Zinc Absorption from Zinc Oxide, Zinc Sulfate, Zinc Oxide + EDTA, or Sodium-Zinc EDTA Does Not Differ When Added as Fortificants to Maize Tortillas[1]

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ABSTRACT The fortification of staple foods with zinc may play an important role in achieving adequate zinc intakes in countries at risk of zinc deficiency. However, little is known about the relative bioavailability of different zinc compounds that may be used in food fortification. The objective of this study was to measure and compare fractional zinc absorption from a test meal that included a maize tortilla fortified with zinc oxide, zinc sulfate, zinc oxide + EDTA, or sodium-zinc EDTA. A double isotopic tracer ratio method (^{67}Zn as oral tracer and ^{70}Zn as intravenous tracer) was used to estimate zinc absorption in 42 Mexican women living in a periurban community of Puebla State, Mexico. The test meal consisted of maize tortillas, yellow beans, chili sauce, and milk with instant coffee; it contained 3.3 mg zinc and had a phytate:zinc molar ratio of 17. Fractional zinc absorption did not differ significantly between the test groups (ANOVA; $P > 0.05$). Percent absorptions were (mean ± SD) zinc oxide, 10.8 ± 0.9; zinc sulfate, 10.0 ± 0.02; zinc oxide + EDTA, 12.7 ± 1.5; and sodium-zinc EDTA, 11.1 ± 0.7. We conclude that there was no difference in zinc absorption from ZnO and $ZnSO_4$ when added as fortificants to maize tortillas and consumed with beans and milk. The addition of EDTA with zinc oxide or the use of prechelated sodium-zinc EDTA as fortificants did not result in higher zinc absorption from the test meal. J. Nutr. 135: 1102–1105, 2005.

KEY WORDS: • *zinc* • *absorption* • *maize* • *fortificants* • *stable isotopes*

In recent years, the importance of zinc for human health and development (1–3) and the possible widespread occurrence of suboptimal zinc intakes (3,4) have gained recognition. The fortification of staple foods with zinc may play an important role in achieving adequate zinc intakes in at-risk populations. Although the relative absorption of iron varies substantially depending on the chemical form of the iron compound used as a fortificant (5), the relative bioavailability of different zinc compounds that may be used in food fortification is not well known, and hence the efficacy of such compounds to improve zinc status.

Two of the most commonly used zinc fortificants to date are zinc oxide and zinc sulfate (6,7). A few earlier studies compared the absorption of zinc between these chemical forms using the oral zinc tolerance test as a measure of bioavailability, but the results were conflicting (8,9). One of those studies reported a much lower plasma appearance from zinc oxide preparations compared with zinc sulfate (9), whereas the other reported no difference (8). A more recent study used stable isotope tracer techniques to measure zinc absorption in a group of Indonesian children; in that study, wheat flour dumplings were fortified with either zinc oxide or zinc sulfate (10) and zinc absorption did not differ between groups.

EDTA is a metal-chelating compound that may facilitate the absorption of nutritionally important minerals by preventing their binding with other compounds that inhibit mineral absorption (e.g., phytate). Various studies showed that iron is better absorbed from NaFeEDTA than from other compounds (11–14). It is also possible that EDTA would have an enhancing effect on zinc bioavailability, as was shown previously in some animal studies (15,16). To date, there are no reports from human studies of the effect of EDTA compounds in improving the absorption of zinc fortificants added to staple foods.

In Mexico, biochemical evidence suggests that the risk of zinc deficiency is high; approximately one third of women and preschool children have low serum zinc concentrations (<9.9 μmol/L) (17). The voluntary fortification of maize flour with zinc oxide and other nutrients was initiated through an agreement between the Mexican federal government and the major maize flour manufacturers (18). The effectiveness of zinc-fortified maize to prevent zinc deficiency may be improved if zinc compounds other than zinc oxide are found to be more efficiently absorbed from maize-based meals. Therefore, the objective of this study was to compare the absorption of zinc from zinc oxide, zinc oxide plus EDTA, prechelated sodium-

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zinc EDTA, and zinc sulfate, when added to maize flour and used to prepare a typical Mexican, maize-based meal.

SUBJECTS AND METHODS

Subjects. Women from Cuautlancingo, a semirural community in Puebla State, Central Mexico were recruited at a community clinic from among those who met the following criteria: 19–44 y of age; not currently pregnant or lactating; reporting to be free from any known chronic disease; not participating in targeted programs providing foods fortified with zinc or iron; and not currently taking any supplements containing zinc or iron. Women meeting these criteria were screened for iron status; those who were nonanemic (hemoglobin > 125 g/L; cutoff value adjusted for altitude) but with low iron stores (serum ferritin ≥ 5 and ≤ 25 μg/L) were invited to participate. Although iron status is not expected to affect zinc absorption, we selected nonanemic women with low iron stores because we planned to repeat the study measuring iron absorption from iron and zinc–cofortified diets, in which case it would be desirable to limit the range of iron status among participants. Details of the study and requirements for participation were explained to the women and written consent was obtained from those who volunteered. Human subjects' approval for this study was obtained from the Ethics Committee of the Instituto Nacional de Salud Pública (INSP; Cuernavaca, Morelos, Mexico) and from the University of California Davis Institutional Review Board (Davis, CA).

It was initially estimated that a group difference of 8% zinc absorption could be detected with 15 subjects/group, based on previous studies (19,20) (2-tailed test, SD = 10.5, $\alpha = 0.05$, 80% power). However, a lower SD was observed for the first subjects completing this study in 2 groups (ZnO and ZnO + EDTA), indicating that fewer subjects were needed.

Preparation of isotopes and study diet. We used the previously validated double isotopic tracer ratio method to measure fractional zinc absorption (21). Zinc oxide, highly enriched with ^{70}Zn, was purchased from Oak Ridge National Laboratory (88.2% enrichment) and from ISOFLEX USA (91.2% enrichment). The orally administered ^{67}Zn compounds were all prepared in powdered form; ^{67}ZnO was purchased from Trace Sciences International (88.6% ^{67}Zn enrichment) and the $^{67}ZnSO_4$ and $^{67}Na_2$ZnEDTA were purchased from ISOFLEX USA.

Nonfortified maize flour was obtained from a local producer (Maseca) and was mixed with water to form a soft dough. Individual oral doses of the ^{67}Zn-enriched powders were weighed accurately ($\pm$0.03 mg) on a microbalance (Sartorius Micro M3P) and placed in the center of 4-g dough balls, which were folded and stored frozen until use. For the group administered ZnO + Na_2EDTA, 2.71 mg of Na_2EDTA (Sigma Chemical) was added to the dough ball to achieve a 0.5:1 mol/L ratio of EDTA:Zn. Additional doses of each isotope were also weighed out and used to verify the zinc content.

For the test diet, the maize flour was mixed with water, and tortillas were formed and cooked on a nonstick pan. For the tortilla that was to include the isotope, the labeled dough ball was thawed slightly, placed in the center of a fresh dough ball, and made into a tortilla. The test meal also included cooked yellow beans, a tomato and chili sauce, and milk with instant coffee (**Table 1**). These foods were chosen because they are representative of a typical rural Mexican meal (17). The quantity of zinc added as ^{67}Zn enriched powder to the flour was equivalent to adding fortificant at a concentration of 40 mg zinc/kg flour. The test meal, including the added zinc, would provide about one third of the current U.S. Recommended Dietary Allowance of zinc for adult women (8 mg/d) (22).

Metabolic study procedures. All participants entered the study during the follicular phase of their menstrual cycle. It is not known whether menstruation causes changes in zinc absorption, but it is known that serum zinc concentration fluctuates during the menstrual cycle (23). An FFQ and a sociodemographic questionnaire, which requested information on the construction of homes, quality of water services, and personal possessions, were administered to the participants by a trained field worker. In the morning hours, a spot-urine pregnancy test was carried out; then participants had blood drawn for biochemical analyses, received the test meal, and finally received an i.v. injection of the ^{70}Zn solution; the i.v. dose of ^{70}Zn (mean $\pm$ SD) was 0.21 $\pm$ 0.05 mg. The plastic bag in which the ^{67}Zn-labeled dough ball was stored was rinsed with water and participants drank the water. The weight of any leftover food items was recorded. Participants were instructed not to consume anything other than water from 2200 h the night before and for 3 h after the test meal. One casual, midstream urine sample was collected into trace element–free, sterile, plastic containers on d 3–5 after isotope administration. The samples were picked up daily from the participants' homes and stored frozen (-70°C) in the nutrition laboratory of INSP in Cuernavaca until purification.

Sample processing and analysis. The urine samples were purified using ion exchange columns (24), and isotopic ratios were measured by inductively coupled plasma MS using the methods and equipment described by Pinna et al. (25). The fractional absorption of the ^{67}Zn dose from the test meal was calculated by the tracer-to tracee method (21,24). If the intrasubject CV for these 3 data points was >20%, purification and enrichment analysis was repeated for urine samples with aberrant results, or the aberrant point was removed. The overall dose of zinc from the ^{67}Zn enriched compounds was (mean $\pm$ SD) 0.96 $\pm$ 0.05 mg as determined by analysis of the duplicate doses.

Biochemical analyses. Serum zinc was measured by flame atomic absorption spectrophotometry (AAnalyst 300, Perkin-Elmer) and hemoglobin was measured using a portable Hemocue. Serum ferritin and C-reactive protein were measured by nephelometry following specifications by the equipment manufacturer (Nephelometer 100, Dade-Behring).

TABLE 1

The content of energy, protein, zinc, phytate, phytate:zinc molar ratio, iron, and calcium from a maize tortilla-based test meal to measure the absorption of zinc

	Maize tortillas (without fortificant)	Yellow beans, cooked	Tomato and chili sauce	Milk with instant coffee and sugar	Zinc from isotopic label[1]	Total
Amount, *g*	64	165	10	240	—	479
Energy,[2] *kJ*	402	996	13	854	—	2265
Protein,[2] *g*	2.4	5.1	0.1	7.4	—	15.0
Zinc,[3] *mg*	0.4	1.0	0.0	0.9	1.0	3.3
Phytate,[2] *mg*	206	347	1	10	—	564
Phytate:zinc molar ratio	51	34	—	0.1	—	17
Iron,[3] *mg*	0.5	2.6	0.0	0.4	—	3.5
Calcium,[3] *mg*	13	67	1	264	—	345

1 The mean amount of zinc added in the form of ^{67}Zn enriched label is shown.
2 Derived from the World Food Dietary Assessment System food composition table for Mexico (Version 2.0).
3 Analyzed values determined by inductively coupled plasma-atomic emission spectrometry.

Data management and statistical analysis. Statistical analyses were performed using SPSS for Windows version 10.1. Data are presented as means ± SD, unless indicated otherwise. Differences among the 4 study groups for baseline characteristics and the percentage absorption of zinc were determined by ANOVA and Tukey's post hoc analysis; the analyses of serum zinc and serum ferritin at baseline were controlled for C-reactive protein. Associations between study variables were tested using Pearson correlations. Group differences or associations were considered to be significant when $P < 0.05$.

RESULTS

A total of 44 participants completed the study. Initially, 9 subjects completed the study in the ZnO group and 9 in the ZnO + EDTA group but data are available for only 7 subjects in the ZnO group because samples for 2 subjects were lost due to an electrical failure in the laboratory. Because the SD for zinc absorption was lower than estimated and the statistical power was adequate to compare the 2 groups, it was unnecessary to recruit further subjects to those groups and the sample size for the remaining 2 groups was conservatively reduced from 15 ($ZnSO_4$, $n = 13$; $Na_2ZnEDTA$, $n = 12$), as originally estimated.

The estimated, daily intakes of energy, zinc, iron, and phytate of the subjects, as derived from the FFQ, were [median (25th, 75th percentile)] 7.5 (5.8, 9.4) MJ/d, 8.9 (6.7, 10.9) mg zinc/d, 11.2 (9.3, 14.0) mg iron/d, and 1564 (1150, 2000) mg phytate/d, respectively, and the phytate:zinc molar ratio was 17. Baseline characteristics of participants did not differ among study groups except for serum ferritin concentration, which was significantly higher in the zinc oxide + EDTA group than in the zinc sulfate group (**Table 2**).

The fractional zinc absorption for each of the 4 fortificant groups were as follows: zinc oxide, 10.8 ± 0.9; zinc sulfate, 10.0 ± 1.9; zinc oxide + EDTA, 12.7 ± 1.5; sodium-zinc EDTA, 12.7 ± 1.5. Zinc absorption from the various compounds tested did not differ. We had sufficient statistical power (85% power, α 0.05) to detect a difference in fractional absorption of zinc of 0.016 (i.e., 1.6% absorption) or less between any 2 test groups. Also, there was no significant correlation between any biochemical indicator and fractional zinc absorption.

DISCUSSION

Zinc absorption from tortillas fortified with isotopically labeled zinc oxide or zinc sulfate did not differ. Further, we did not observe an enhancing effect of EDTA on zinc absorption from tortillas fortified with either sodium-zinc EDTA, or zinc oxide + EDTA among the women tested in this study. The mean absorption of zinc from all groups was 11%, which is in the lower range of dietary zinc absorption (3) but consistent with previous reports of zinc absorption from meals with a similar phytate:zinc molar ratio (26,27).

Our results comparing the absorption of zinc from tortillas fortified with either zinc oxide or zinc sulfate are consistent with results of similar studies reported since we initiated the present study. A study by Lopez de Romaña et al. (28) measured zinc absorption from both bread and porridge prepared from wheat using a ^{65}Zn label and whole-body counting. Zinc absorption did not differ between bread fortified with either zinc oxide or zinc sulfate (14 vs. 14%, respectively) or between porridge fortified with either zinc oxide or zinc sulfate (6 vs. 7%, respectively). Preliminary results reported by Rosado et al. (7) indicated no difference in zinc absorption from tortillas fortified with zinc oxide (37%) or zinc sulfate (37%) using a single zinc stable isotope method and fecal monitoring. Herman et al. (10) also reported no difference in zinc absorption by children from wheat dumplings fortified with either zinc oxide (24%) or zinc sulfate (24%).

The consistency among the above-mentioned studies strongly suggests that the water-insoluble zinc oxide is not absorbed to a lesser degree than the water-soluble zinc sulfate, when added to staple food products. Because zinc oxide is soluble in dilute acid, it likely dissolves in the hydrochloric acid secretions of the stomach. One study demonstrated that the intestinal absorption of zinc from zinc oxide was reduced with an experimental reduction in gastric acid secretion and a resultant increased pH (≥5), compared with that at the lower, normal pH of the stomach (29). No such inhibition by reduced stomach acidity was found with water-soluble zinc acetate (29). It was therefore suggested that there may be an advantage to using zinc sulfate as a fortificant among populations in which hypochlorydria, a condition that occurs frequently among the elderly and with some bacterial infections, is widespread. Therefore, although it appears that either zinc oxide or zinc sulfate can be recommended for food fortification programs among healthy populations, further epidemiologic research is required to determine the extent of hypochlorydria and whether it poses a concern with regard to selection of a soluble zinc salt over zinc oxide.

EDTA appeared to confer no advantage to the absorption of zinc from the test meals in this study. This is in contrast to the ample evidence that EDTA enhances the absorption of iron in humans (11–14). The discrepancy may be related to differences in stability constants and optimal pH for the binding of different minerals with EDTA. For example, EDTA complexes of Fe^{3+} and Cu^{2+} have a higher stability constant

TABLE 2

Baseline characteristics of participants in a study of zinc absorption from a tortilla-based meal with 1 of 4 different zinc fortificant groups[1]

	ZnO	ZnO + Na_2EDTA	$Na_2ZnEDTA$	$ZnSO_4$
n	7	10	12	13
Age, *y*	24.0 ± 3.4	27.6 ± 4.9	29.0 ± 5.2	27.6 ± 5.5
Body weight, *kg*	59.1 ± 6.1	55.7 ± 8.8	57.9 ± 10.8	61.4 ± 10.2
Serum zinc, *μmol/L*	10.3 ± 1.0	10.2 ± 1.2	10.8 ± 0.9	10.3 ± 1.2
Hemoglobin, *g/L*	142 ± 13	148 ± 14	148 ± 14	145 ± 10
Serum ferritin,[2] *μg/L*	12.8 ± 9.0[ab]	21.6 ± 13.6[b]	12.5 ± 5.1[ab]	12.0 ± 4.2[a]
Serum C-reactive protein, *mg/L*	2.3 ± 3.9	2.7 ± 2.6	1.9 ± 1.6	4.3 ± 5.5

1 Values are means ± SD.
2 Means in a row with superscripts without a common letter differ, $P < 0.05$.

than with Zn^{2+}. However, stability of mineral chelation with EDTA is also dependent on pH. It is possible that at the pH (6–7) of the upper small intestine, where the majority of mineral absorption occurs, EDTA may chelate more readily with Ca^{2+}, whose optimum pH for chelation is 7.5, than with Zn^{2+}, whose optimum pH for chelation is 4.0 (30). Because our test meal included milk and a total of 345 mg of calcium compared with only 3.3 mg of zinc, preferential binding with calcium may have occurred. On the other hand, a study of zinc and calcium absorption among women demonstrated an increase in zinc absorption, but no change in calcium absorption, from a test diet when bread was fortified with iron EDTA (31). The interaction of EDTA with minerals and its ultimate effect on intestinal mineral absorption is complex and will depend on the concentration of other ions, other competing mineral ligands, as well as pH. The effect of EDTA on zinc fortificant absorption may thus be dependent on the composition of the diet.

Some studies showed that high ratios of EDTA:zinc may have an inhibitory effect on zinc absorption, such that the enhancing effect diminishes at higher EDTA:zinc molar ratios. The inhibitory effect shown by Hempe and Cousins (32) in their rat study occurred with an EDTA:zinc ratio of 0.43:1. On the other hand, Hurrell et al. (16) did not observe an inhibitory effect of EDTA on zinc retention in rats with EDTA:zinc molar ratios of up to 37:1. Solomons et al. (33) suggested that the inhibitory effect of EDTA on zinc absorption from aqueous solutions occurred when the EDTA:zinc ratio was > 0.33:1, as determined by oral zinc tolerance tests. However, in the latter study, EDTA was provided in the form of iron-EDTA and all tests were done using aqueous solutions with pharmacologic doses; thus, the nature of the inhibitory effect is difficult to interpret. In our study the EDTA:zinc molar ratio of 0.5:1 in the test diet did not appear to inhibit zinc absorption. Dose-response trials of increasing EDTA:zinc molar ratios on zinc absorption in humans using isotopic tracer techniques and standard test meals would be useful to further understand this relation.

In conclusion, our study suggests that zinc oxide and zinc sulfate are equally well absorbed when added as fortificants to a maize-based diet and that EDTA did not increase zinc absorption in this diet type. The possibility that EDTA could enhance zinc absorption in other diet types requires further investigation.

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