The Poor Bioavailability of Elemental Iron in Corn Masa Flour Is Not Affected by Disodium EDTA[1]

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ABSTRACT The most sustainable way to eradicate iron deficiency is through food fortification. Elemental iron powders are commonly utilized as fortificants due to their low cost and few sensory problems. However, their bioavailability is unknown. Our goals were to measure the bioavailability of elemental iron in Mexican style corn masa flour tortillas and to evaluate the effects of Na_2EDTA. We used a stable isotope of H_2-reduced iron powder, with and without Na_2EDTA in tortillas prepared with corn masa flour. Two groups of 5- to 7-y-old children (n = 12/group) were fed tortillas to which was added 3 mg/100 g of H_2-reduced ^{58}Fe with a mean particle size of 15 μm. In one group, Na_2EDTA was incorporated at a ratio of 1:2 mol/mol. The next day, ^{57}Fe ascorbate was given as a reference dose. After 14 d, blood samples were analyzed for isotopic enrichment. When normalized to 40% absorption of the reference dose, the geometric mean (±range 1 SD) bioavailability of reduced iron in tortilla was 3.8% (2.7–5.3). The addition of Na_2EDTA, tended to increase it (P = 0.18) to 5.1% (2.8–9.2). This observed low absorption was compounded by the use of iron isotopes with smaller particle size (mean diameter 15 μm) than typical of commercial elemental iron powder (<45 μm). We conclude that H_2-reduced iron powder is an ineffective fortificant in corn tortillas.

KEY WORDS: • elemental iron • iron bioavailability • corn masa flour • disodium EDTA • stable isotopes

Iron deficiency continues to be the most prevalent single nutrient deficiency worldwide, leading to adverse effects on the health of infants, children, pregnancy and women of childbearing age (1,2). Although dietary modification and medicinal supplements have been proposed, these have generally been unsuccessful. To date, the best accepted, most cost-effective and sustainable means to eradicate iron deficiency is food fortification in which iron is added to a commonly consumed product without affecting the characteristics of the food or its price (3). Corn masa flour is a popular, affordable, widely consumed staple in Mexico and most of Central America, offering an appealing vehicle for iron fortification (4). The selection of an iron fortificant for corn masa flour is a challenge due to its abundance of phytates, which are potent inhibitors of iron absorption (5,6). The use of soluble salts such as ferrous sulfate or, to a lesser extent, ferrous fumarate, is unsuitable not only due to the strong inhibition of absorption exerted by phytates but also to the common organoleptic changes rendering it unacceptable for consumers. Thus, many countries have elected to use elemental iron powders in their fortification of cereal staple flours. Their main advantages are lower cost and lack of chemical reactivity. The latter decreases the risk of organoleptic changes, thereby increasing consumer acceptability and shelf life of the flour. Several forms of elemental iron are available, depending on the method of preparation and suppliers (7). Reduced iron is a form of elemental iron that is provided commercially in small particle size (<45 μm or 325-mesh). Because of its inertness, reduced iron is poorly soluble in water or weak acids, which is very likely to hamper its bioavailability. This is compounded by the currently unresolved consideration of the solubility of elemental iron and the unpredictable rate at which it is solubilized in the acid milieu of the stomach, yielding an unknown fraction available for absorption. Only soluble iron will enter the "common pool" of nonheme iron that interacts with inhibitors or enhancers in the gastrointestinal tract and eventually determines how much is absorbed.

In contrast, traditional radioiron bioavailability studies utilize a "tracer," i.e., a minute amount of added radioactive iron that is assumed to enter the common pool and behave identically to the dietary soluble nonheme iron. This tenet has been well demonstrated for the native iron in many foodstuffs and soluble iron salts such as ferrous sulfate and fumarate (8–12). Elemental iron powders cannot be labeled radioactively and will solubilize incompletely, rendering bioavailability figures unreliable because the tracer labels only the soluble moiety (13,14). The alternative, only recently available, is to utilize stable isotopes of elemental iron in which the bioavailability of this nonradioactive tracer can be measured reliably without the aforementioned drawbacks. Stable isotopes have the further benefit of a lack of radiation exposure to the subject. Nevertheless, there are two potential limitations to using stable isotopes for this research. The first is that the particle size, shape, porosity and other physical characteristics may not be identical to commercial iron sources. The second is that the cost of these isotopes and their analysis in biological

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samples is higher than that of radioiron. This may preclude studies in an optimal number of individuals to reach statistical power. Recently, however, the cost of iron stable isotopes has decreased and they have been more widely used in nutritional research.

The addition of chelates such as NaFeEDTA (iron, sodium ethylene diamine tetraacetate) resulted in good iron bioavailability and apparently fewer organoleptic adverse effects (15–18). This approach, however, despite the provisional approval of an international expert panel (19), has not been implemented in a sustainable program due to the cost and safety concerns with the use of NaFeEDTA (18). On the other hand, Na_2EDTA, which is less costly and devoid of safety considerations, has been shown to enhance bioavailability as an additive to ferrous salts in corn masa flour tortillas (20), but it has never been tested with elemental iron powders.

Our aim was to measure the bioavailability of stable-isotope labeled elemental hydrogen-reduced iron obtained in corn masa flour tortillas and to assess the effect of the addition of Na_2EDTA on the bioavailability of iron in that flour.

SUBJECTS AND METHODS

Subjects. Two groups of fifteen children, 5–7 y old (weight 20–25 kg) were recruited by a trained nursing team from a public school in Santiago, Chile. After oral and written explanations of the potential risks to children and their families, informed written consent was obtained from all families who participated in the study. The study was reviewed and approved by the Institutional Review Boards for experimentation in human subjects of the University of Chile, Santiago and Baylor College of Medicine, Houston, TX.

Reduced iron isotope and meal preparation. The concentration of iron added was 5.1 mg/100 g flour such that the children consumed 3 mg/100 g of tortilla. This was necessary because 100 g of dry flour makes ~170 g of tortilla, which is too much for a 5-y old child to consume. This concentration is well within the recommended fortification range of 3–6 mg iron/100 g dry flour. The children consumed 91.0 ± 11.2 g (mean ± SD) of tortilla with 25.2% moisture.

We chose not to add the reduced iron to dry flour because, although this would have been closer to what occurs in industrial mills, the iron dose becomes impossible to control and measure accurately due to unavoidable loss of flour in the tortilla preparation process. Roe and Fairweather-Tait (21) added the isotope iron powder in a thin gelatin capsule that would melt upon baking the dough and liberate the isotope. The drawback of this system is that the iron is not thoroughly mixed with the flour and remains concentrated where the capsule melts. Nevertheless, knowing the exact amount of iron added into the capsule within the flour matrix is crucial for the estimation of absorption. In our study, after both sides of the tortilla had been grilled, the capsule was added to a small amount of dough and the tortilla folded over. Therefore, the third grilling, intended to seal the tortilla, served to melt the capsule into a limited area of the tortilla. Because the melted capsule was identifiable by sight and touch, we ensured that it was taken in the first bite.

Tortillas were prepared from fresh (<3 mo from manufacture) nonfortified corn masa flour provided by MINSA, S.A. de C.V, a large industrial miller in Mexico. Iron isotopes, ^{57}Fe and ^{58}Fe, with enrichments >97% were purchased from Trace Sciences (Toronto, Canada). All isotopes were produced via calutrons in Russia. The material was provided as reduced iron powder (^{58}Fe) and ferric chloride (^{57}Fe), in argon gas ampules without further chemical alteration.

For iron measurement, the blood sample of 0.5 mL was digested in 10 mL of concentrated HNO_3 in a titration flask on a hot plate at sub-boiling temperature for 24 h. The sample was then dried and redissolved in 1–2 mL of 6 mol/L HCl to make the sample solution for the ion exchange procedure. An anion exchange column method was used to isolate the iron as previously described (22). The solution collected from the column was dried and resuspended in 30 μL of HNO_3 and loaded onto the filament for MS analysis. All samples were analyzed for isotopic enrichment using a Finnigan MAT 261 magnetic sector thermal ionization MS (San Jose, CA) as described previously (23).

Of the 30 children scheduled initially, 6 dropped out, 4 due to acute febrile illnesses within a week of the study and 2 refused to follow the protocol. Therefore, 24 children, whose normal health and nutrition status were verified by a pediatrician, were divided randomly into two equal groups to complete the study.

Protocol

Study Group 1: labeled iron with flour without Na_2EDTA. *Day 1.* Approximately 100 g of tortilla with 3 mg of ^{58}Fe was consumed by each child after an overnight fast. No food or beverage, other than deionized water, was consumed during the next 4 h. The melted isotope-containing capsule was located and fed as the first bite of the tortilla meal.

Day 2. A total of 5 mg of ^{57}Fe-ascorbate (1:2 mol/mol) in water was consumed after an overnight fast. More ^{57}Fe was needed because of its greater natural abundance. Only deionized water was permitted during the next 4 h.

Day 16. Whole blood (7 mL) was extracted by venipuncture and collected in heparinized vacutainer tubes for measurement of iron status and red cell stable isotope enrichment.

Study Group 2: Stable iron with Na_2EDTA. The clinical protocol was the same on d 1, 2 and 16 as for group 1 except for the use of flour prepared with a 1:2 EDTA:^{58}Fe (mol/mol).

We assessed the iron status of the subjects by measuring hemoglobin, mean cell volume (Cell Dyn 1700, Abbott Laboratories, IL), free erythrocyte protoporphyrin (Aviv Hematofluorometer, Lakewood, NJ) and serum ferritin (24).

Statistical analysis. Serum ferritin and iron bioavailability data were logarithmically transformed and geometric means reported. Pearson's test was used to correlate the ferrous ascorbate reference dose absorption with bioavailability of the test meals. Absorption ratios were compared using Student's t test with prior logarithmic transformation to linear data. The data reported are the antilogs. An α value of 0.05 was considered to be significant. Statistical analyses were performed using Statistica for Windows 4.5, StatSoft, Tulsa, OK.

RESULTS

Children in groups 1 and 2 did not differ in age, weight, height, hemoglobin or free erythrocyte protoporphyrin. Serum ferritin of group 1 was significantly lower than that of group 2 ($P = 0.03$). This difference has no bearing on the results because they were normalized with the reference dose, a better indicator of iron status (**Table 1**).

When Na_2EDTA was added in a 1:2 mol/mol to corn masa flour, the bioavailability of ^{58}Fe reduced iron increased by 34%, a nonsignificant difference ($P = 0.18$). The means of the uncorrected absorptions and that of the reference dose did not differ (Table 1). Normalization to 40% of the reference dose is done to correct for the different iron nutrition of the subjects; this determines their individual percentage of iron absorption. This correction allows for proper comparison of studies between subjects with different iron status such as groups 1 and 2 in this study. The absorption of the reference dose (^{57}Fe) and the bioavailability of the stable isotope (^{58}Fe) were correlated ($r = 0.64$, $P < 0.01$), underlining the fact that the percentage of iron absorbed from the meal depends on iron status, which is best measured with the reference dose.

DISCUSSION

There was very poor iron bioavailability of fortified iron in the corn masa flour tortilla. This finding is particularly disquieting because it is likely that the absorption values described for the stable isotope iron used here are somewhat higher than

TABLE 1

Iron nutrition status of children and bioavailability of corn-masa tortilla fortified with reduced iron with or without EDTA[1]

Reduced Fe[58]		Weight	Height	Hb	MCV	FEP[2]	SF[3]	Iron absorption		
								Corn-masa Fe[58]	Ascorbate Fe[57]	Corrected to 40%
		kg	*cm*	*g/L*	*fL*	*μmol/L RBC*	*μg/L*	%		
Group										
1	Alone	20.8 ± 3.2	11[4] ± 6	126 ± 5	79 ± 2	1.02 ± 0.43	13 (7–25)	2.0 (1.1–3.8)	21.5 (11.9–39.0)	3.8 (2.7–5.3)
2	+EDTA	22.2 ± 4.3	113 ± 7	127 ± 9	80 ± 2	1.00 ± 0.28	23 (14–40)	2.3 (1.0–5.0)	18.1 (8.7–37.8)	5.1 (2.8–9.2)
P[4]		0.37	0.74	0.91	0.30	0.91	0.03	0.68	0.53	0.18

[1] Values are geometric means ± SD or geometric mean (range of 1 SD), $n = 12$.
[2] To convert to μg/dL, divide by 0.0177.
[3] Abbreviations: Hb, hemoglobin; MCV, mean cell volume; FEP, free erythrocyte protoporphyrin; SF, serum ferritin.
[4] Student's *t* test *P*-value.

would be found in the field. This is due to the likelihood that the smaller particle size of the stable isotope improved its absorption relative to available commercial iron (25).

We found additionally that Na_2EDTA did not significantly enhance the absorption of iron. This study had sufficient power to detect a doubling in absorption, which would be considered necessary for clinical benefit, and this was not seen. It is likely that the use of gelatin capsules led to inadequate mixing of the iron and the EDTA. In previous studies in our laboratory we found a clear favorable effect of Na_2EDTA when iron fumarate and EDTA were added and mixed into the dry flour before adding water, kneading and resting the dough; the iron was then expected to interact with the EDTA at this stage, maximizing the effect of chelation (20).

In a recent study from the United Kingdom (21), stable isotope bioavailability was measured in wheat flour products. These investigators obtained a strikingly high absorption of reduced iron (65%) from their wheat bread rolls. This value was very high compared with the 10% iron absorption we obtained in similarly high extraction Chilean bread fortified with ferrous sulfate (26) and the 6.5% obtained in a recent study with stable isotopes (27). This discrepancy remains unexplained but it is likely due to methodological differences. In our view, it is extremely unlikely that absorption values approaching 65% can be obtained in clinical settings, and the findings from the current study support this view.

A study in Guatemala using commercial corn masa flour tortillas fortified with stable isotope ferrous fumarate also did not show an enhancing effect of Na_2EDTA on iron bioavailability (16) despite a higher EDTA:Fe (1:1 mol/mol) than in the current study. This result is consistent with the lack of efficacy of Na_2EDTA in the present study with elemental iron. Studies in our laboratories, however, found significant enhancement of iron absorption of fumarate with the addition of Na_2EDTA (20). The preparation of the isotope and the Na_2EDTA was different. In our study, we mixed the Na_2EDTA into the dry flour and added the isotope during the kneading of the dough, whereas in the Guatemalan study, the isotope was added to the bean paste spread on the baked tortilla. This may have not allowed sufficient time for complete mixing and hindered contact between the EDTA and iron moieties in the gastrointestinal tract.

To allow for valid conclusions, it is important to use a study fortificant as close as possible in physical and chemical characteristics to the commercial fortificant and mix the fortificants with the vehicle as similarly as possible to the industrial process. Unfortunately, commercial type elemental iron cannot be obtained in stable isotope form to completely resolve this issue at the present level of technology. Nevertheless, the low absorption values we found indicate that for comparative and practical purposes, this approach is useful for evaluating the relative value of fortification strategies. Combining field efficacy trials (28) and stable isotope bioavailability studies may allow for a reasonably complete understanding of the benefits and limitations of proposed iron fortification approaches. The experimental use of intestinal cell lines is another promising advance (29). The high cost and long time required for field efficacy trials to be conducted (3) limit this approach and make it necessary to use multiple strategies in assessing proposed interventions.

In summary, our data cast a dismal outlook on the likelihood that use of this fortificant in this matrix would improve the iron status of the population. Furthermore, elemental iron in wheat bread is also poorly absorbed (27). Elemental iron is used in cereal-based staple diets in most of the American continent (30), Europe and Asia. Accordingly, these data should be used in the planning of fortification programs and research is required to overcome the problems of fortification of cereal staples.

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