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Bioavailability of zinc oxide added to corn tortilla is similar to that of zinc sulfate and is not affected by simultaneous addition of iron

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Abstract

Background—Corn tortilla is the staple food of Mexico and its fortification with zinc, iron, and other micronutrients is intended to reduce micronutrient deficiencies. However, no studies have been performed to determine the relative amount of zinc absorbed from the fortified product and whether zinc absorption is affected by the simultaneous addition of iron.

Objective—To compare zinc absorption from corn tortilla fortified with zinc oxide versus zinc sulfate and to determine the effect of simultaneous addition of two doses of iron on zinc bioavailability.

Methods—A randomized, double-blind, crossover design was carried out in two phases. In the first phase, 10 adult women received corn tortillas with either 20 mg/kg of zinc oxide added, 20 mg/kg of zinc sulfate added, or no zinc added. In the second phase, 10 adult women received corn tortilla with 20 mg/kg of zinc oxide added and either with no iron added or with iron added at one of two different levels. Zinc absorption was measured by the stable isotope method.

Results—The mean (± SEM) fractional zinc absorption from unfortified tortilla, tortilla fortified with zinc oxide, and tortilla fortified with zinc sulfate did not differ among treatments: 0.35 ± 0.07, 0.36 ± 0.05, and 0.37 ± 0.07, respectively. The three treatment groups with 0, 30, and 60 mg/

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kg of added iron had similar fractional zinc absorption (0.32 ± 0.04, 0.33 ± 0.02, and 0.32 ± 0.05, respectively) and similar amounts of zinc absorbed (4.8 ± 0.7, 4.5 ± 0.3, and 4.8 ± 0.7 mg/day, respectively).

Conclusions—Since zinc oxide is more stable and less expensive and was absorbed equally as well as zinc sulfate, we suggest its use for corn tortilla fortification. Simultaneous addition of zinc and iron to corn tortilla does not modify zinc bioavailability at iron doses of 30 and 60 mg/kg of corn flour.

Keywords

Corn tortillas; food fortification; stable isotopes; zinc absorption; zinc oxide

Introduction

Corn tortilla is the staple food of Mexico and is consumed in significant amounts by a majority of the population, especially in areas where deficiencies of zinc and other micronutrients are common. We have proposed and carried out fortification of corn tortillas with zinc, iron, and other micronutrients as a strategy to reduce micronutrient deficiencies in these populations [1–5]. No studies have been performed to determine the relative amount of zinc absorbed from the fortified product and whether zinc absorption is affected by the simultaneous addition of iron. Several compounds have been used for zinc fortification. Zinc oxide is the cheapest and is more stable than several alternatives, and it has been widely used for fortification of wheat flour for many years [6, 7]. Because of its lower cost and higher stability, zinc oxide is the most commonly used zinc source in the fortification of cereal-based foods [8]. However, because of its lower solubility, initial studies suggested that its absorption was lower than that of more soluble compounds, such as zinc sulfate [6, 9, 10]. Moreover, it was suggested that besides solubility, other factors, such as gastric pH, are also important in the oral absorption of zinc compounds [11, 12]. Others have proposed that to predict nutrient bioavailability based only on the solubility of the compound could be misleading [8].

Zinc and iron have similar absorption and transport mechanisms in the human intestine and thus may compete for absorptive pathways [13]. Studies that have investigated the inhibitory effect of iron on zinc absorption have concluded that such an inhibitory effect occurs only when both minerals are administered simultaneously and the iron molar ratio is at least two times that of zinc [14]. Other studies have suggested that the effect of iron on zinc absorption when administered with other foods occurs only at a very high Fe:Zn molar ratio of 25:1 [15].

In a first phase of the present study, we evaluated zinc absorption from unfortified corn tortilla, corn tortilla fortified with zinc oxide, and corn tortilla fortified with zinc sulfate. In a second phase of the study, we compared zinc absorption from corn tortilla fortified with zinc oxide with no added iron, with 30 mg/kg of iron, and with 60 mg/kg of iron.

Materials and methods

Subjects

Ten subjects were involved in each phase of the study. They were healthy women ranging in age from 21 to 51 years. All were staff or students of the Instituto Nacional de Ciencias Médicas y Nutrición (INCMN), where the study was carried out. Only women were included in both studies to avoid differences due to sex and to make our results comparable to our previous zinc bioavailability studies in Mexican women [3, 8]. A clinical evaluation of the subjects was carried out before the study. All were healthy with no history of recent disease, and none were taking any medication or had ingested zinc supplements for 6 months before the study. The characteristics of the subjects on each phase of the study are described in table 1. Participation of the subjects in the study was voluntary. All signed consent forms after the nature and potential risks of the study were explained to them. The study protocol was reviewed and accepted by the Committee on Biomedical Research with Human Subjects of the INCMN.

Experimental design

The subjects in phase 1 of the study received three different test meals: corn tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide (CT + ZnO), tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc sulfate (CT + $ZnSO_4$), and tortilla made from flour with no zinc added (CT). The subjects in phase 2 also received three different treatments: tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide (CT + ZnO), tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide + 30 mg/kg of extra fine reduced elemental iron (CT + ZnO + 30Fe), and tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide + 60 mg/kg of extra fine reduced elemental iron (CT + ZnO + 60Fe). Each type of tortilla was extrinsically labeled with a different zinc stable isotope. The subjects were randomly assigned to each consecutive latin square design sequence by a blind random selection of subject number to avoid any effect of treatment order. The total amount of zinc absorbed and fractional absorption of zinc (FAZ) were measured with a zinc stable isotopic extrinsic label technique requiring labeling of all feeds with different tracers on the 3 consecutive days and measurement of isotopic enrichment levels in 24-hour fecal samples. After ingestion of the first standardized test meal, each subject collected all fecal samples for 13 days.

Isotope preparation and labeling of tortillas

Zinc stable isotopes were obtained from the Oak Ridge National Laboratory (Oak Ridge, Tennessee, USA). Commercially available isotopes were in oxide form. In order to include the isotope into the fortificant salt in the same chemical form, zinc-68 was chemically transformed to zinc sulfate by having zinc-68-oxide and sulfuric acid in stoichiometric ratio, heated by moderate boiling until total conversion to zinc sulfate was reached in about 5 hours. Since the amount of isotope was very low, several beakers with elemental zinc oxide were converted simultaneously to be tested as the obtained zinc sulfate. Identification tests included the addition of methyl orange to the solution with no pink color developing and tests of solubility in water (very soluble) and alcohol (not soluble at all). Zinc sulfate must be odorless, colorless, transparent prisms or small needles and water soluble (Pharmacopeia of Mexico, 8th Edition). With these tests, we assured that the salt was 100% transformed to zinc sulfate.

Isotopes in the appropriate form were included in the fortificant zinc salt, which was mixed into corn flour using a “V” blender adapted with an intensifier bar for optimum mixing (Patterson Kelly Co.; Liquid-solids Blender, Model 1-B-16-P). The mixing time and procedure were validated previously to reach a completely homogeneous mixing of the fortificants into the corn flour.

The tortillas were hand-made by adding deionized water to flour at a 1:1.5 ratio. The obtained dough is used to prepare tortillas by making small balls that are pressed by a press machine and cooked on a hot surface made from aluminum. The dough was weighed to obtain tortillas of about 30 g each. In order to eliminate contamination of minerals, all materials used for tortilla preparation were previously washed with nitric acid at 30% and then rinsed with deionized water.

Standardized meals

In phase 1, the tortillas in each of the three standardized test meals were labeled with a different stable isotope. Tortillas fortified with zinc oxide were labeled with Zn-67 at a dose of 1 mg/day/subject, tortillas fortified with zinc sulfate were labeled with Zn-68 at a dose of 1 mg/day/subject, and nonfortified tortillas were labeled with Zn-70 at a dose of 0.5 mg/day/subject.

In phase 2, tortillas fortified with zinc oxide were labeled with Zn-70 at a dose of 0.6 mg/day/subject, tortillas fortified with zinc oxide + 30 mg/kg of reduced iron were labeled with Zn-67 at a dose of 1.3 mg/day/subject, and tortillas fortified with zinc oxide + 60 mg/kg of reduced iron were labeled with Zn-68 at a dose of 1.3 mg/day/subject.

The average intakes of energy, zinc, and phytate content with each of the standardized test meals are shown in table 2. Tortillas ($n = 8$) in each of the standardized test meals were added with small amounts of other foods to increase palatability and easy ingestion as follows: 245.4 ± 9.3 g of corn tortilla in phase 1 and 235 ± 12 g in phase 2 of corn tortilla with the corresponding isotope, 100 g of tomato, 5 g of onions, 5 g of hot pepper, and 300 mL of fruit juice. Standardized test meals were given at breakfast and lunch during three consecutive days. The evening meal on each of the 3 days was also standardized to include 25 g of white bread, 40 g of ham, 20 g of cheese, 100 g of cantaloupe, and 1 cup of tea, but it was not labeled with any zinc isotope. The total energy intake from the three meals was 8,294 ± 21 kJ in phase 1 and 8,487 ± 11 kJ in phase 2. All meals were eaten under supervision to assure that they were completely eaten, and no other food was allowed between meals.

A sample of each batch of tortillas was taken for analysis of isotope and zinc concentrations to determine the precise amount of isotope and zinc ingested by each subject. The methods are described below. The phytate content of tortillas was estimated with an ion-exchange HPLC as reported previously [16, 17].

Sample collection, processing, and isotope analysis

Blood samples were taken after an overnight fast on the first day of the study and then analyzed for plasma zinc concentration by atomic absorption spectrophotometry (AAS) (Perkin Elmer 2380) [18]. Zinc deficiency was considered to be present when the plasma zinc concentration was < 70 μg/dL [19]. Feces were collected for 13 days after ingestion of the first experimental meal in plastic containers previously washed with 30% nitric acid. The subjects were asked to record the date and time at which each sample was collected and to store them in containers with blue ice refrigerated until the next morning when they were collected to be analyzed. For analysis, the samples were homogenized in 24-hour pools, dried overnight at 100°C, and dry-ashed in a muffle furnace (Thermolyne 53600, Model FA1740) by a gradual increase in temperature (200°C for 3 hours, 300°C for 1 hour, 400°C for 1 hour, and 450°C for 24 hours). After being cooled to room temperature, the samples were reconstituted with 6N hydrochloric acid. The reconstituted samples were analyzed for total zinc concentration by AAS and then purified in an ionic exchange resin to obtain a zinc aqueous solution. The purified samples were then vaporized and analyzed for isotope ratios by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Plasma Quad 3; VG Elemental) [18, 20, 21]. Isotope enrichment was determined as previously described [20]. Zinc content in tortillas and test meal samples was determined by following the same procedure as isotopes zinc determination.

Data analysis

FAZ with each of the three treatments was determined by the fecal monitoring method. The quantity of isotope tracer present in each daily fecal pool was analyzed, and the fraction of administered dose was calculated. The cumulative values of these measurements were plotted against the time from isotope administration. Correction for absorbed isotope subsequently secreted into the intestinal lumen and excreted in the feces was made by extrapolating to time zero a linear regression line through the final points of the cumulative fecal excretion plot after excretion of unabsorbed isotope was apparently complete. FAZ was calculated by subtracting the cumulative fractional fecal excretion from the total amount of tracer consumed. Total absorbed zinc was determined by multiplying FAZ by total daily dietary zinc, considering total zinc in the meal as well as zinc isotope in tortillas. Treatments FAZ, total absorbed zinc, and the amount of zinc consumed from tortilla were compared with a paired *t*-test. Since the the amount of zinc consumed from tortilla was different between treatments in phase 2, an additional analysis was performed to compare treatments adjusting for consumed zinc with analysis of variance in a linear mixed model considering the subject random effect. For statistical analysis, SPSS, version 10, was used.

Results

Age, weight, body mass index (BMI), and baseline fasted plasma zinc concentration of the subjects who participated in both phases are shown in table 1. The prevalence of plasma zinc levels < 70 μg/dL was 70% in the first phase of the study and 80% in the second phase, and 67% of women had erythrocyte zinc < 12 μg/mL in both phases of the study. Mean FAZ and absorbed zinc for both phases are shown in table 3. In phase 1, there were no significant differences between treatment groups in FAZ. The subjects absorbed significantly less zinc with CT than with fortified tortillas, and absorption from both fortified tortillas (CT + ZnO and CT + $ZnSO_4$) was similar. In phase 2, there were no significant differences between treatment groups in FAZ. The subjects absorbed statistically the same amount of zinc with the three treatments.

Discussion

In the present study, FAZ from zinc oxide added to corn tortillas was similar to that from zinc sulfate. Our results are similar to those found in a study in which zinc oxide and zinc sulfate were equally absorbed from maize tortillas in Mexican women [22]. Similar results were also found in children [23] and adults [7], in which both zinc compounds were equally absorbed from wheat flour. Thus, it seems consistent that the bioavailability of zinc oxide is similar to the bioavail-ability of more soluble compounds such as zinc sulfate when added to wheat and corn foods. It is common to anticipate mineral absorption based on the solubility of the mineral salt [24]. Our results with corn tortillas and the previous results with wheat products suggest that several factors interact to determine the bioavailability of mineral salts added to cereals, and that predicting bioavailability solely on the basis of the water solubility of the compound could be an oversimplification.

In both phases of this study, FAZ was relatively high, from 32% to 37%; these values are somewhat higher than those found in similar studies [17, 22]. This difference cannot be attributed to low zinc intake, as the zinc intake from these test meals was quite high. The relatively low plasma zinc concentration of the participants suggests that low zinc status may have contributed, although the evidence is controversial [25–27].

FAZ in the two zinc-fortified groups was similar to that in the control group. As a result, less zinc was absorbed in the control group. Since less zinc was ingested in this group, a higher FAZ could have been expected [28]; however, the phytate:zinc ratio in this group was almost double that in the other two groups. Thus, the effect of a low amount of zinc may have been counteracted by the much higher phytate:zinc molar ratio in the control group.

The second part of this study evaluated a possible inhibition of zinc absorption when administered along with different doses of iron. The results showed similar FAZ values when tortillas were not iron fortified and when they were fortified with iron in doses of 30 or 60 mg/kg of corn flour. Our results agree with the findings of the studies of Davidsson et al. [29] and Harvey et al. [30] that evaluated zinc absorption in women consuming a fortified meal and did not find changes in zinc absorption in the presence of iron. Davidsson et al. [29] found similar FAZ values of 0.38 and 0.30, respectively, when cereal was given with 25 mg of iron added as ferrous sulfate and when the same cereal was given with no iron added. They also compared the same cereal fortified with 10 mg of iron and nonfortified cereal, resulting in a FAZ of 0.31 for both treatments. Harvey et al. [30] evaluated FAZ at different stages of pregnancy and found that at week 16, FAZ was 0.22 with meals fortified with 100 mg of iron as ferrous gluconate and 0.21 with nonfortified meals; at week 24, both FAZ values were 0.24, and at week 36 both FAZ values were 0.31. In contrast, other studies have reported that fortification with different doses of iron inhibits zinc absorption. Chung et al. (31) concluded that intake of lemonade with 60 mg of iron as ferrous sulfate significantly decreased FAZ by 5% in breastfeeding women, and Troost et al. [32] found a decrease in FAZ from 0.44 in the absence of iron to 0.26 with 100 mg of iron as ferrous gluconate in subjects with ileostomy. Sandstrom et al. [33] measured FAZ in adults consuming 0, 40, 100, and 1,000 mg/day of iron as ferrous sulfate in a water solution, and found a significant reduction in FAZ as iron doses increased. Studies that found a decreased FAZ generally administered higher amounts of iron relative to zinc than the amounts administered in the present study. The amounts of zinc and iron administered in the present study were the amounts that are recommended for corn flour fortification in Mexico [2]. Just for comparison, in one of the experimental groups, iron was added at a dose of two times the concentration that is currently recommended. Thus, it seems that fortification with small amounts of iron is less likely to have any negative effect on zinc absorption.

In the studies that found a decreased FAZ in the presence of iron [31–34], iron and zinc were added to a water solution with ascorbic acid, a maltodextrin solution, or flavored deionized water. In our study and other studies that did not find an effect of iron on zinc absorption [29, 30], zinc and iron were added to solid foods or meals. This suggests that a negative effect of iron on zinc absorption occurs when the minerals are not accompanied by foods. Simultaneous addition of iron to zinc-fortified foods does not interfere with zinc absorption when both minerals are added in small amounts to solid foods.

During tortilla preparation, calcium hydroxide is added for liming, and therefore tortillas in this study and in regular diets have a high concentration of calcium (~ 1.042 mg/g of tortilla) [35]. Calcium is known to reduce iron absorption [36, 37], but its effect on zinc absorption is unknown. Ingested calcium in experimental meals (~ 510 mg/day) [35] could have played a role in zinc and iron absorption; however, the purpose of this study was to measure zinc absorption in zinc- and iron-fortified tortillas made as they are typically prepared in Mexico. Thus, the effect of zinc and calcium on iron absorption in maize tortillas needs to be addressed in future studies.

Conclusions

The present study determined that zinc from zinc oxide and zinc sulfate is equally absorbed in zinc-fortified corn tortillas. Since fortification of corn flour, and perhaps other cereals as well, with zinc oxide produces highly stable food products, and the cost of zinc oxide is less than that of other zinc compounds, we suggest that zinc oxide be the compound of choice for fortification of corn tortillas. In this study, we did not find evidence that iron fortification of corn tortillas decreases zinc absorption, even at double the dose that is currently used for fortification; thus, simultaneous fortification of corn tortillas with zinc and iron is feasible and is recommended.

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TABLE 1

Characteristics of subjects in both phases of the study before experimental meals[a]

Characteristic	Phase 1	Phase 2
N	10	10
Age (yr)	35.1 ± 8.6	34.3 ± 9.7
Weight (kg)	58.7 ± 5.5	57.7 ± 10.0
Body mass index (kg/m^2)	23.2 ± 1.4	22.7 ± 2.0
Plasma zinc (μg/dL)	67.8 ± 8.6	64.9 ± 5.8
Plasma zinc < 70 μg/dL (%)	70	80
Erythrocyte zinc < 12 μg/mL (%)	67	67

[a]Plus–minus values are means ± SD.

TABLE 2

Characteristics and energy content of experimental meals in each phase of the study

	Phase 1			Phase 2		
Meal composition	**CT + ZnO**	**CT + $ZnSO_4$**	**CT**	**CT + ZnO + 30Fe**	**CT + ZnO + 60Fe**	**CT + ZnO**
Energy (kJ/day)	8,209.5 ± 280.4	8,345.4 ± 197.1	8,326.7 ± 127.0	8,413.1 ± 63.3	8,591.8 ± 101.1	8,456.9 ± 89.3
Tortilla consumed (g/day)	459.9 ± 31.3	475.0 ± 22.0	473.0 ± 14.2	482.6 ± 7.1	502.6 ± 11.3	487.5 ± 10.0
Zinc consumed (mg/day)	13.0 ± 1.5	13.1 ± 2.3	6.5 ± 0.3	14.8 ± 1.0	14.1 ± 1.2	13.5 ± 0.6
Tortilla dietary zinc (mg)	5.9 ± 2.1	5.9 ± 2.1	5.4 ± 1.9	7.0 ± 0.1	6.9 ± 0.2	6.4 ± 0.1
Added zinc (mg)	6.4 ± 1.3	6.6 ± 2.4	0.5 ± 0.3	7.8 ± 0.9	7.3 ± 1.1	7.1 ± 0.7
Phytate consumed from tortilla (mg/day)	2,220.5 ± 151.2	2,293.8 ± 106.3	2,283.7 ± 68.5	2,353.9 ± 48.1	2,330.3 ± 34.1	2,426.7 ± 54.5
Phytate:zinc[a]	17.2 ± 2.2:1	18.1 ± 5.0:1	34.9 ± 1.6:1	15.8:1	16.4:1	17.9 1:1
Iron:zinc[a]	—	—	—	1.8:1	3.5:1	—

CT, corn tortilla made from flour with no zinc added; CT + ZnO, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide; CT + $ZnSO_4$, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc sulfate; CT + ZnO + 30Fe, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide + 30 mg/kg of extra fine reduced elemental iron; CT + ZnO + 60Fe, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide + 60 mg/kg of extra fine reduced elemental iron

[a] Molar ratio: zinc was measured in tortilla samples, phytate was estimated from previous studies, and iron amount was estimated from added iron.

TABLE 3

Fractional absorption of zinc (FAZ) and absorbed zinc from experimental meals in each phase of the study (mean ± SD)

Treatment	FAZ	Absorbed zinc (mg/day)
Phase 1		
CT + ZnO	0.36 ± 0.05	4.8 ± 0.8[a]
CT + $ZnSO_4$	0.37 ± 0.07	5.0 ± 1.0[a]
CT	0.35 ± 0.07	2.3 ± 0.5[b]
Phase 2		
CT + ZnO + 30Fe	0.33 ± 0.02	4.5 ± 0.3
CT + ZnO + 60Fe	0.32 ± 0.05	4.8 ± 0.7
CT + ZnO	0.32 ± 0.04	4.8 ± 0.7

CT, corn tortilla made from flour with no zinc added; CT + ZnO, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide; CT + $ZnSO_4$, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc sulfate; CT + ZnO + 30Fe, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide + 30 mg/kg of extra fine reduced elemental iron; CT + ZnO + 60Fe, tortilla made from flour fortified with 20 mg/kg of elemental zinc as zinc oxide + 60 mg/kg of extra fine reduced elemental iron

[a,b]Values with different superscript letters are significantly different (paired *t*-test), $p < 0.05$.