Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA[1–3]

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ABSTRACT

Background: Reducing the phytate content in grains by genetic manipulation is a novel approach to increasing nonheme-iron absorption from mixed diets. Fractional iron absorption from a genetically modified strain of low-phytate maize (LPM) increased significantly, by 50%.

Objective: We assessed iron absorption from porridges prepared from the same LPM (*lpa-1-1* mutant) and unmodified wild-type maize (WTM), both of which were fortified with either ferrous sulfate or sodium iron EDTA.

Design: Porridges providing 3.4 mg Fe were fortified with either ferrous sulfate or sodium iron EDTA to provide an additional 1 mg Fe/serving. In 14 nonanemic women, iron absorption was measured as the amount of radioiron incorporated into red blood cells (extrinsic tag method) 12 d after consumption of the study diets.

Results: No significant effect of phytate content on iron absorption was found when porridge was fortified with either sodium iron EDTA or ferrous sulfate. Fractional absorption of iron from WTM porridge fortified with sodium iron EDTA (5.73%) was 3.39 times greater than that from the same porridge fortified with ferrous sulfate (1.69%). Fractional absorption of iron from the sodium iron EDTA–fortified LPM porridge (5.40%) was 2.82 times greater than that from LPM porridge fortified with ferrous sulfate (1.91%) ($P < 0.0001$ for both comparisons, repeated-measures analysis of variance). Thus, the previously identified benefit of LPM was no longer detectable when maize porridge was fortified with additional iron.

Conclusion: Iron was absorbed more efficiently when the fortificant was sodium iron EDTA rather than ferrous sulfate, regardless of the type of maize. *Am J Clin Nutr* 2001;73:80–5.

KEY WORDS Iron absorption, iron deficiency, anemia, phytate, phytic acid, food fortification, ferrous sulfate, sodium iron EDTA, maize, corn, genetically modified food

INTRODUCTION

Iron deficiency is one of the major nutritional problems in the developing world, affecting primarily women of childbearing age, infants, and children (1–3). The main causative factors are poor iron content, low bioavailability of iron, or both in the largely plant-based diets that are typically consumed in many low-income countries (4). Food components such as phytates, tannins, and selected dietary fibers, which bind iron in the intestinal lumen, can impair iron absorption (5). Phytate is the component that probably has the greatest effect on iron status because many plant foods have high phytate contents that can severely impair iron absorption (5–7).

A novel approach to limiting the phytate content of mixed diets is to reduce the amount of phytate contained in grains (seeds) by inducing genetic mutations that interfere with phytate synthesis (8, 9). Earlier studies found that a reduction in phytate content had minimal effects on the nutrient composition of flint corn (10).

Food fortification is generally considered to be a potentially beneficial long-term strategy for reducing the prevalence of iron deficiency in developing countries (11, 12). Both the dietary components and the type of fortificant used can affect iron absorption (13). When deciding on the appropriate fortificant to use in different situations, one must consider the composition of meals and the overall diet, the efficiency of absorption of the dietary iron and the fortificant iron, and associated cost factors and sensory properties.

Sodium iron EDTA is an iron chelate that was used successfully as a dietary fortificant in several trials in the developing world (14–16). When foods have high contents of substances such as phytate that inhibit mineral absorption, the iron in sodium iron EDTA is absorbed more efficiently than are other forms of nonheme iron. Moreover, the use of sodium iron EDTA has the advantage of making the total nonheme iron pool, including intrinsic nonheme food iron, as absorbable as the iron in the sodium iron EDTA (17–21).

The long-term objective of the current research effort is to assess the nutritional effect of substituting genetically modified, low-phytate maize (LPM) for unmodified maize as a strategy for

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TABLE 1
General characteristics of the subjects

Characteristic	Value
Weight (kg)	60.7 ± 12.3 (47.8–97.7)[1]
Height (m)	1.68 ± 0.09 (1.54–1.82)[1]
Hemoglobin (g/L)	138.1 ± 8.6 (129–160)[1]
Hematocrit	0.41 ± 0.02 (0.37–0.46)[1]
Serum ferritin (μg/L)	21.9 ± 2.4 (14.1–34.3)[2]

[1] $\bar{x}$ ± SD; range in parentheses. n = 14.
[2] Geometric $\bar{x}$ ± SD; range in parentheses. 95% CI: 6.5, 140.

improving the iron status of low-income, maize-consuming populations. In a previous study, we found that the fractional absorption of iron from maize tortillas increased by ≈50% when the phytate content was reduced to one-third the amount originally contained in the unmodified (wild type) strain of flint corn (22). The specific purpose of the present study was to assess iron absorption from test meals composed of LPM or wild-type maize (WTM) porridges fortified with either sodium iron EDTA or ferrous sulfate.

SUBJECTS AND METHODS

Materials

In all studies, a parent strain of WTM (nonmutant) and an LPM (*lpa*-1-1 mutant) were provided by the US Department of Agriculture–Agricultural Research Service National Small Grains Germplasm Research Facility at Montana State University. The study material, which was produced during the summer of 1995, was a flint corn with 0.99 or 0.35 g phytate/100 g dry wt of maize for the wild-type and *lpa*-1-1 genotypes, respectively.

Subjects

Healthy, nonpregnant adult female volunteers (n = 14) aged 19–42 y who were not habitually consuming iron-containing nutritional supplements were recruited from the University of California Davis student population. The sample size estimate for this group was calculated to be sufficient to detect an increase of 50% in iron absorption, assuming a level of significance of 5%, a statistical power of 80%, and a CV of ≈33%. The research protocol was approved by the University of California Davis and the University of California Berkeley Human Subject Committees and Radiation Use Committees. Written, informed consent was obtained from all subjects before the study.

The general characteristics of the subjects are shown in **Table 1**. At the beginning of the study, their mean hemoglobin concentration was 138 g/L and their geometric mean serum ferritin concentration was 21.9 μg/L. Four women had serum ferritin concentrations <12 μg/L, which indicates depleted iron stores. These baseline values did not change significantly during the course of the study.

Diets

Each type of maize was subjected to nixtamalization (23), the traditional method of preparing the dough to make tortillas from maize. Briefly, maize is boiled in 0.5% $Ca(OH)_2$ and water (5:1 proportion of liquid to maize) for 60 min and is then cooled to room temperature. The maize is rinsed with water and is ground to prepare the wet dough. Traditionally, this dough would be made into tortillas. In this study, a measured amount of the dough providing 3.4 mg Fe was then cooked with water, sugar, and condiments to make porridge. Each type of porridge was fortified with either ferrous sulfate or sodium iron EDTA to provide an additional 1 mg Fe/serving. The different types of porridge were offered in random order. During the study, each subject consumed 2 types of porridge for 2 consecutive days each.

The composition of the maize doughs used to make the porridges is shown in **Table 2**. The phytic acid content of the diets was determined by HPLC (24) and the iron, zinc, and calcium contents were determined by flame atomic absorption spectrophotometry (model 3030 B; Perkin-Elmer, Norwalk, CT) (25). The following molar ratios of the diets were calculated: phytate:iron, phytate:zinc, calcium:phytate, and [calcium] × [phytate]/[zinc] (Table 2). The final experimental diets and their components are shown in **Table 3**.

Procedures

Each portion of the study porridges was incubated with 2 μCi (74 kBq) ^{55}Fe or 1.5 μCi (55.5 kBq) ^{59}Fe (as $FeCl_3$) overnight in a refrigerator at 4 °C before consumption. The volunteers came to the laboratory after fasting overnight. Before they ate the porridge on day 1, we obtained 30 mL blood to estimate baseline ^{55}Fe and ^{59}Fe radioactivity in red blood cells. We used the double radioisotope technique described by Viteri and Kohaut (26).

After the blood sample was obtained, the reheated diets were offered at breakfast on that day and the next day. The porridge was served with disposable dishes and spoons. The dishes and spoons were rinsed twice with distilled water, and the residual water was consumed by the subjects. No additional food was permitted for ≥4 h after the test meals.

TABLE 2
Approximate composition and fiber, phytate, and mineral contents of nonfortified doughs prepared from wild-type maize (WTM) with a normal phytate content and low-phytate maize (LPM)[1]

	Dough	
Component[2]	WTM	LPM
Residual moisture (g)	1.8 ± 0.0 [a,3]	2.5 ± 0.1[b]
Protein (g)	8.5 ± 0.1[a]	9.0 ± 0.0[b]
Lipids (g)	4.7 ± 0.0[a]	4.8 ± 0.0[b]
Ash (g)	2.5 ± 0.0[a]	2.2 ± 0.0[b]
Total carbohydrate (g)[4]	84.3	84.0
Phytate (mg)	817 ± 21[a]	361 ± 3[b]
Iron (mg)	4.6 ± 0.1[a]	3.8 ± 0.2[b]
Calcium (mg)	439 ± 3[a]	318 ± 1[b]
Magnesium (mg)	180 ± 4	186 ± 4
Zinc (mg)	2.6 ± 0.1	2.5 ± 0.1
Phytate:iron[5]	15.0	8.0
Phytate:zinc[5]	31.7	14.5
Calcium:phytate[5]	8.9	14.6
[Ca] × [Phytate]/[Zn][5]	3.5	1.2

[1] Three samples of each type of maize were analyzed as dough. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (ANOVA).
[2] Per 100 g dry matter.
[3] $\bar{x}$ ± SD.
[4] Total carbohydrate by difference [100 − (mean protein + mean ether extract + mean ash)].
[5] Mean molar ratio.

TABLE 3
Composition of the study diets per serving[1]

Ingredient	WTM porridge: With NaFeEDTA	WTM porridge: With $FeSO_4$	LPM porridge: With NaFeEDTA	LPM porridge: With $FeSO_4$
Dough (g dry wt)	76.2	76.2	90.3	90.3
Water (mL)	240	240	240	240
Sugar (g)	22.8	22.8	22.8	22.8
Cinnamon (g)	2.9	2.9	2.9	2.9
Margarine (g)	13.0	13.0	13.0	13.0
Iron (mg)				
From dough	3.4	3.4	3.4	3.4
From NaFeEDTA	1.0	—	1.0	—
From $FeSO_4$	—	1.0	—	1.0
Total	4.4	4.4	4.4	4.4
Phytate:iron	16	16	6.8	6.8
Radioiron				
^{55}Fe as $FeCl_3$				
(μCi)	2.0	—	2.0	—
(kBq)	74	—	74	—
^{59}Fe as $FeCl_3$				
(μCi)	—	1.5	—	1.5
(kBq)	—	56	—	56

[1] WTM, wild-type maize (normal phytate content); LPM, low-phytate maize; NaFeEDTA, sodium iron EDTA; $FeSO_4$, ferrous sulfate.

On day 12, a second blood sample (30 mL) was drawn to estimate iron absorption from the first set of diets; iron absorption was determined by measuring the amount of isotope incorporated into red blood cells. These same samples also provided baseline values for the next set of absorption studies. The second set of test diets was administered to the subjects on the 2 d after the blood sample was obtained. A third sample of blood (30 mL) was drawn on day 24 of the study. Radioactivity in triplicate samples of porridges and in duplicate samples of blood was analyzed at the University of California Berkeley. Iron absorption was calculated by assuming 85% and 90% incorporation of radioiron into red blood cells for subjects with serum ferritin values > or <15 μg/L, respectively (26). Hemoglobin, hematocrit (27), and serum ferritin concentrations (Magic Ferritin [^{125}I] radioimmunoassay; CIBA-Corning, Pittsfield, MA) were measured in all samples.

Statistical analyses

Descriptive statistics (minimum, maximum, mean, and SD) were calculated for all variables at baseline and after consumption of the study diets. Serum ferritin and iron absorption values were natural log transformed to normalize their distribution. Correlation analysis was used to relate iron status (natural log serum ferritin concentration) to the natural log of absorbed iron (%). Within-subject differences in iron absorption from the different porridges were compared by using repeated-measures analysis of variance. The significance of these comparisons was determined by using Tukey's studentized range test with a procedure-wise error rate of 0.05. Data were analyzed with PC-SAS (release 6.04; SAS Institute Inc, Cary, NC).

RESULTS

The approximate composition and mineral contents of the nixtamalized LPM and WTM doughs, expressed per 100 g dry wt, are shown in Table 2. Dough prepared from WTM had significantly lower residual moisture, protein, and lipid (by ether extraction) contents ($P < 0.05$) than did dough prepared from LPM. In contrast, the ash, phytic acid ($P < 0.01$), iron ($P < 0.05$), and calcium ($P < 0.05$) contents of the WTM dough were significantly greater than those of the LPM dough. The ingredients used to prepare the study diets are listed in Table 3.

The composition of the inositol phosphates in the dough prepared from WTM and LPM is shown in **Table 4**. Inositol pentaphospate and inositol hexaphosphate together constituted 99% of the inositol phosphates in the WTM dough and 94% of the inositol phosphates in the LPM dough.

The percentage of iron absorbed from the diets and the absorption ratios of the 2 types of maize and the 2 fortificants are shown in **Table 5**. The geometric means of iron absorption, expressed as percentages of the dose administered, were 5.73% and 1.69% for the WTM diets fortified with sodium iron EDTA and ferrous sulfate, respectively, and 5.40% and 1.91% for the LPM diets containing the same fortificants, respectively. There was no significant effect of the phytate content of the diets on the absorption of iron from each fortificant source of iron ($P = 0.81$). Iron absorption from sodium iron EDTA–fortified diets was significantly higher ($P < 0.0001$) than that from ferrous sulfate–fortified diets, which agrees with several other studies (18–21).

There was a significant inverse relation between the ln of serum ferritin concentration and the ln of iron absorption for all diets combined ($r = -0.53$, $P < 0.001$) and for the individual diets fortified with sodium iron EDTA ($r = -0.64$, $P < 0.05$ for LPM; $r = -0.81$, $P < 0.001$ for WTM). The correlations between ln serum ferritin concentration and ln of iron absorption were not significant for the diets fortified with ferrous sulfate, possibly because of the low absorption of iron and limited variability of absorption with these diets.

DISCUSSION

In a previous study, we found that the fractional absorption of iron was significantly greater, by ≈50%, when tortillas were

TABLE 4
Inositol phosphate content of dough made from wild-type maize (WTM) and low-phytate maize (LPM)[1]

Component	Dough WTM	Dough LPM
IP_3		
(μmol/g)[2]	Trace	Trace
(% of total)[3]	—	—
(μg/kg)	Trace	Trace
IP_4		
(μmol/g)	0.11 ± 0.02	0.34 ± 0.01
(% of total)	0.8 ± 0.2	6.1 ± 0.0
(μg/kg)	52 ± 11	167 ± 4
IP_5		
(μmol/g)	1.41 ± 0.07	0.63 ± 0.01
(% of total)	11.2 ± 0.3	11.2 ± 0.3
(μg/kg)	816 ± 41	362 ± 4
IP_6		
(μmol/g)	11.06 ± 0.24	4.63 ± 0.11
(% of total)	88.0 ± 0.4	82.8 ± 0.3
(μg/kg)	7301 ± 159	3053 ± 70
Total		
(μmol/g)	12.58 ± 0.33	5.59 ± 0.11
(% of total)	100	100
(μg/kg)	8169 ± 210	3607 ± 34

[1] $\bar{x}$ ± SD. Inositol phosphate content was analyzed in duplicate. IP_3, inositol triphosphate; IP_4, inositol tetraphosphate; IP_5, inositol pentaphosphate; IP_6, inositol hexaphosphate.
[2] Per g dry matter.
[3] Percentage of total mmol inositol phosphate/g dough.

prepared from genetically modified LPM in place of the unmodified parent strain of flint corn (22). The present study was conducted to determine whether the combined use of LPM and different iron fortificants would have a synergistic effect on iron absorption. We found no synergistic effect.

Although flint corn is not the type of maize typically consumed by Latin American populations, it was the only form of LPM available in sufficient quantity at the time of these studies. The results are probably applicable to other strains of genetically modified LPM.

Iron sulfate is the fortificant used most commonly in developing countries to control iron deficiency (12). Sodium iron EDTA is an iron chelate that has been proposed as an alternative fortificant for use when diets contain large amounts of inhibitors of mineral iron absorption (18, 19, 28–31). Previous studies showed that sodium iron EDTA forms a common pool with nonheme iron and partially protects this iron from being bound intraluminally by inhibitors of absorption, thereby increasing the fractional absorption of the entire nonheme-iron pool (18, 20, 28, 31). Fortification of sugar with sodium iron EDTA successfully increased iron reserves in a rural Guatemalan population (14, 15).

In the present study, iron from the porridges prepared with sodium iron EDTA was absorbed ≈3 times more efficiently than was iron from the porridges containing ferrous sulfate. It has been hypothesized that fortification with sodium iron EDTA may be advantageous only when there are large amounts of phytates in the diet. Most of the iron (94%) from the original sodium iron EDTA is released from the EDTA complex before absorption, resulting in free EDTA that can then form chelates with diet-derived iron; these chelates then become available for absorption (19, 20, 31). The results of the present study are consistent with this hypothesis in that the ratio of iron absorption from the sodium iron EDTA–fortified diet to iron absorption from the ferrous sulfate–fortified diet was greater with WTM porridge (3.39) than with LPM porridge (2.82), although the difference was not significant (Table 5).

Unlike our earlier study, in which iron absorption from tortillas prepared with LPM was 50% higher than that from tortillas prepared with WTM, there was no apparent advantage of the LPM with regard to iron absorption when the porridges were fortified with additional iron from sodium iron EDTA. The different results in these 2 studies, in which the same 2 varieties of maize were used, may be related to *1*) the characteristics of the subjects in each study, *2*) differences in the preparation of the maize, *3*) the amount of iron in the diets, and *4*) the ingredients of the respective study diets.

The subjects in the previous study had higher iron reserves than those in the present study (serum ferritin concentrations of 67.0 compared with 21.9 μg/L, respectively). However, after adjustment for serum ferritin concentration, iron absorption from WTM porridge fortified with ferrous sulfate in the present study was still significantly lower than iron absorption from the same form of maize in the earlier study. This suggests that iron status does not explain the differences in iron absorption between the studies, possibly because few individuals in either study had low iron stores (32).

In the former study, tortillas were formed from maize dough and then heated on a griddle, whereas in the present study the porridge was prepared by mixing the dough with water and heating it. Although it is conceivable that heating tortillas on a hot griddle could alter the composition of inositol phosphates, thereby affecting iron absorption, we found little difference in the composition of inositol phosphates between the tortillas and the porridge (22). Thus, the difference in preparation method probably did not explain the difference between the 2 studies in relative iron absorption.

In the present study, the total amount of iron administered was higher than in our previous study (4.4 mg/portion of porridge

TABLE 5
Iron absorption from wild-type maize (WTM) and low-phytate maize (LPM) porridges fortified with either sodium iron EDTA (NaFeEDTA) or ferrous sulfate ($FeSO_4$)[1]

	Value
Iron absorption (% of dose)	
WTM porridge	
NaFeEDTA	5.73[a] (1.31, 25.05)
$FeSO_4$	1.69[b] (1.05, 2.70)
LPM porridge	
NaFeEDTA	5.40[a] (1.21, 24.11)
$FeSO_4$	1.91[b] (0.61, 5.98)
Iron absorption ratio	
LPM:WTM	
NaFeEDTA	0.94 (0.34, 2.62)
$FeSO_4$	1.13 (0.39, 3.27)
NaFeEDTA:$FeSO_4$	
WTM porridge	3.39 (1.21, 9.50)
LPM porridge	2.82 (1.10, 7.21)

[1] Geometric $\bar{x}$; 95% CI in parentheses. Values with different superscript letters are significantly different, $P < 0.05$ (repeated-measures ANOVA followed by post hoc Tukey's analyses).

compared with 0.93 mg/portion of tortilla). When iron intake is high, a lower percentage of iron is absorbed. Using data reported by Layrisse et al (33), we estimated that fractional iron absorption may have decreased by ≈18% in the present study as a result of the higher amount of iron administered to the subjects.

Lastly, in the present study, cinnamon was added to the porridges to enhance the flavor of the final product. Tannins are a major constituent of cinnamon (34–36) and are known to form insoluble complexes with divalent metal ions such as iron, rendering them less available for absorption (37–39). On the basis of the reduction in iron absorption observed with increasing ratios of tannins to iron as reported by Tuntawiroon et al (39), we estimate that the tannins from the cinnamon in the present study may have reduced iron absorption by 24%. Thus, iron absorption from the maize porridges in the present study may have been affected not only by the phytate content of the meal, as in the previous study, but also by the higher amounts of iron and tannins in the meal.

In summary, these results indicate that iron absorption from sodium iron EDTA–fortified maize porridges was more efficient than was iron absorption from similar porridges fortified with ferrous sulfate. Under the study conditions with these iron-fortified maize porridges, no additional advantages of genetically modified LPM were detected.

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