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Effect of Increasing Levels of Zinc Fortificant on the Iron Absorption of Bread Co-Fortified with Iron and Zinc Consumed with a Black Tea

Manuel Olivares · Carla Castro · Fernando Pizarro · Daniel López de Romaña

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Abstract Iron (Fe) and zinc's (Zn) interaction at the absorptive level can have an effect on the success of co-fortification of wheat flour with both minerals on iron deficiency prevention. The aim of the study was to determine the effect of increasing levels of zinc fortificant on the iron absorption of bread co-fortified with iron and zinc consumed with a black tea. Twelve women aged 33–42 years participated in the study. They received on four different days 200 mL of black tea and 100 g of bread made with wheat flour (70 % extraction) fortified with either 30 mg Fe/kg alone, as ferrous sulfate (A), or with the same Fe-fortified flour, but with graded levels of Zn, as zinc sulfate: 30 mg/kg (B), 60 mg/kg (C), or 90 mg/kg (D). Fe radioisotopes (^{59}Fe and ^{55}Fe) of high specific activity were used as tracers, and Fe absorption iron was measured by the incorporation of radioactive Fe into erythrocytes. The geometric mean and range of ±1 SD of Fe absorption were as follows: A=6.5 % (2.2–19.3 %), B=4.6 % (1.0–21.0 %), C=2.1 % (0.9–4.9 %), and D=2.2 % (0.7–6.6 %), respectively; ANOVA for repeated measures $F=10.9$, $p<0.001$ (Scheffè's post hoc test: A vs. C, A vs. D, B vs. C, and B vs. D; $p<0.05$). We can conclude that Fe absorption of bread made from low-extraction flour fortified with 30 mg/kg of Fe, as ferrous sulfate, and co-fortified with zinc, as zinc sulfate consumed with black tea is significantly decreased at a zinc fortification level of ≥60 mg/kg flour.

Keywords Wheat flour · Food fortification · Iron absorption · Iron · Zinc · Tea · Humans

M. Olivares (✉) · F. Pizarro · D. López de Romaña
Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile
e-mail: molivare@inta.uchile.cl

C. Castro
Faculty of Veterinary and Animal Sciences, School of Veterinary Sciences, University of Chile, Santiago, Chile

Introduction

Mass fortification has proven to be an effective and sustainable strategy for iron deficiency prevention. Currently, 78 countries have mandatory or voluntary programs of wheat flour fortification [1]. Since iron deficiency coexists with zinc deficiency in the developing world, an increasing number of these countries are also enriching the wheat flour with zinc [2]. However, the mutual negative interaction that both minerals have on their intestinal absorption is a potential factor that can affect the overall success of this fortification strategy [3, 4]. Studies carried out by Herman et al. and by us have shown that the addition of zinc, as zinc sulfate, to wheat flour fortified with iron, as ferrous sulfate, significantly decreases iron absorption [5, 6].

Non-hem iron absorption is greatly affected by enhancers and inhibitors contained within the diet. It is favored by compounds such as ascorbic acid, meat, and some organic acids, and it is inhibited by substances such as phytates, polyphenols/tannins, casein, and oxalates [7]. On the other hand, zinc absorption is promoted by animal protein, the amino acids histidine and methionine, and organic acids and is strongly inhibited by phytates [8]. The interaction between Zn and Fe might be modified by the amount of dietary inhibitors and enhancers of the absorption of these minerals. Bread is usually consumed with other foods, such as herbal infusions, and especially with tea. These beverages are rich in polyphenols that can chelate non-hem iron, thus potentiating the inhibitory effect that phytates contained in wheat flour already have [9]. However, it is unknown if tea modifies the interaction that iron and zinc added to wheat flour have at the absorptive level.

The aim of our study was to determine the effect of increasing levels of zinc fortificant on the iron absorption of bread fortified with iron and zinc consumed with a black tea.

Subjects and Methods

Subjects

Twelve women between 33 and 42 years of age participated in the study. None were pregnant, as confirmed by a negative test for human chorionic gonadotropin in urine; all were using a birth control method (e.g., intrauterine device, oral contraceptive, or tube ligation) at the time of the study; were in apparent good health; and none had consumed vitamin or mineral supplements in the previous 6 months. A written informed consent was obtained from each volunteer before participation in the study. The protocol was reviewed by 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

All breads were prepared by study personnel at the Micronutrient Laboratory of the Institute of Nutrition and Food Technology. Wheat flour (70 % extraction), produced by an industrial mill, was used to prepare all breads used for the iron absorption tests. In Chile, by legislation, wheat flour is fortified with iron, as ferrous sulfate, (30 mg/kg), thiamine (6.3-mg/kg), riboflavin (1.3 mg/kg), niacin (13.0 mg/kg), and folic acid (2.2 mg/kg). The iron content of the wheat flour was corroborated by atomic absorption spectrophotometry after acid wet digestion. Four batches of iron and zinc-fortified bread dough of ~2 kg each were prepared by mixing 1.26 kg flour, 17.64 g yeast, 9.24 g sugar, 12.6 g salt, without or with either 166.3, 332.5, or 498.8 mg of $ZnSO4 \cdot 7H2O$ (30, 60, and 90 mg Zn/kg; Merck, Darmstadt, Germany) per batch. Zinc sulfate was added first to water to allow for homogenization. All ingredients were mixed by hand in polyethylene recipients during 30 min, and the mixture was then fermented at 22 °C for 60 min. The bread dough was shaped into buns and left to ferment for another 30 min. Finally, the buns were baked at 230 °C for 25 min. The final product offered to the subjects was French-type bread buns of ~100 g each. All breads were prepared the day before administration.

The black tea infusion was prepared by adding 15 teabags (31.5 net weight of Te Supremo "1875 Original Blend"; Cambiaso Hnos. SAC, Valparaíso, Chile) in 3,000 mL of boiling water which were extracted after 2 min. Then 150 g of sugar was added. Two hundred milliliters of the beverage was offered at each meal.

Isotopic Studies

Fe radioisotopes (^{59}Fe and ^{55}Fe) of high specific activity were used as tracers for the bioavailability studies (Du Pont de Nemours, Wilmington, DE). Isotopes were incorporated into the dough. The radioactivity doses were 37 kBq of ^{59}Fe and ^{111} kBq of 55Fe per 100 g of bread.

The isotopically labeled breads were consumed after an overnight fast, and no food or beverages other than water were allowed for the following 4 h. The amount of labeled bread intake was calculated by differential weight of the plate before and after the intake. For the calculation of total radioactivity ingested, radioactivity from solution aliquots was counted in sextuplicate as standards. Measurement of blood radioactivity was performed from duplicate venous samples following the method by Eakins and Brown [10]. Samples were counted the sufficient number of times to ensure <3 % counting error. A liquid scintillation counter (TRICarb 2000, Canberra Packard, Downers Grove, IL, USA) was used for all the isotope measurements. Percentage of absorption was calculated based on the blood volume, as estimated from height and weight [11] and assuming 80 % red cell use of radioactive iron [12].

Study Protocol

An experimental cross-over design was performed, where iron absorption was compared within the same subjects. On day 1 (A), the subjects received ~100 g of bread (~70 g of wheat flour) fortified only with iron (control bread; Zn/Fe weight ratio 0:1) labeled with 111 kBq of ^{55}Fe and 200 mL of tea, and on day 2 (B), they received ~100 g of bread fortified with 30 mg of Fe and 30 mg of Zn/kg of wheat flour (Zn/Fe weight ratio 1:1) labeled with 37 kBq of ^{59}Fe and 200 mL of tea. A venous blood sample was obtained on day 14 to measure circulating radioactivity and to determine the iron status of the subjects. Subjects were then given ~100 g of bread fortified with 30 mg of Fe and 60 mg of Zn/kg wheat flour (Zn/Fe weight ratio 2:1) labeled with 111 kBq of ^{55}Fe and 200 mL of tea (C). The following day (day 15), they received ~100 g of bread fortified with 30 mg Fe and 90 mg of Zn/kg wheat flour (Zn/Fe weight ratio 3:1) labeled with 37 kBq of ^{59}Fe and 200 mL of tea (D). A final venous sample was obtained on day 28 to measure the increase in red blood cell radioactivity.

Radioactivity from labeled bread aliquots and venous samples was counted simultaneously at the end of the study to avoid an error in the calculation of Fe absorption due to decay that has occurred between the administration of the isotopes and the absorption measurement 14 days later. In addition, the absorption of Fe administered on days 14 and 15 was corrected for the isotope that had been administered on days 1 and 2 by subtracting the radioactivity of the blood sample of day 14 from red blood cell radioactivity of day 28.

Table 1 General characteristics and iron nutritional status of subjects

Age (years)	37.5±3.4
Weight (kg)	67.2±8.8
Height (m)	1.55±0.05
Hemoglobin (g/L)	141±8
Mean cell volume (fL)	85.8±5.4
Zn-protoporphyrin (μg/dL RBC)	78.8±16.0
Transferrin saturation (%)	23.1±8.1
Serum ferritin (μ/L)[a]	46.5 (31.0–69.8)

[a] Geometric mean and range of ±1 SD

Percentage of iron absorption was=(^{59}Fe or ^{55}Fe counts per minute/milliliter of blood × volemia) / (^{59}Fe or ^{55}Fe counts per minute ingested) × 100. The result is multiplied by 0.8 because 80 % of absorbed iron is incorporated into red blood cells [12].

Blood Analyses

Hemoglobin and mean cell volume (CELL-DYN 3200, ABBOTT Diagnostics, Abbott Park, IL), serum iron, and total iron binding capacity [13], Zn protoporphyrin (ZP Hematofluorometer model 206D, AVIV Biomedical Inc., Lakewood, NJ), and serum ferritin [14] were measured to evaluate the iron status of the subjects. Fe status was considered to be normal when all of these laboratory indexes were within the reference range. Depleted iron stores were defined as serum ferritin <12 μg/L; Fe deficiency without anemia was defined as normal hemoglobin with at least two abnormal laboratory results (transferrin saturation <15 %, Zn-protoporphyrin > 70 μg/dL RBC, serum ferritin <12 μg/L); and Fe deficiency anemia was defined as hemoglobin <120 g/L with two or more abnormal other iron status measurements.

Statistical Methods

Because Fe absorption and serum ferritin have skewed distributions, the values were first converted to their logarithms. The results were then retransformed to their antilogarithms to recover the original units and were then expressed as geometric means and ±1 SD ranges. An ANOVA for repeated measures (Statistica for Windows, release 4.5; StatSoft Inc., Tulsa, OK) was used to compare the absorption of iron from the four bread formulations administered. ANOVA and Scheffè's multiple comparison test were calculated on logarithmically transformed data. All comparisons were done at the 5 % level of significance.

Results

The general characteristics and iron status laboratory indicators of the volunteers are shown in Table 1. The majority of subjects had a normal iron nutritional status, and only two women presented Fe deficiency without anemia.

Fe absorption of bread fortified with Fe (30 mg/kg) alone or in combination with Zn at different Zn/Fe ratios consumed together with 200 mL of black tea is shown in Table 2. Zn fortification levels of 60 and 90 mg/kg significantly inhibited Fe absorption (ANOVA for repeated measures, $F=10.9$, $p<0.001$). The geometric mean (range ± SD) absorption of bread fortified with Fe alone (A) was 6.5 % (2.2–19.3 %), and at 0:1 (B), 1:1 (C) 2:1, and (D) 3:1 Zn/Fe weight ratios, it was 4.6 % (1.0–21.0 %), 2.1 % (0.9–4.9 %), and 2.2 % (0.7–6.6 %), respectively (Scheffè's post hoc test: A vs. C, A vs. D, B vs. C, and B vs. D; $p<0.05$).

Discussion

Scarce studies have evaluated the effect of zinc fortification on iron absorption from wheat flour fortified with iron. Ferrous sulfate is one of the compounds recommended by WHO to fortify low-extraction wheat flour, while Zn oxide and Zn sulfate are the most used zinc fortificants because of their low cost [15]. Herman et al. [5] found in children who had consumed wheat flour dumplings made from non-fermented refined wheat flour fortified with 60 mg Fe/kg that co-fortification with 60 mg Zn/kg, as zinc sulfate, significantly inhibited iron absorption. However, this inhibitory effect was not observed when zinc oxide, a less water-soluble compound,

Table 2 Effect of increasing levels of zinc fortificant on the iron absorption of bread fortified with iron and zinc consumed with a black tea ($n=12$)

Minerals	Fortification level (mg/kg wheat flour)			
Zinc	0	30	60	90
Iron	30	30	30	30
Zn/Fe ratio	0/1	1/1	2/1	3/1
Iron absorption (%)[a]	6.5a (2.2–19.3)	4.6a (1.0–21.0)	2.1b (0.9–4.9)	2.2b (0.7–6.6)

[a] Geometric mean (range ± 1 SD). One-way repeated measures ANOVA ($F=10.9$, $p<0.001$). Scheffè's post hoc test: different lowercase letter indicate statistically significant difference ($p<0.05$)

was used as fortificant [5]. In another unpublished study, no harmful effect of Zn fortification was observed on iron absorption in children who received at breakfast and lunch 100 g of non-fermented wheat flour fortified with 30 mg Fe/kg, as ferrous sulfate and either 30 or 90 mg Zn/kg, as zinc sulfate [16]. We have observed in women receiving bread prepared from low-extraction wheat flour fortified with 30 mg Fe/kg, as ferrous sulfate, and graded doses of zinc (0, 30, 60, and 90 mg Zn/kg, as zinc sulfate) that 90 mg Zn/kg significantly inhibited iron absorption [6].

However, products made from wheat flour are usually consumed with other foods that can influence iron and zinc absorption. Foods with a high content of inhibitors or enhancers of iron and/or zinc absorption could affect the negative interaction of these minerals at the absorptive level. In many countries of Latin America, it is a custom that bread is eaten together with herbal infusions, such as tea. Tea has a high content of polyphenols, compounds that have a well-known inhibitory effect on the absorption of non-hem iron [9]. Previous studies of our group have found a 15.1 and 19.8 % of iron absorption of a bread made with iron fortified low-extraction wheat flour; however, when this bread was simultaneously consumed with tea, this percentage decreases to 9.1 % [17], a comparable value to the 6.5 % found in the current study. In the current study, using a similar design to our previous study, but with the difference that the bread co-fortified with iron and zinc was consumed with a cup of black tea and not with water, we found that iron absorption was significantly inhibited at zinc concentrations of 60 and 90 mg/kg flour. Thus, the threshold for the inhibitory effect of zinc on iron absorption is observed at Zn/Fe ratio of 2:1 when the co-fortified bread is consumed with tea, whereas when it is consumed with water, this inhibitory effect was appreciated at a ratio of 3:1 [6]. This discrepancy could be explained by the fact that it is likely that when co-fortified bread is consumed with tea, there is a higher proportion of Zn to Fe cations available for uptake by the enterocyte due to the chelating effect polyphenols have on non-hem iron and a no effect or a lesser effect of these compounds on zinc cations [9, 18, 19], thereby increasing zinc competition with iron for a common intestinal transporter pathway.

Conclusion

Fe absorption of bread made from low-extraction flour fortified with 30 mg/kg of Fe, as ferrous sulfate, and co-fortified with zinc, as zinc sulfate, is significantly decreased at a zinc fortification level of ≥60 mg/kg flour when this food is simultaneously eaten with black tea. This information may be useful for the definition of the proper iron and zinc fortification levels of low-extraction wheat flour. Tea consumption together with fortified bread should be discouraged.

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