Bioavailability of elemental iron powder in white wheat bread

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Objective: Iron fortification of wheat flour is widely used. In most cases, elemental iron powders are utilized as fortificants due to their lower cost and few, if any, sensory problems. However, their bioavailability is unknown. We aimed to measure the bioavailability of H_2-reduced elemental iron powder in white wheat bread made from 72% extraction flour.
Design: A stable isotope of H_2-reduced iron powder (mean particle size 15 μm) was used as fortificant in bread prepared from unfortified wheat flour. In all, 12 5- to 7-y-old children were fed bread with 4 mg of H_2-reduced ^{58}Fe /100 g of flour. The next day ^{57}Fe ascorbate was given as reference dose. After 14 days, erythrocytes were analyzed for isotopic enrichment using mass spectrometry.
Results: When normalized to 40% absorption of the reference dose, the geometric mean (±range of 1 s.d.) bioavailability of reduced ^{58}Fe in wheat bread rolls was 6.5% (3.7–11.8).
Conclusions: When compared to previous radioiron studies of ferrous sulfate showing 10% absorption from an identical meal in adult women, the relative bioavailability can be estimated at about 65%. However, the bioavailability of this smaller particle size ^{58}Fe (15 μm) is likely to be higher than that of commercial iron powder (45 μm) although the precise difference cannot be ascertained with current methods. Thus, the bioavailability of commercial elemental iron powders currently used in fortification programs is likely to be substantially lower than that of ferrous sulfate.
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Keywords: elemental iron; bioavailability; wheat bread

Introduction

Wheat flour bread is an appealing vehicle for iron fortification because of its popularity, relative low cost, and widespread consumption in many countries of both hemispheres. The selection of an iron fortificant for wheat flour is a challenge due to its abundance of phytates, potent inhibitors of iron absorption (Hallberg *et al*, 1987). Apart from the inhibition exerted by phytates, the use of soluble salts such as ferrous sulfate and, to a lesser extent, fumarate can be accompanied by organoleptic changes that render this practice unacceptable for consumers. Exceptionally, in countries with mild heat and humidity climate and rapid mill-to-baking turnaround time (<3 months) ferrous sulfate has been used successfully (Peña *et al*, 1991; Walter *et al*, 2001). Experiences in Central America with ferrous fumarate have also been reported (Dary, 2002; Dary *et al*, 2002). However, if these latter conditions are not met, ferrous salts become unsuitable. Consequently, many countries have turned to elemental iron powders in their fortification programs of cereal staple flours (Hurrell, 1997). Its main advantages are lower cost and lack of chemical reactivity. This chemical inertness virtually eliminates the risk of organoleptic changes, thereby increasing consumer acceptability and shelf life.

Several forms of elemental iron are available depending on the method of preparation and suppliers (Hurrell *et al*, 2002).

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H_2-reduced iron is a form of elemental iron that is commonly provided commercially in small particle size (<45 μm or 325 mesh). Owing to its low reactivity, reduced iron is poorly soluble in water or weak acids, which is very likely to hamper its bioavailability. Moreover, the absolute solubility of elemental iron and the rate at which it dissolves in the acid milieu of the stomach is unpredictable, so that an uncertain fraction will be available for absorption. Only soluble iron will enter the 'common pool' of nonheme iron that interacts with inhibitors or enhancers in the diet and eventually determines how much is absorbed. This caveat is not evident in traditional radioiron bioavailability studies that utilize an extrinsic tag or tracer—a minute amount of added radioactive iron—that is assumed to enter the common pool and behave identically to the dietary soluble nonheme iron therein. This has been well demonstrated for the native iron in many foodstuffs and soluble iron salts such as ferrous sulfate (Layrisse *et al*, 1969, 1973; Bjorn-Rasmussen *et al*, 1972, 1973, 1974; Cook *et al*, 1972).

With elemental iron powders that cannot, in a practical fashion, be radioactively labeled, bioavailability figures become unreliable (Hurrell, 1984, 1997, 1998, 1999). For these reasons, radioiron tracers are unsuitable to evaluate bioavailability of elemental iron products. As an alternative, elemental iron stable isotopes have recently become available; however, stable isotope iron reagents may not be identical to the commercial products. In fact, for this work, the iron stable isotope obtained was of smaller particle size than the commercial reduced iron.

With this caveat in mind, the aim of this study is the measurement of the bioavailability of elemental H_2-reduced iron obtained as a stable isotope in 72% extraction wheat flour bread. To the best of our knowledge, this is the first study evaluating stable isotope iron bioavailability in bread using erythrocyte iron incorporation.

Subjects and methods

Subjects

A group of 15 children, 5- to 7-y-old (weight 20–25 kg), were brought in by a trained nursing team from a municipal school in Santiago, Chile. Informed written consent was obtained from all families in accordance with the University of Chile's Institutional Review Board for research in human subjects and The Institutional Review Board for Human Subjects of Baylor College of Medicine, Houston, TX.

Reduced iron stable isotope addition

We elected not to add the reduced iron to dry flour, although this would have been closer to what occurs in the industrial mill, because the iron dosage becomes impossible to control due to unavoidable loss of flour in the bread preparation process. Researchers in the UK (Roe & Fairweather-Tait, 1999) added the isotope iron powder in a thin gelatin capsule that would melt upon baking of the bread 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, the iron is indeed within the flour matrix and the exact dose of iron can be added into the capsule, a crucial step for the estimation of absorption. In our study we replicated this procedure. Since the melted capsule was identifiable by touch, we made sure it was taken in the first bite. French-type bread rolls were prepared from fresh (less than 3 months from manufacture) nonfortified 72% extraction wheat flour (GRANOTEC, Santiago, Chile).

The protocol was:

Day 1: Approximately 100 g of bread (75 g of flour, taking the iron to 4.0 g/100 g of flour) with 3 mg of ^{58}Fe was consumed by each child after an overnight fast. The melted isotope-containing capsule was located and fed as the first bite of the bread roll. All children then ate the whole roll. No food or beverage, other than tap water, was given over the following 4 h.

Day 2: A total of 5 mg of $^{57}FeCl_3$-ascorbate in water was consumed also after an overnight fast. Only water was permitted during the following 4 h. This larger amount of ^{57}Fe is necessary to detect enrichment, because it is a naturally more abundant stable isotope.

Day 15: Venipuncture and extraction of 7 ml of whole blood collected in heparinized vacutainer mineral-free tubes for measurement of iron status and red cell stable isotope enrichment.

Iron status of the subjects included: hemoglobin, mean cell volume (Cell Dyn 1700, Abbott labs, IL, USA), Protoporphyrin (Aviv Hematofluorometer, Lakewood, NJ, USA) and serum ferritin (Arredondo *et al*, 1991).

Preparation and measurement of stable isotopes

Iron isotopes, ^{57}Fe and ^{58}Fe, with enrichments >90% were purchased from Trace Sciences, Inc. (Toronto, Canada). The material was provided as reduced iron powder in argon gas ampoules without further chemical alteration. Apart from the particle size (average 15 μm), no other specifications were available. For the measurement of erythrocyte iron enrichment, a 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 dissolved in 1–2 ml of 6 N 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 (Abrams *et al*, 1996). The solution collected from the column was dried and suspended in 30 μl of 3% HNO_3 and loaded onto the filament for mass spectrometric analysis. All samples were analyzed for isotopic enrichment using a Finnigan MAT 261 magnetic sector thermal ionization mass spectrometer (San Jose, CA, USA) as previously described (Ames *et al*, 1999).

Data on serum ferritin and iron bioavailability were expressed as geometric means (±range of 1 s.d.) reported. Pearson's test was used to correlate ferrous ascorbate

Table 1 Bioavailability of wheat bread fortified with elemental H_2-reduced ^{58}Fe

UPN	Sex	Age (y)	Weight (kg)	Height (cm)	Hb (g/l)	MCV (fl)	FEP (μmol/l)	SF (μg/l)	Iron absorption (%)	
									Bread reduced Fe^{58}	Ferrous ascorbate Fe^{57}
1	1	4	26.1	118	130	77	1.06	16	2.7	28.5
2	1	4	33.1	125	145	81	1.27	25	2.8	15.5
3	1	4	25.8	110	122	76	0.96	18	2.2	23.3
4	1	6	21.9	108	138	80	1.88	17	1.2	14.0
5	1	6	27.1	111	145	80	0.96	26	3.5	27.5
6	1	6	23.0	109	138	83	0.96	24	5.0	11.3
7	2	5	23.5	107	122	76	1.82	20	3.4	29.6
8	2	4	23.6	105	126	77	1.47	14	4.2	35.3
9	2	5	21.7	108	123	83	1.42	25	2.9	28.8
10	2	6	21.1	110	134	77	1.17	27	8.3	12.3
11	2	5	19.1	106	129	83	1.01	10	5.7	34.8
12	2	5	24.6	127	129	81	1.12	20	10,4	37.3
Mean			21.9	112	132	79	1.26	19[a]	3.7[a]	23.0[a]
s.d.					8	3	0.33	14–26	2.1–6.7	14.9–35.5
Normalized 40%									6.5 (3.7–11.8)	**40.0**

[a]Geometric mean and range ± 1 s.d.
Sex 1=male; 2=female.

reference dose absorption with bioavailabilities of the test meals (Statistica for Windows 4.5, StatSoft Inc., Tulsa, OK, USA).

Results

Of the 15 children scheduled initially, three became ineligible (two due to acute febrile illnesses within a week of the study and one refused the protocol). The results reported are for the remaining 12 suitable subjects, an equal number of girls and boys. On average (±s.d.), these children consumed 91.0 ± 11.2 g of bread of 25.2% moisture.

The uncorrected bioavailability (geometric mean ± range of 1 s.d.) for ^{58}Fe H_2 reduced iron was 3.7% (2.1–6.7%). When it was normalized taking the reference dose to 40%, bioavailability in wheat flour bread became 6.5% (3.7–11.8%) (Table 1). Normalization to the reference dose has two purposes: (1) to report data that can be compared to other studies, because iron status is a strong determinant of absorption and individuals with different iron status could not be compared otherwise, and (2) placing the bioavailability of ferrous ascorbate at 40% mimics the absorption of marginally iron-deficient individuals. The bioavailability in subjects with good iron status, as were these children, was of only 3.7%. Had they been marginal in iron nutrition they would have absorbed 6.5%. Another method for normalization is comparing results with the bioavailability of ferrous sulfate in the same matrix. Previous studies in our lab, with the same kind of bread in adult women—also normalized to 40% of the reference dose (Peña *et al*, 1991)—showed that radioactive ferrous sulfate's bioavailability was 10%; thus the relative bioavailability of the reduced iron compared to ferrous sulfate can be estimated as approximately 65%.

The correlation between percent bioavailability and absorption of the reference dose and with serum ferritin values were highly significant ($P<0.01$) (data not shown). This gives technical credence to the results.

The children had very good iron nutrition; nevertheless, the serum ferritins had a sufficient spread (range 10–26 μg/l) to obtain a variety of bioavailability values (range 10.4–1.2%)-useful to better interpret the results (Table 1).

Discussion

The relative bioavailability of ^{58}Fe H_2-reduced iron in white bread manufactured with 72% extraction wheat flour was estimated to be about 65% that of ferrous sulfate by comparing the results of the present study with those of a previous study that measured iron absorption from ferrous sulfate in adult women consuming the same bread meal.

It would be reasonable to assume that the values for bioavailability of H_2-reduced ^{58}Fe should be considerably higher than those expected of the commercial iron due to its smaller particle size. However, there can be no certainty regarding the magnitude of the actual difference between the two. Unfortunately, currently available stable isotopes, of H_2-reduced iron due to their production in calutrons, have a smaller size than the commercial product used in food fortification programs and have not been further characterized. The industrial specification for flour fortification is that all iron particles be ≤45 μm in diameter and capable of

passing through a #325 mesh. The stable isotope iron was outside that standard in that it was an average of 15 μm. The smaller size of the isotope compared to industrial iron is likely to lead to results that represent a maximum rather than a typical value for H_2-reduced iron absorption. Unfortunately, iron particles smaller than 15 μm are an explosion hazard and cannot be used in industrial processes.

The only prior study using stable isotopes in bread is from the UK (Roe & Fairweather-Tait, 1999), where stable isotope bioavailability in wheat flour bread buns was measured. These investigators obtained a strikingly high absorption of reduced iron (65%) in their wheat bread rolls. This is very high if compared to the 6.5% from the H_2-reduced ^{58}Fe reported here and the 10% iron absorption we have obtained in a similar extraction Chilean bread fortified with radioiron-labeled ferrous sulfate (Peña *et al*, 1991). This discrepancy may be due to the use of the inherently less precise fecal retention method (rather than erythrocyte incorporation). Also, they gave the bread with a cola drink, which could have facilitated dissolution of the elemental iron in the gastric juice and so increased absorption (S Fairweather-Tait, personal communication). Otherwise, it remains unexplained.

An estimation of the absolute amount of iron absorbed from 100 g of bread (75 g flour) with 3.0 mg of H_2-reduced ^{58}Fe, that is, 4.0 mg/100 g of flour at the normalized bioavailability of 6.5%, would yield only 0.195 mg. A daily consumption of 300 g of bread would bring this amount to a more meaningful 0.58 mg. However, this quantity of bread is seldomly consumed in most of the countries that use white bread in America and Europe. Consumption of larger quantities in Asia and Africa is of bread manufactured with higher extraction flour, which yields a matrix much higher in phytates that would strongly inhibit bioavailability. Furthermore, the figure of 6.5% is obtained with the small particle H_2-reduced ^{58}Fe and should be considerably lower with commercial iron. Nevertheless, these results show that H_2-reduced iron, which is commonly used to fortify wheat flour, has about half the bioavailability of ferrous sulfate in a human feeding study. This confirms the opinion of the expert iron group of SUSTAIN (Hurrell, 2002) and more recently the WHO fortification guidelines that when such iron is used in wheat flour fortification, it should be added at twice the rate of ferrous sulfate to adjust for it being half as bioavailable, with the caveat that this reduced iron is not exactly similar to commercial elemental iron.

Future studies of this nature must consider that there are different sorts of elemental iron powders and that they may vary substantially in their characteristics depending on many diverse factors. Considering these caveats, though these results cannot be directly applied to estimate the impact of a fortification program, we can say with confidence that bioavailability is lower than 6.5%, and this gives us a practical starting point until appropriate studies can be carried out with stable isotopes that are similar to commercial elemental iron.

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