Iron absorption from wheat flour: effects of lemonade and chamomile infusion

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Abstract

Objective: We measured iron bioavailability of meals based on wheat flour consumed by a vulnerable population in Latin America.

Methods: Bioavailability of iron (ferrous sulfate) from fortified noodles, noodle soup, noodle soup eaten with lemonade sweetened with *panela* (unrefined whole cane sugar), bread alone, and bread consumed with a chamomile infusion sweetened with *panela* was studied using the double isotopic method in 13 women.

Results: Iron bioavailabilities from bread, noodles, and noodle soup were not significantly different (7.4%, 6.3%, and 6.0%, respectively). Iron absorption from noodle soup was significantly higher when given with lemonade (11.0%) compared with absorption of the same meal without lemonade ($P < 0.02$) or with the absorption of noodles ($P < 0.04$). Iron absorption of bread given alone or with chamomile infusion sweetened with *panela* (8%) was not significantly different.

Conclusion: Iron bioavailability of meals based on wheat flour, fortified with ferrous sulfate, is improved when given with lemonade. The consumption of this beverage may be an alternative to further increase the effectiveness of wheat flour fortification in preventing iron deficiency in low-income Latin American populations.

Keywords: Iron absorption; Wheat flour; Ferrous sulfate; Ascorbic acid; Citric acid

Introduction

Iron deficiency is the single most common nutritional disorder worldwide. It is prevalent in most of the developing world and it is probably the only nutritional deficiency of consideration in industrialized countries [1]. Because of their high iron requirements, the most commonly affected groups are infants, children, adolescents, and women of child-bearing age. In the developing world the prevalence of iron deficiency is due mainly to a low intake in bioavailable iron [2].

Non-heme iron is the main form of dietary iron. Its absorption is greatly affected by enhancers and inhibitors contained within the diet [3]. Most foods consumed in undeveloped countries have a predominance of inhibitors of non-heme iron absorption. The balance between enhancers and inhibitors of non-heme absorption becomes the determinant in the possibility of obtaining the required iron for the vast majority of people in the world whose diet contains very little or no heme iron. Therefore, knowledge of iron absorption from various representative meals of vulnerable populations is critical to the design of effective strategies to combat iron deficiency. Two previous studies have studied iron absorption from typical Latin American diets [4,5].

Staple foods such as wheat, corn, rice, or potatoes make up the largest proportion of food supply in developing countries. Some of these foods are suitable for iron fortification. However, they contain inhibitors of non-heme iron absorption such as phytates, polyphenols, and tannins. The proportion of absorbable iron can be increased by promoting its consumption with foods rich in promoters of non-heme absorption (i.e. ascorbic acid, citric acid, and meat).

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In some rural low-income populations of Latin America meals based on wheat flour or potato are consumed with fruit juice, mainly lemonade, and/or herbal infusions. In many cases these beverages are sweetened with *panela*. This is a pure, wholesome, traditional, unrefined, non-centrifugal whole sugar cane.

Fortification has been identified as one of the most cost-effective and sustainable approaches for controlling iron deficiency [6]. However, it is difficult to achieve good results regarding iron status through iron fortification, if the original diet has low bioavailability. Other measures, which also enhance iron bioavailability or protect the fortificant from dietary inhibitors, are needed. Wheat flour fortification is the most widely used strategy to prevent and control iron deficiency in Latin America [7].

This study was designed to measure iron bioavailability in meals consumed by vulnerable populations in Latin America. This was done by measuring the bioavailability of iron from fortified noodles, noodle soup, noodle soup eaten with lemonade sweetened with *panela*, bread, and bread consumed with a chamomile infusion sweetened with *panela*.

Materials and methods

Subjects

Iron absorption studies were performed in 13 women 32 to 50 y of age. None were pregnant and all used contraceptive intrauterine devices and were in apparent good health. Written, informed consent was obtained from each volunteer before participation in the study. The protocol was reviewed and was in accordance with the standards set by the Institute of Nutrition and Food Technology's ethics committee on human research; the Chilean Commission of Nuclear Energy approved radioactive doses.

Test meals

Noodles

The noodles consisted of 1200 g of wheat flour (70% extraction), 28 g of whole egg, and 360 mL of distilled deionized water mixed by hand for 15 min. A solution containing 36 mg of elemental iron as ferrous sulfate labeled with radioiron was added, and the dough was kneaded again for 15 min. Noodles were cut by hand and later dried in a stove for 24 h.

Noodle soup

Fifty-five grams of onion fried in 80 g of fat, 1 g of *achiote*, and 15 g of salt were boiled in 640 mL of water for 10 min. Then 640 g of chopped raw potatoes and 464 g of raw noodles labeled with radioiron were added and the mix was boiled again for 10 min. Later 20 mL of whole cow's milk was added. One whole egg (62.5 g) was added to each serving (150 g) before it was eaten.

Bread

The bread consisted of 1750 g of wheat flour (70% extraction), 437.5 g of fat, 252.5 g of granulated sugar, 175 g of whole egg, 105 g of yeast, 17.5 g of salt, 262.5 g of whole cow's milk, and 437.5 mL of distilled deionized water mixed by hand for 15 min. A solution containing 52.5 mg of elemental iron as ferrous sulfate labeled with radioiron was added, and the dough was kneaded again for 15 min. The dough was baked for 45 min. A total of 3300 g of bread was obtained.

Lemonade was composed of 10% of lemon juice, water, and *panela* (2%).

Chamomile infusion was prepared by boiling 22.4 g of chamomile (*Matricaria chamomila*) in 3000 mL of water plus 150 g of *panela*.

Iron contents of bread, raw noodles, and *panela* were 3.12, 4.84, and 4.15 mg per 100 g, respectively. Soluble tannins content of chamomile infusion was 24.5 mg/100 mL.

Isotope studies

Iron isotopes (^{55}Fe and ^{59}Fe) of high specific activity were used as tracers; both isotopes were iron (III) chlorides as purchased (New England Nuclear, Boston, MA, USA). Isotopes were incorporated into the dough (bread, noodles) or mixed with the dose of reference, lemonade, or infusion immediately before administration.

A solution containing 0.27 mmol/L (15 mg/L) of elemental iron as ferrous ascorbate (molar ratio 1:2 iron to ascorbic acid) labeled extrinsically with tracer amount of ^{59}Fe chloride (New England Nuclear) was prepared (reference dose). The specific radioactivity of this aqueous solution was 12.3 kBq of ^{59}Fe/mg of elemental iron.

The test meals were consumed after an overnight fast, and no food or beverages other than water was permitted during the following 4 h. The amounts of aqueous solution and test meals ingested were calculated by differential weight of the glasses or plates. For the calculation of total radioactivity ingested, aliquots of the aqueous solution and test meals were counted in sextuplicate as standards. Measurement of blood radioactivity was performed in duplicate venous samples according to the method of Eakins and Brown [8]; these were counted a sufficient number of times to allow a 3% counting error. A liquid-scintillation counter (Beckman LS 5000 TD, Beckman Instruments, Fullerton, CA, USA) was used for the double isotope measurements.

Calculations assumed that 80% of the absorbed radioactivity would be incorporated into the hemoglobin of circulating erythrocytes. Blood volume was estimated on the basis of sex, weight, and height [9].

On day 1 the subjects received 157.5 g of cooked noodles labeled with 111 kBq of ^{55}Fe; on day 2 they ate 150 g of

Table 1
General characteristics and iron status of subjects

Subject	Age (y)	Height (cm)	Weight (kg)	Hemoglobin (g/L)	Transferrin saturation (%)	Serum ferritin (μg/L)
1	39	151.0	66.4	136	29.4	24.8
2	40	149.0	56.6	129	23.5	54.4
3	38	148.8	57.3	133	39.1	60.7
4	42	148.7	53.7	139	33.4	29.1
5	32	158.3	91.7	126	17.5	22.7
6	43	155.0	67.7	130	41.3	26.9
7	36	151.5	88.9	132	23.8	51.9
8	37	150.5	55.0	149	28.4	41.3
9	35	144.5	56.5	134	44.4	79.2
10	39	149.5	61.4	157	32.1	135.2
11	37	142.4	59.5	142	48.8	84.2
12	40	154.5	79.0	140	42.5	64.1
13	50	153.5	65.3	133	29.5	117.3
Mean	39	150.6	66.1	137	33.4	52.2*
SD	4	4.3	12.7	9	9.3	29.0–93.7

* Geometric mean ± 1 SD range.

noodle soup (containing ~85 g of cooked noodles tagged with 37 kBq of ^{59}Fe). A venous blood sample was obtained 2 wk later (day 14) to measure the circulating radioactivity and to determine the iron status of the subjects. This same sample also provided baseline values of ^{55}Fe and ^{59}Fe radioactivity in red blood cells for the next set of absorption studies. On day 14 subjects were given 150 g of noodle soup (containing ~85 g of cooked noodles labeled with 37 kBq of ^{59}Fe) with 150 mL of lemonade sweetened with *panela*; on day 15 they received 70 g of bread labeled with 111 kBq of ^{55}Fe. A venous sample was obtained on day 28 to measure the increase in red blood cell radioactivity. This sample also provided baseline values for the next set of absorption studies. On day 28 the subjects received 70 g of bread labeled with 111 kBq of ^{55}Fe given with 150 mL of chamomile infusion sweetened with *panela*. On day 29 they drank 50 mL of a reference dose of 0.27 mmol/L (15 mg/L) of elemental iron as ferrous ascorbate (molar ratio 1:2 iron to ascorbic acid) labeled with 37 kBq of ^{59}Fe. A final venous sample was obtained on day 42 to determine the increase in red blood cell radioactivity.

Hemoglobin, mean cell volume, serum iron, total iron-binding capacity, and serum ferritin were determined in venous blood obtained on day 14 [10].

For comparative studies of iron bioavailability, the absorption of the reference dose of ferrous ascorbate is used to offset the effect of differences in iron status among individuals. For purposes of comparison, all studies currently refer to 40% absorption of the reference dose of ferrous ascorbate. This absorption percentage is used because it corresponds to that obtained in borderline iron-deficient populations.

Because the percentages of iron absorption and serum ferritin have a skewed distribution, these values were converted to logarithms before performing mean ± SD analysis; the results were retransformed to antilogarithms to recover the original units and expressed as geometric means ± 1 SD. Absorption data were analyzed with repeated measures analysis of variance and Pearson's correlation coefficients. Post hoc comparisons were made by using Tukey's test. Statistical analysis was performed on logarithmically transformed data with Statistica 4.5 for Windows (StatSoft, Inc., Tulsa, OK, USA).

Results

The general characteristics and iron statuses of subjects are presented in Table 1. None of the subjects had anemia (hemoglobin <120 g/L) or iron depletion (serum ferritin <12 μg/L).

Table 2 lists iron absorption of test meals and the reference dose of ferrous ascorbate. The iron bioavailabilities from bread, noodles, and noodle soup were not significantly different (7.4%, 6.3%, and 6.0%, respectively). Iron absorption from noodle soup was significantly higher when given with lemonade sweetened with *panela* compared with the same meal without lemonade ($P < 0.02$). The iron absorption of bread given alone or with a chamomile infusion sweetened with *panela* was not significantly different.

The iron absorption of the reference dose of ferrous ascorbate was negatively correlated with serum ferritin ($r = -0.74$, $P < 0.05$).

Discussion

There is substantial evidence that food fortification is the best long-term approach for preventing iron deficiency at a population level [6]. One of the strategies to further improve iron nutrition is to consume the fortified food with foods that improve non-heme absorption and decrease the intake of foods or beverage that inhibit non-heme iron absorption.

Cereals contain phytates and other components such as

Table 2
Iron absorption of test meals and reference dose

Subject	Iron absorption (%)					
	Noodles*	Noodle soup*	Noodle soup + lemonade*	Bread*	Bread + chamomile infusion*	Ferrous ascorbate
1	15.7	17.8	35.3	12.6	22.2	85.0
2	10.0	10.6	22.4	8.6	20.2	83.1
3	3.0	5.1	4.0	2.2	6.5	74.4
4	9.5	6.1	18.6	8.9	7.1	69.9
5	17.5	21.5	16.7	13.5	25.5	59.6
6	4.6	1.3	11.4	11.8	12.2	53.0
7	36.0	31.5	44.3	37.3	12.6	33.3
8	4.7	3.5	8.6	3.0	5.7	27.0
9	22.8	28.3	35.6	19.6	12.0	20.2
10	1.0	2.9	2.7	2.2	1.9	15.0
11	12.0	6.6	10.0	7.7	3.6	13.1
12	0.9	1.1	3.5	3.7	3.1	7.1
13	1.8	1.3	2.3	4.0	4.4	5.0
Mean†	6.3	6.0	11.0	7.4	8.0	29.8
SD	1.9–20.7	1.9–19.6	4.0–30.4	3.1–17.6	3.5–17.9	11.4–78.0
Mean adjusted‡	8.5	8.1	14.7	9.9	10.7	40.0

* Repeated measures analysis of variance (F = 3.21, $P = 0.02$). Tukey's test: noodle versus noodle soup + lemonade, $P < 0.04$; noodle soup versus noodle soup + lemonade, $P < 0.02$).

† Geometric mean ± 1 SD range.

‡ Corrected to 40% absorption of the reference dose.

polyphenols that are inhibitors of non-heme iron absorption [3]. They are the staple foods in most diets of the developing world, consisting of wheat flour of 70–80% extraction consumed in Latin America. In the past decade, flour fortification has been implemented in many countries. Currently, 19 countries are fortifying wheat flour with iron, mainly as reduced iron and a lesser proportion as ferrous fumarate or ferrous sulfate [11]. Our results on iron absorption of bread fortified with ferrous sulfate (9.9%) confirm prior work indicating similar bioavailability of ferrous sulfate when it is used to fortify bread [12–14]. However, iron absorption from iron-fortified noodles given alone (8.5%) or as soup (8.1%) was comparable to that obtained in bread.

Iron absorption of noodle-based soup was significantly increased when given with lemonade sweetened with *panela*. This effect may be attributable to a complementary or additive promoter effect on non-heme absorption of some components of the beverage, such as ascorbic acid and citric acid. The approximate molecular ratios of ascorbic acid to iron and citric acid to iron in this meal were 1.3:1 and 32.3:1, respectively. Many studies have shown that ascorbic acid is a potent enhancer of non-heme iron absorption [15]. This vitamin can counteract the inhibitory effects of dietary phytates and polyphenols on iron absorption [15]. Studies on the effect of ascorbic acid on non-heme iron absorption performed in different meals have shown an exponential dose-response curve, with the enhancing effect being proportional to the molar ratio of ascorbic acid to iron [16–18]. This effect is relatively less marked at high molar ratios of ascorbic acid to iron, reaching a plateau at very high molar ratios. The promoter effect of vitamin C on iron absorption affects native food iron and fortification iron. An evident effect on iron absorption has been observed beginning at a molar ratio ≥1:1 of ascorbic acid to iron [15]. However, in most meals with a low concentration of inhibitors of non-heme-iron absorption, a biologically significant effect on non-heme iron absorption is observed at molar ratios ≥2:1 [15,16,18]. Molar ratios equal to or higher than this are required to observe a significant effect in meals with a high content of phytate or polyphenols [15,19]. Some organic acids such as lactic acid, citric acid, and malic acid have a weaker promoter effect on iron absorption compared with ascorbic acid. The promoter effect on iron absorption is observed at molar ratios >100:1 [20,21]. However, in one study the addition of 1 g of citric acid reduced the iron absorption from a Latin America–type meal [5]. There is no explanation for this contradictory result. Sugars such as fructose and lactose have some enhancing effect on iron absorption [3]. It is improbable that fructose from *panela* has a promoting effect on iron absorption from the noodle soup because of the low concentration of this reducer sugar (1.5–7 mg/100 g of *panela*).

Polyphenols and phytate are two of the most important dietary inhibitors of non-heme iron absorption. Previously we reported that tea reduced iron absorption from bread by 39% [12]. However, in our study chamomile infusion did not inhibit iron absorption from bread. This finding may be attributable to the lower content of tannins in chamomile than in tea (9.7 ± 1.0 and 100.2 ± 7.6 mg/g of dry weight, respectively) [22]. However, we cannot exclude the existence in the *panela* of a non-identified compound with a promoter effect on non-heme iron absorption that could have counteracted the inhibitory effect of the tannins in the infusion.

Conclusions

Iron bioavailability of wheat flour, fortified with ferrous sulfate, is improved when given with lemonade sweetened with *panela*. The consumption of this beverage may be an alternative to further increase the effectiveness of wheat flour fortification in preventing iron deficiency in low-income Latin American populations.

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