different except for their phytic acid and magnesium concentrations. Magnesium concentrations differed only in the unprocessed grain and not in the dough or tortillas.

The preparation of the dough, which included soaking the grain in lime-treated water, resulted in generally higher concentrations of ash and minerals in the dough than in the unprocessed grain. Dough prepared from WTM had significantly greater concentrations of iron and calcium and a lower concentration of protein than dough prepared from LPM.

The composition of the inositol phosphates in the unprocessed grain, dough, and tortillas prepared from WTM and LPM are shown in Table 3. Most of the inositol phosphates were present as inositol penta- and hexaphosphates. The traditional process of making tortillas changed the proportions of inositol tri-, tetra-, penta-, and hexaphosphates and the total inositol phosphate content slightly. Specifically, some of the hexaphosphate was converted to pentaphosphate and to smaller amounts of tetra- and triphosphate.

Tortillas made from 100% WTM had higher concentrations of iron and calcium than those made from 100% LPM (Table 2). Tortillas prepared from 100% WTM had a lower moisture content (42.3%) than tortillas prepared from LPM (52.7%). The LPM tortillas had a slightly softer texture than those prepared from WTM. As a consequence of the mineral and phytic acid composition, the molar ratios of phytic acid to iron were expected to favor greater fractional absorption of iron from the LPM tortilla

TABLE 2

Chemical composition of wild-type maize (WTM) and low-phytic acid maize (LPM) strains and effects of processing<sup>1</sup>

Component <sup>2</sup>	Unprocessed grain		Dough		Tortillas	
	WTM	LPM	WTM	LPM	WTM	LPM
Protein (g)	8.1 ± 0.03 <sup>d,3</sup>	8.2 ± 0.03 <sup>d</sup>	8.5 ± 0.06 <sup>c</sup>	9.0 ± 0.01 <sup>a</sup>	8.6 ± 0.07 <sup>c</sup>	8.9 ± 0.03 <sup>b</sup>
Ether extract (g)	4.6 ± 0.12 <sup>a</sup>	4.8 ± 0.10 <sup>a</sup>	4.7 ± 0.01 <sup>a</sup>	4.8 ± 0.04 <sup>a</sup>	4.1 ± 0.01 <sup>b</sup>	4.0 ± 0.03 <sup>b</sup>
Ash (g)	1.6 ± 0.12 <sup>c</sup>	1.6 ± 0.04 <sup>c</sup>	2.5 ± 0.01 <sup>a</sup>	2.2 ± 0.01 <sup>b</sup>	2.6 ± 0.02 <sup>a</sup>	2.2 ± 0.02 <sup>b</sup>
Total carbohydrate (g) <sup>4</sup>	85.8	85.5	84.3	84.0	84.8	85.0
Iron (mg)	2.7 ± 0.21 <sup>c</sup>	2.7 ± 0.32 <sup>c</sup>	4.6 ± 0.07 <sup>a</sup>	3.8 ± 0.21 <sup>b</sup>	4.5 ± 0.14 <sup>a</sup>	3.7 ± 0.14 <sup>b</sup>
Calcium (mg)	9.1 ± 2.12 <sup>c</sup>	6.7 ± 0.31 <sup>c</sup>	439.0 ± 3.27 <sup>a</sup>	318.0 ± 1.42 <sup>b</sup>	438.0 ± 1.09 <sup>a</sup>	316.0 ± 0.20 <sup>b</sup>
Magnesium (mg)	165 ± 0.03 <sup>b</sup>	157 ± 3.52 <sup>c</sup>	180 ± 4.38 <sup>a</sup>	186 ± 4.39 <sup>a</sup>	180 ± 4.18 <sup>a</sup>	180 ± 4.43 <sup>a</sup>
Zinc (mg)	2.2 ± 0.32 <sup>a,b</sup>	1.8 ± 0.06 <sup>b</sup>	2.6 ± 0.07 <sup>a</sup>	2.5 ± 0.07 <sup>a</sup>	2.6 ± 0.07 <sup>a</sup>	2.7 ± 0.14 <sup>a</sup>
Phytic acid (mg)	988 ± 6.13 <sup>a</sup>	348 ± 7.53 <sup>b</sup>	817 ± 21.0 <sup>a</sup>	361 ± 3.41 <sup>b</sup>	847 ± 20.35 <sup>a</sup>	368 ± 6.25 <sup>b</sup>
Phytic acid:Fe <sup>5</sup>	30.8	11.0	15.0	8.0	16.1	8.4
Phytic acid:Zn <sup>5</sup>	45.2	18.8	31.7	14.5	32.9	14.0
Ca:phytic acid <sup>5</sup>	0.2	0.3	8.9	14.6	8.5	14.1
(Ca × phytic acid):Zn <sup>5</sup>	0.1	0.1	3.5	1.2	3.6	1.1

<sup>1</sup> Three samples of each type of maize were analyzed as unprocessed grain, dough, and tortilla. Means within a row with different superscript letters are significantly different,  $P < 0.05$  (ANOVA).

<sup>2</sup> Per 100 g dry matter.

<sup>3</sup>  $\bar{x} \pm$  SD.

<sup>4</sup> Total carbohydrate by mean difference:  $100 - (\text{mean protein} + \text{mean ether extract} + \text{mean ash})$ .

<sup>5</sup> Mean molar ratios.

compared with the WTM tortillas. The lower calcium content of the LPM tortillas and the consequent reduction of the molar ratios of (calcium × phytic acid) to zinc also theoretically favored greater fractional absorption from the LPM tortillas, although this was not tested directly.

#### Iron absorption studies

Iron absorption from the diets and the reference dose is shown in Table 4. The geometric means of iron absorption were 1.93%, 1.65%, and 2.88% of intake for 100% WTM, 50% WTM + 50%

LPM, and 100% LPM tortillas, respectively. Repeated-measures ANOVAs followed by post hoc Tukey's tests indicated that iron absorption from 100% LPM tortillas was significantly higher than that from 100% WTM and 50% WTM + 50% LPM tortillas ( $P < 0.001$ ). Iron absorption from 100% WTM and 50% WTM + 50% LPM tortillas was not significantly different ( $P = 0.34$ ).

The geometric mean of iron absorption from the reference dose was only 14.1% (95% CI: 10.88, 18.40), which is consistent with the fact that none of the subjects was iron deficient. When adjusted to 40% iron absorption from the reference dose of fer-

TABLE 3

Inositol phosphate content of wild-type maize (WTM) and low-phytic acid maize (LPM) strains<sup>1</sup>

Component	Unprocessed grain		Dough		Tortillas	
	WTM	LPM	WTM	LPM	WTM	LPM
IP3						
( $\mu\text{mol/g}$ ) <sup>2</sup>	Tr	Tr	Tr	Tr	0.07 ± 0.03	0.05 ± 0.01
(% of total) <sup>3</sup>	—	—	—	—	0.5 ± 0.23	0.8 ± 0.11
( $\mu\text{g/g}$ )	Tr	Tr	Tr	Tr	29 ± 11.8	19 ± 3.0
IP4						
( $\mu\text{mol/g}$ )	0.07 ± 0.01	0.04 ± 0.01	0.11 ± 0.02	0.34 ± 0.01	0.10 ± 0.01	0.07 ± 0.01
(% of total)	0.4 ± 0.04	0.7 ± 0.15	0.8 ± 0.15	6.1 ± 0.01	0.7 ± 0.07	1.2 ± 0.14
( $\mu\text{g/g}$ )	32 ± 3.5	17 ± 3.5	52 ± 10.6	167 ± 3.5	47 ± 3.5	32 ± 3.5
IP5						
( $\mu\text{mol/g}$ )	0.83 ± 0.02	0.19 ± 0.01	1.41 ± 0.07	0.63 ± 0.01	1.37 ± 0.01	0.56 ± 0.03
(% of total)	5.5 ± 0.17	3.5 ± 0.06	11.2 ± 0.27	11.2 ± 0.34	10.5 ± 0.18	9.9 ± 0.33
( $\mu\text{g/g}$ )	478 ± 12.3	107 ± 4.1	816 ± 40.9	362 ± 4.1	790 ± 4.1	324 ± 16.4
IP6						
( $\mu\text{mol/g}$ )	14.20 ± 0.11	5.09 ± 0.11	11.06 ± 0.24	4.63 ± 0.11	11.52 ± 0.33	5.01 ± 0.07
(% of total)	94.1 ± 0.13	95.9 ± 0.09	88.0 ± 0.41	82.8 ± 0.33	88.3 ± 0.48	88.2 ± 0.29
( $\mu\text{g/g}$ )	9370 ± 70.0	3360 ± 74.7	7301 ± 158.7	3053 ± 70.0	7604 ± 214.7	3307 ± 46.7
Total						
( $\mu\text{mol/g}$ )	15.08 ± 0.09	5.31 ± 0.11	12.58 ± 0.33	5.59 ± 0.11	13.05 ± 0.30	5.68 ± 0.10
(% of total)	100	100	100	100	100	100
( $\mu\text{g/g}$ )	9880 ± 61.3	3484 ± 75.3	8169 ± 210.2	3607 ± 34.1	8471 ± 203.5	3682 ± 62.5

<sup>1</sup>  $\bar{x} \pm$  SD. Inositol phosphate was analyzed in duplicate. IP3, inositol triphosphate; IP4, inositol tetraphosphate; IP5, inositol pentaphosphate; IP6, inositol hexaphosphate; Tr, trace.

<sup>2</sup> Per g dry matter.

<sup>3</sup> Percentage of total  $\mu\text{mol/g}$ .

rous ascorbate, the adjusted iron absorption for tortillas prepared from 100% LPM was 8.15%, compared with 5.48% for tortillas prepared from 100% WTM. There was a significant negative association between the serum ferritin concentration and absorption of iron from the study diets ( $r = -0.23$ ,  $P < 0.05$ )

## DISCUSSION

The LPM used in the current study was produced by inducing a genetic mutation to block the synthesis of phytic acid in the germ and aleurone, resulting in a final seed phytic acid content that was about one-third that of the parent WTM strain. The total phosphorus content of the seed was unaffected. With minor exceptions, the remaining macronutrient and mineral profile of LPM was similar to that of WTM. As a result of this isolated change in seed phytic acid content, the molar ratios of phytic acid to iron, phytic acid to zinc, calcium to phytic acid, and (calcium  $\times$  phytic acid) to zinc were theoretically more favorable for trace element absorption. Although unprocessed LPM had a significantly lower magnesium content than WTM, the magnitude of this difference was small and the difference was no longer detectable in the processed dough or tortillas.

During processing of the respective types of maize into dough and tortillas, several changes in nutrient composition were identified. The relative concentrations of protein and fat increased slightly in both types of dough, presumably because of a loss of soluble carbohydrate into the liquid used to soak the grains. The fat content of the tortillas decreased slightly, probably because of loss of volatile fats during cooking. During processing of the doughs, there were considerable increases in the concentrations of calcium and iron and small increases in the concentrations of zinc and magnesium. These changes persisted after preparation of the tortillas. The increases in calcium contents were undoubtedly due to the addition of lime [ $0.5\% \text{Ca(OH)}_2$ ] to the water during the process of nixtamalization. The increased concentrations of other minerals may have occurred either because of their presence in the soaking solutions or as a result of leaching from the grinding equipment or cooking pans. These changes are consistent with data reported previously (5), although earlier studies noted substantial variability in the calcium content of tortillas depending on how the dough was prepared (11). Tortillas are an important source of calcium in Mexican (11) and Central American (5) diets, although the bioavailability of calcium from this source may be limited (12).

According to the International Nutritional Anemia Consultative Group (13), absorption of iron is lower from maize than from other cereals. Mean absorption values of 1.8% and 2.6% were reported previously for maize porridge and maize tortillas, respectively, for subjects with adequate iron status (14, 15). These findings are similar to the values measured for WTM in the current study. Notably, the uptake of iron was  $\approx 50\%$  greater with the LPM tortillas than with the WTM tortillas. Although the magnitude of the difference in absolute amount of iron absorbed by these iron-replete subjects was small, the effect of LPM on the total amount of absorbed iron would be substantially greater in iron-depleted individuals. When iron absorption from tortillas was adjusted to a reference value of 40% absorption of iron from iron ascorbate, the 50% greater absorption from LPM implied an important contribution to total iron absorption in populations with high rates of iron deficiency. For example, if an iron-defi-

cient woman consumes an average of  $\approx 650$  g tortillas/d, as has been reported for the central highlands of Mexico (16), an additional 0.45 mg Fe would be absorbed daily from LPM tortillas, which corresponds to about one-third of the daily requirement.

Several studies have found that the absorption of minerals is directly related to their solubility in the intestinal lumen (3, 17) and that phytic acid (2, 11, 18) and calcium (19–21) are 2 dietary components that can reduce the solubility of nonheme iron. The negative effect of phytates on iron absorption in particular depends on the number of phosphate groups bound to the inositol ring, inositol hexaphosphate having the major effect (22). Almost all of the phytic acid in both types of maize used in the present study was inositol hexaphosphate, although the total amounts differed substantially.

Presumably, modification of the total phytic acid content accounted for the observed differences in iron absorption. It is conceivable, however, that the minor differences in calcium and iron contents of the study diets also affected iron absorption. Hallberg et al (20) reported the results of a study in which different amounts of calcium (ranging from 40 to 600 mg) were added to 80 g of wheat rolls, which were prepared from 55% extraction flour and contained a total of 3.8 mg Fe. The additional calcium affected iron absorption by 2 different mechanisms. Calcium inhibited the enzymatic degradation of phytic acid during the fermentation of the dough, resulting in both higher final phytic acid concentrations and reduced iron absorption. Calcium also interfered directly with iron absorption when added to the bread after baking. The first mechanism is not relevant to the present study because fermentation cannot occur under the conditions of nixtamalization. The direct interference of calcium with iron absorption in the study by Hallberg et al was dose related up to 300 mg Ca per 3.8 mg Fe (weight ratio of calcium to iron, 79:1), with no additional effect on iron absorption when the concentration of calcium was increased further. In the present study, the weight ratios of calcium to iron in the LPM and WTM diets were 87:1 and 97:1, respectively. Thus, the relative concentrations of calcium and iron were in a range in which the slightly greater calcium-to-iron ratio of the WTM diet should not have exerted any further influence on iron absorption. Likewise, it seems unlikely that the relatively small differences in dietary iron content would have influenced the efficiency of iron absorption (23). Thus, the most likely explanation for the observed differences in iron absorption from the LPM and WTM tortillas was their respective phytic acid contents.

It is worth noting that despite the reduction in phytic acid content, the molar ratio of phytic acid to iron in the LPM tortilla was still  $\approx 8.4$ . Wise (24) suggested that molar ratios of phytic acid to iron  $> 6$  are likely to reduce iron absorption. Thus, even greater benefits for iron absorption might be expected if the phytic acid content of LPM could be reduced further. Similar benefits can also be anticipated for absorption of zinc. Whereas the (calcium  $\times$  phytic acid) to zinc molar ratio of fresh tortilla prepared from WTM was  $\approx 2.1$ , this ratio in the LPM tortilla was  $\approx 0.5$ , which is the level at which absorption of zinc reportedly begins to be affected (25).

The fact that the total phosphorus contents of LPM and WTM were similar indicates that there was a substantial increase in inorganic phosphorus in LPM of  $\approx 1.7$  mg/g tortilla. Thus, concern might be raised about possible adverse effects of increased inorganic phosphorus consumption on calcium metabolism.

However, investigators have found that the addition of 1200 mg P to a basal diet that contained 800 mg P had no effect on calcium absorption or balance, regardless of the amount of calcium in the diet (26). Thus, it seems unlikely that the increased amount of inorganic phosphorus in LPM will have undesirable metabolic consequences.

In summary, we found that the reduction in the phytic acid content of maize achieved through genetic engineering yielded minimal changes in the content of other components of the cereal grain; that the production of dough changed the contents of nutrients from unprocessed corn for both WTM and LPM, accentuating the differences in maize strains; and that fractional iron absorption increased by  $\approx 50\%$  when human volunteers consumed tortillas prepared from the genetically modified LPM. Thus, null hypotheses 2 and 3 were rejected in favor of the LPM strain, regarding its potential to improve iron nutrition. Given the possible importance of these results for human trace element nutrition in populations that are dependent primarily on plant-derived diets, further efforts are warranted to develop and evaluate a wider range of low-phytic acid cereals with even greater reductions in phytic acid contents.  $\square$

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