Influence of Phytase, EDTA, and Polyphenols on Zinc Absorption in Adults from Porridges Fortified with Zinc Sulfate or Zinc Oxide

Marica Brnić, Rita Wegmüller, Christophe Zeder, Gabriela Senti, and Richard F. Hurrell

Abstract

Fortification of cereal staples with zinc is recommended to combat zinc deficiency. To optimize zinc absorption, strategies are needed to overcome the inhibitory effect of phytic acid (PA) and perhaps polyphenols. Five zinc absorption studies were conducted in young adults consuming maize or sorghum porridges fortified with 2 mg zinc as zinc sulfate (ZnSO₄) or zinc oxide (ZnO) and containing combinations of PA or polyphenols as potential inhibitors and EDTA and phytase as potential enhancers. Fractional absorption of zinc (FAZ) was measured by using the double isotopic tracer ratio method. Adding phytase to the maize porridge immediately before consumption or using phytase for dephytinization during meal preparation both increased FAZ by >80% (both P < 0.001). Adding Na₂EDTA at an EDTA:zinc molar ratio of 1:1 increased FAZ from maize porridge fortified with ZnSO₄ by 30% (P = 0.01) but had no influence at higher EDTA ratios or on absorption from ZnO. FAZ was slightly higher from ZnSO₄ than from ZnO (P = 0.02). Sorghum polyphenols had no effect on FAZ from dephytinized sorghum porridges but decreased FAZ by 20% from PA-rich sorghum porridges (P < 0.02). The combined inhibitory effect of polyphenols and PA was overcome by EDTA. In conclusion, ZnSO₄ was better absorbed than ZnO, phytase used to degrade PA during digestion or during food preparation substantially increased zinc absorption from ZnO-fortified cereals, and EDTA at a 1:1 molar ratio modestly enhanced zinc absorption from ZnSO₄-fortified cereals but not ZnO-fortified cereals, and sorghum polyphenols inhibited zinc absorption in the presence, but not absence, of PA. This trial was registered at clinicaltrials.gov as NCT01210794. J. Nutr. 144: 1467–1473, 2014.

Introduction

Zinc deficiency is widespread in low-income countries, causing impairments in the immune system, in physical growth, and in pregnancy outcomes (1). The main cause of zinc deficiency is an inadequate zinc intake or absorption from plant-based diets low in bioavailable zinc that contain few if any zinc-rich animal-source foods (2). Fortification of cereal staples with zinc is recognized as a safe and appropriate strategy to treat and prevent zinc deficiency (3). However, a good knowledge of the dietary factors that influence zinc absorption is essential for an effective fortification approach.

Phytic acid (PA) is a known inhibitor of zinc absorption, and it is naturally present in cereal-based staples (4), with maize, wheat, sorghum, and millet having high PA contents (5). PA binds minerals, including zinc, in the human gastrointestinal tract, forming insoluble complexes that prevent zinc absorption (6,7); and the PA to zinc molar ratio (PA:Zn) of a meal has been used to estimate the fractional absorption of the ingested zinc (8,9).

PA can be degraded by phytase enzymes leading to an increase in the bioavailability of zinc and other minerals (10,11). PA degradation was reported to occur on fermentation of cereals such as wheat, which contain endogenous phytases (12,13), and on addition of exogenous phytases (14) during the preparation of cereal flours for complementary feeding. Both processes, however, require long preparation in slurry form with adjustments of pH and temperature for efficacious dephytinization. A simpler procedure, suitable for in-home fortification mixtures, would be to add the phytase to the cereal porridge at the time of consumption so that PA degradation can take place during the digestive process. Two iron-absorption studies showed that the addition of a microbial phytase, active at gut pH, to a high-PA cereal meal immediately before consumption substantially increased iron absorption (15,16). A similar effect would be expected for zinc but has yet to be demonstrated.

EDTA and polyphenols are 2 other food components that might influence zinc absorption from zinc-fortified cereals. EDTA has been repeatedly shown to increase iron absorption...
(17) and polyphenols have been reported to decrease iron absorption (18), but data on their effect on zinc absorption in humans are scarce and inconsistent (19). Two human studies with stable isotopes reported increased zinc absorption when the meals were fortified with zinc and EDTA (20,21); and in a recent animal study, rats fed a PA-rich diet enriched with Na₂₃ZnEDTA had a higher zinc concentration in plasma and femur than those fed a diet enriched with other organic or chelated zinc compounds including oxide, sulfate, and gluconate (22). Other studies, however, did not observe significant improvements in zinc absorption when EDTA was added with zinc fortification compounds (23–25); and it has been suggested that the inconsistency might be explained by the EDTA:Zn molar ratio (3,19). Another possibility is an influence of the solubility of the zinc fortification compound in gastric acid, because EDTA increases the bioavailability of only water-soluble iron compounds but not of water-insoluble compounds (26).

A wide variety of polyphenolic compounds, of varying chain length and composition, are naturally present in fruits, vegetables, beverages, including wine and tea, and in colored cereals and beans (27). The largely polymeric polyphenols in tea, cocoa, wine, some vegetables, beans, and sorghum bind iron in the gastrointestinal tract and inhibit its absorption (17). However, there are no human isotope studies evaluating the influence of polyphenols on zinc absorption, and a single zinc balance study in humans reported a small, but not significant (P = 0.63), decrease in zinc balance when tea was consumed together with the meal (28). The rat studies in the literature reported contradictory findings including that tea, wine, coffee, and other polyphenols decrease zinc absorption (29,30), have no effect (30–33), or increase zinc concentration in some tissues (34–36) but decrease it in others (35,37). Caco-2 cell studies with tea and other polyphenols also reported contradictory results on the effect on zinc uptake (38,39). The widely consumed staples sorghum and beans may contain high amounts of both polyphenols and PA, so there is a need to know the influence of polyphenols on zinc absorption in the presence of PA as well as in defluorinated products.

The stable-isotope zinc absorption studies reported here used the double isotope tracer methodology in adult human participants. They were designed to evaluate factors that could have an influence on the design of efficacious zinc-fortified foods. These factors include the following: the addition of an exogenous phytase to a cereal porridge immediately before consumption (28). The rat studies in the literature reported a small, but not significant (P = 0.63), decrease in zinc balance when tea was consumed together with the meal (28). The rat studies in the literature reported contradictory findings including that tea, wine, coffee, and other polyphenols decrease zinc absorption (29,30), have no effect (30–33), or increase zinc concentration in some tissues (34–36) but decrease it in others (35,37). Caco-2 cell studies with tea and other polyphenols also reported contradictory results on the effect on zinc uptake (38,39). The widely consumed staples sorghum and beans may contain high amounts of both polyphenols and PA, so there is a need to know the influence of polyphenols on zinc absorption in the presence of PA as well as in defluorinated products.

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**Participants and Methods**

**Participants.** Seventy-five apparently healthy men and women (non-pregnant, nonlactating) aged between 18 and 45 y with normal BMIs who reported being free from any metabolic, gastrointestinal, and chronic disease; not currently taking long-term medication (except contraceptives); and not smoking were recruited among the students and employees of the ETH Zurich (Switzerland). Participants were allocated to 5 studies, with 15 participants per study (Table 1). They were asked not to take any vitamin/mineral supplements 2 wk before and during the study. Eligibility to participate and written informed consent from all participants were obtained at the ETH Zurich before the start of the study. The study was conducted at the Clinical Trials Center of the University Hospital Zurich. The study was approved by the Ethics Committee of the Canton of Zurich (KEK Zurich).

**Study design.** Five separate single-blind zinc absorption studies were carried out (Table 1). Three test meals per study were served in a crossover design and in a random order. The double isotope tracer ratio technique applied to spot urine samples was used to measure the fractional absorption of zinc (FAZ) (40,41). On study day 1, the participants provided the first baseline spot urine sample and had a venous blood sample drawn after an overnight fast for the analyses of plasma zinc (PZn) and C-reactive protein (CRP). They received the first test meal labeled with the oral isotopic tracer ⁶⁷Zn, and immediately afterward another stable isotopic tracer (⁶⁷Zn) was administered i.v. over 5 min. No foods or liquids were allowed during the following 3 h. Five days after the tracer administration, the first enriched urine sample was collected. This time was identified in a pilot study as the most suitable time for urine collection, because from that time both isotopes declined in a proportional manner. The second and the third test meals were administered, each after a 4-wk washout period on days 29 and 57, respectively, by using the same procedure and the same tracers. New baseline samples were collected before each test meal to account for residual enrichment from the previous administration.

**Test meals.** Different combinations of potential zinc absorption enhancers and inhibitors were investigated in each of the 5 studies (Table 1). Whole maize (Zea mays L.) flour was used to prepare PA-rich porridges for studies 1–3 (yellow whole maize, purchased as flour from Saska Grain). Sorghum [Sorghum bicolor (L.) Moench] porridges in studies 4 and 5 were prepared with decorticated polyphenol-rich brown sorghum flour (Variety Framida, ICSV 1001; purchased as whole grains in Burkina Faso; decorticated in France at the Institut de recherche pour le développement (IRD), Montpellier, using the DMS500 decorticator; Electra SAS; decortication yield: 90%) and white sorghum flour with low polyphenols (Variety Kossa, CSM 485; purchased as whole grains in Mali; decorticated in Mali at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Bamako, using the TADD decorticator; Venables Machine Works; decortication yield: 86%). The decorticated sorghum grains were milled at the Human Nutrition Laboratory, ETH Zurich, by using the centrifugal mill (Retsch GmbH). The final test meal portions contained 50 g flour, 170 g high-purity water (18 MΩ·cm), and 5 g sugar for maize or 15 g sugar for sorghum porridges.

Each study meal (A, B, or C) was prepared in 1 multiple-portion batch, and the same cooking procedure was used for all the study meals. The ingredients were mixed, cooked for 10 min, and cooked down to 55°C and their pH was adjusted to between 5.0 and 5.5 by adding a 0.5 mol/L HCl solution. In case of dephytination (test meal C in study 1 and all test meals in study 4), phytase was added immediately after this step. For all test meals, the batch was kept at 55°C for 60 min under continuous stirring. The meal was then warmed to 80°C to inactivate the added phytase and cooled to room temperature. Water that had evaporated during this process was replaced, and portions of 225 g for the maize and 235 g for sorghum porridges were weighed into plastic bowls. They were stored frozen at −25°C and defrosted overnight before consumption. They were heated to −40°C just before consumption by using a microwave oven.

All test meals were fortified with 1 mg unlabeled zinc as zinc sulfate (ZnSO₄; studies 1, 2, 4, and 5) or as zinc oxide (ZnO; study 3; Lohmann GmbH) and with 1 mg labeled zinc as ⁶⁷ZnSO₄ or as ⁶⁷ZnO, respectively. ZnSO₄ was prepared as a solution, whereas ZnO was homogeneously mixed with the powdered sugar to avoid imprecise doses. In studies 2–5, Na₂EDTA - 2H₂O (Sigma Aldrich) was used as the source of EDTA. Unlabeled and labeled zinc and EDTA were added and thoroughly mixed with the warm test meals just before serving. The enzyme phytase (DSM FS Phytase 20.000 G; DSM Nutritional Products) was added during meal preparation [study 1 (test meal C) and study 4 (all meals)] or just before serving (study 1, test meal B). The phytase was derived from a genetically modified culture of Aspergillus niger and had pH optima at 5 and slightly below 3. More detailed properties of this phytase are described elsewhere (15). The activity of the phytase is measured as the amount of enzyme that liberates 1 μmol of inorganic phosphorous per minute and is expressed in phytase units (FTUs).

The amount of phytase for the dephytination during meal preparation was estimated considering 100% activity at the adjusted cooking
Preparation of stable-isotope labels. Zinc oxide, highly enriched in $^{67}$Zn (90.6% enrichment) and $^{70}$Zn (95.4% enrichment), was purchased from Chemgas. $^{67}$ZnSO$_4$ for oral administration was prepared from $^{67}$ZnO by dissolution in stoichiometric amounts of diluted H$_2$SO$_4$. The resulting $^{67}$ZnSO$_4$ solution was diluted to a zinc concentration of 1 mg/g, and 1 g of the solution was weighed onto the test meal. $^{67}$ZnO for oral administration was prepared in powdered form by mixing $^{67}$ZnO with powdered sugar to a zinc concentration of 1 mg/g, so as to avoid an imprecise dose of the small quantity of isotope. For a homogeneous distribution in the mixture was checked by atomic absorption spectroscopy (AAS) measurements. Individual 1-g doses were weighed in advance into polypropylene cryovials and sprinkled over the test meal. The empty tubes were then rinsed with additional high-purity water (18 MΩ - cm), then dried. They were instructed to consume the entire meal and the water. The empty bowl was then rinsed with additional high-purity water (18 MΩ - cm; 2 × 10 mL), which was then drunk.

### Table 1

Overview of the 5 zinc absorption studies with respective effect investigated, zinc compound used, and brief description of the meals

<table>
<thead>
<tr>
<th>Study, investigated effect</th>
<th>Zinc compound</th>
<th>Meal A</th>
<th>Meal B</th>
<th>Meal C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, Effect of phytase during digestion</td>
<td>ZnSO$_4$</td>
<td>Maize porridge</td>
<td>Maize porridge + phytase</td>
<td>Maize porridge, dephytinized</td>
</tr>
<tr>
<td>2, Effect of EDTA$^2$ at 2 ratios using a water-soluble zinc compound</td>
<td>ZnSO$_4$</td>
<td>Maize porridge</td>
<td>Maize porridge + EDTA:Zn, 1:1</td>
<td>Maize porridge + EDTA:Zn, 2:1</td>
</tr>
<tr>
<td>3, Effect of EDTA at 2 ratios using a water-insoluble zinc compound</td>
<td>ZnO</td>
<td>Maize porridge</td>
<td>Maize porridge + EDTA:Zn, 1:1</td>
<td>Maize porridge + EDTA:Zn, 2:1</td>
</tr>
<tr>
<td>4, Effect of polyphenols alone and effect of EDTA</td>
<td>ZnSO$_4$</td>
<td>White sorghum porridge, dephytinized</td>
<td>Red sorghum porridge, dephytinized</td>
<td>Red sorghum porridge dephytinized + EDTA:Zn, 1:1</td>
</tr>
<tr>
<td>5, Combined effect of polyphenols and phytic acid and effect of EDTA</td>
<td>ZnSO$_4$</td>
<td>White sorghum porridge</td>
<td>Red sorghum porridge</td>
<td>Red sorghum porridge + EDTA:Zn, 1:1</td>
</tr>
</tbody>
</table>

1 $^{67}$ZnO, zinc oxide; $^{70}$ZnSO$_4$, zinc sulfate.
2 EDTA administered in the form of Na$_2$EDTA.

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TABLE 2

Total zinc, PA, and polyphenols in maize and sorghum porridges administered to young adults in the 5 zinc absorption studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Meal</th>
<th>Zinc$^2$</th>
<th>PA</th>
<th>PA:Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/meal</td>
<td>mg/meal</td>
<td>mol/mol</td>
</tr>
<tr>
<td>1–3</td>
<td>Maize porridge</td>
<td>2.6 ± 0.10</td>
<td>259 ± 19.3</td>
<td>&lt;8</td>
</tr>
<tr>
<td>4, 5</td>
<td>White sorghum porridge</td>
<td>3.0 ± 0.02</td>
<td>356 ± 3.7</td>
<td>&lt;8</td>
</tr>
<tr>
<td>4, 5</td>
<td>Brown sorghum porridge</td>
<td>2.8 ± 0.02</td>
<td>281 ± 5.7</td>
<td>&lt;8</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs unless otherwise indicated; n = 3. NA, not analyzed; PA, phytic acid.
2 Zinc includes native zinc, 1 mg unlabeled zinc, and 1 mg $^{67}$Zn.
3 Meal C in study 1 and all meals in study 4 were completely dephytinized with PA concentrations below the limit of detection (<8 mg/meal) and PA:Zn molar ratios of 0.

### Analytical methods

Freeze-dried meals, and all labeled and unlabeled zinc solutions, were analyzed for their zinc content by flame AAS (AA240FS; Varian) after microwave digestion (MLS-ETHOS plus; MLS GmbH) by using an HNO$_3$/H$_2$O$_2$ mixture. The PA content was determined in the freeze-dried porridges by the modified Makower method (42), in which iron was replaced by cerium in the precipitation step. After the hydrolyzation of the precipitate (Digest Automat K-438; Büchi Labortechnik AG) in concentrated H$_2$SO$_4$, inorganic phosphate was determined according to Van Veldhoven and Mannaerts (43) and converted into PA concentrations. The total polyphenol content was measured in thawed liquid porridges by the modified Folin-Ciocalteu method, as recommended by Singleton and Rossi (44) and was expressed as gallic acid equivalents.

Blood samples, drawn into zinc-free heparinized tubes, were refrigerated at 4°C immediately after collection. The plasma was separated within 1 h, separated into aliquots in acid-washed plastic vials, and frozen at −25°C until analysis. PZn concentration was measured by flame AAS (AA240FS; Varian) after dilution with HNO$_3$, 10% by using commercial aqueous standards (Titrisol; Merck) for external calibration. Accuracy was checked by analysis of commercially available serum controls (Seronorm Trace Elements Serum L-1 and L-2; Sero). CRP was measured on an Immulite 2000 automatic system (Siemens Healthcare Diagnostics).

Spot urine samples, collected in polyethylene containers, were stored at −25°C until analysis. Their zinc isotopic composition was analyzed in duplicate under chemical blank monitoring at the Human Nutrition Laboratory. Urine samples were concentrated by freeze-drying, then mineralized by microwave digestion (MLS-ETHOS plus; MLS GmbH) by using a mixture of HNO$_3$ and H$_2$O$_2$ and finally zinc was isolated from
sample matrix by ion-exchange chromatography (45). All acids used for the preparation of samples were ultrapure. 70Zn:66Zn and 67Zn:66Zn isotopic ratios were measured to determine 70Zn and 67Zn enrichment in the urine samples by using a high-resolution double-focusing magnetic sector field multicollector inductively coupled plasma mass spectrometer (Finnigan Neptune; Thermo Electron).

**Calculation of fractional zinc absorption.** The fractional absorption of the 67Zn dose from the test meal was calculated by using the oral to i.v. tracers ratio method applied to spot urine samples, according to principles described by Friel et al. (40). Enrichment observed in the baseline samples from the second and third study were used as the new natural abundance in subsequent calculations.

**Statistical analysis.** Sample size was calculated on the basis of FAZ data from previous studies (46,47). A sample size of 15 participants provided 80% power to detect a minimum relative difference of 10% in FAZ (α = 0.05) between test meals consumed in different studies by allowing a dropout rate of 20%.

The statistical analyses were performed only with the per-protocol participants by using SPSS software (version IBM SPSS Statistics 20). FAZ values are presented as geometric means and SDs. Group data were not normally distributed; hence, log-transformed values were used for the statistical analysis. Comparisons of zinc absorption values within 1 study were made by repeated-measures ANOVA. Bonferroni adjustment for multiple comparisons was applied. Unpaired t tests were used to compare FAZs between the single meals from studies 2 and 3 as well as to check for differences in age, BMI, and zinc status between participants of the 2 studies. Significance was defined at the P = 0.05 level.

**Results**

**Participants.** A total of 60 participants completed the 5 studies as per protocol (Table 3). Fifteen enrolled participants were withdrawn from the studies, and none of their data were considered for analysis (2 voluntary withdrawals, 11 incomplete or missing test meal consumption data, 2 incomplete i.v. dose administrations). Five male and 10 female participants had a PZn concentration slightly below the suggested lower cutoffs, indicating mild zinc deficiency (74 mg/L) (48), but concentrations of the 67Zn dose from the test meal was calculated by using the oral to i.v. Calculation of fractional zinc absorption. (Finnigan Neptune; Thermo Electron).

**Discussion**

In study 1, consuming the active phytase enzyme together with the cereal meal increased zinc absorption to the same extent as when the meal was completely dephytinized before consumption. Other studies with stable isotopes in children (49) and adults (50) also reported that enzymatic PA degradation before consumption increases zinc absorption. In our study, both treatments almost doubled zinc absorption (Table 4) and would be expected to substantially improve the efficacy of zinc-fortified foods. This suggests that the phytase enzyme, with reported maximum activity at a pH of 2 and 5, successfully degraded most if not all of the PA during the digestion process in the stomach and intestinal tract. Adding phytase to cereal porridges immediately before consumption was also previously reported to approximately double iron absorption (15,16).

The amount of zinc absorption (FAZ) from cereal porridges in our studies as well as the increase with phytase treatment (~80%) are similar to those from earlier studies with radioisotopes (51,52). Sandström et al. (52) reported an FAZ value of 8.2% from a meal based on whole wheat (PA:Zn molar ratio of 8.6 compared with 9.1 in our study) and 13.2% from a meal based on refined wheat (PA:Zn molar ratio of 0.4). Similarly, López de Romana et al. (51) reported an FAZ value of 6.4% from an unfermented whole-wheat porridge (PA:Zn molar ratio of 12) and 13.8% from a yeast-fermented refined-wheat bread (PA:Zn molar ratio of 0.5). The meals were fortified with ZnCl2.

### Table 3

<table>
<thead>
<tr>
<th>Study (male)</th>
<th>Age</th>
<th>BMI</th>
<th>PZn</th>
<th>Plasma CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>10</td>
<td>22.6 ± 2.3</td>
<td>22.6 ± 1.7</td>
<td>88.5 ± 19.0</td>
</tr>
<tr>
<td>Study 2</td>
<td>12</td>
<td>23.2 ± 2.8</td>
<td>21.7 ± 1.9</td>
<td>96.0 ± 8.3</td>
</tr>
<tr>
<td>Study 3</td>
<td>13</td>
<td>22.3 ± 2.2</td>
<td>22.5 ± 2.0</td>
<td>89.3 ± 10.7</td>
</tr>
<tr>
<td>Study 4</td>
<td>11</td>
<td>23.5 ± 2.2</td>
<td>22.8 ± 2.0</td>
<td>72.7 ± 9.9</td>
</tr>
<tr>
<td>Study 5</td>
<td>16</td>
<td>23.5 ± 2.4</td>
<td>21.9 ± 1.9</td>
<td>71.1 ± 8.8</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs unless otherwise indicated. CRP, C-reactive protein; PZn, plasma zinc.

2 None of the baseline characteristics of participants in studies 2 and 3 (for which fractional absorptions of zinc between study comparisons were determined) differed (P > 0.05, unpaired t tests).

### Composition of the test meals

The basic composition of the maize and sorghum test meals in studies 1–5 is reported in Table 2. In study 1, 190 FTUs of phytase were added to the maize meal just before consumption. Test meal C in study 1 and all of the test meals in study 4 were completely dephytinized during meal preparation, and the PA concentrations were below the limit of detection (<8 mg/mg). In studies 2 and 3, 12.5 mg EDTA was added as Na2EDTA to the respective maize meals B to reach the EDTA:Zn molar ratios of 1:1, and 25 mg EDTA was added to the respective test meals C to reach the EDTA:Zn molar ratios of 2:1. In studies 4 and 5, 12.5 mg EDTA was added to the respective brown sorghum test meals C to reach an EDTA:Zn molar ratio of 1:1.
TABLE 4  Fractional absorption of zinc from maize and sorghum porridges consumed by young adults1

<table>
<thead>
<tr>
<th>Meal</th>
<th>ZnSO4</th>
<th>MAIZE PORRIDGE</th>
<th>Absorption</th>
<th>MAIZE PORRIDGE</th>
<th>Absorption</th>
<th>MAIZE PORRIDGE</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Maize porridge</td>
<td>8.7 (6.7, 11.1)</td>
<td>Maize porridge, dephytinized</td>
<td>16.5 (13.5, 20.2)</td>
<td>Maize porridge + phytase</td>
<td>15.8 (13.5, 18.4)</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>Maize porridge</td>
<td>10.0 (7.5, 13.2)</td>
<td>Maize porridge + EDTA (1:1)</td>
<td>13.2 (9.5, 18.3)</td>
<td>Maize porridge + EDTA (2:1)</td>
<td>9.0 (7.3, 11.0)</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>Maize porridge</td>
<td>7.34 (5.4, 10.0)</td>
<td>Maize porridge + EDTA (1:1)</td>
<td>9.6 (6.4, 14.4)</td>
<td>Maize porridge + EDTA (2:1)</td>
<td>5.9 (3.7, 9.4)</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>White sorghum porridge</td>
<td>15.0 (9.7, 23.4)</td>
<td>Brown sorghum porridge, dephytinized</td>
<td>16.6 (13.0, 21.2)</td>
<td>Brown sorghum porridge, dephytinized + EDTA (1:1)</td>
<td>14.3 (10.6, 19.4)</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>White sorghum porridge</td>
<td>10.7 (8.0, 14.3)</td>
<td>Brown sorghum porridge</td>
<td>8.4 (6.5, 10.9)</td>
<td>Brown sorghum porridge + EDTA (1:1)</td>
<td>12.7 (9.3, 17.3)</td>
</tr>
</tbody>
</table>

Values are geometric means (95% CIs) unless otherwise indicated. Values in a row with superscripts without a common letter differ, *P* < 0.05. ZnO, zinc oxide; ZnSO4, zinc sulfate.

Administered in the form of Na2EDTA.

Test meals A from studies 2 and 3 were compared by using unpaired *t* tests, and the difference was significant (*t* *P* = 0.02).

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acceptable and similarly priced, it would be the preferred zinc fortification compound. This result, however, is different from previous studies that reported no difference between absorption from the 2 compounds by using either radio- (51) or stable isotopes (23,59). However, in all of the 3 previous studies the fortificants were added to the meal before or during the cooking procedure, possibly facilitating the ZnO dissolution when compared with the addition of the fortificant just before consumption as in our studies. Another possibility is that the composition of the meal influences the dissolution of the more insoluble compound in the gastric juices, thus changing its absorption relative to the more soluble compound. This has been reported for ferric pyrophosphate whose absorption relative to ferrous sulfate was reported to vary between 21% and 75%, depending on the meal composition (60).

Our study is the first to our knowledge to investigate the effect of cereal polyphenols on human zinc absorption. Previous studies in rats and in vitro reported contradictory results on the effect of tea, wine, and coffee polyphenols on zinc balance and zinc uptake (29–39). In 1 human study, a small, but not statistically significant decrease in zinc balance occurred when tea was consumed together with the meal (28). The results from our study 4, in which dephytinized brown and white sorghum meals were administered, indicate that there is no independent effect of polyphenols from brown sorghum on FAZ. However, when the similar meals containing their native PA contents were served in study 5, FAZ from the high-polyphenol brown sorghum was significantly lower than that from the white sorghum with low polyphenol contents, even though the PA contents were comparable or even higher in the white sorghum. The reason for this finding is unclear. It is possible that the lower absorption from brown sorghum was caused by an interaction of PA with polyphenols or with other sorghum components, or it could be due to the presence of other components in the brown sorghum that inhibit zinc absorption. Possible polyphenol-PA interactions have not been described in the literature, although previous in vitro studies reported the formation of zinc chelating protein-PA complexes (61). The Framida brown sorghum cultivar used in this study has a high protein content compared with other cultivars (62), and peptides or proteins from this variety may more strongly influence zinc binding in the presence of PA and polyphenols. With phytase treatment, however, these potential complexes seem to be destroyed, because FAZs from dephytinized meals with high or low polyphenol contents (study 4) are equal.

For the design of efficacious zinc-fortified foods, it is clear that the choice of the zinc compound is of less importance and that the fortificant can be selected on the basis of price and sensory properties. The fortification level can be defined by the needs for absorbed zinc and by the expected absorption. Our study confirms that PA is the major inhibitor of zinc absorption in cereal foods and suggests that, although cereal polyphenols per se do not influence zinc absorption, they may interact with PA and cause a small additional inhibition. Overcoming the negative effect of PA is key to optimizing absorption from cereal foods, and this can be achieved by the use of a phytase during food preparation or by adding the phytase to the cereal food at the time of consumption. If soluble zinc fortification compounds are used, the addition of EDTA may provide a useful additional amount of absorbed zinc.

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