Zinc absorption from a low–phytic acid maize

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ABSTRACT

Background: Phytic acid reduction in cereal grains has been accomplished with plant genetic techniques. These low–phytic acid grains provide a strategy for improving the mineral (eg, zinc) status in populations that are dependent on grains, including maize (Zea mays L.), as major dietary staples.

Objective: The objective was to compare the fractional absorption of zinc from polenta prepared from maize low in phytic acid with that prepared from a wild-type isohybrid maize (control) after short-term consumption by adults whose habitual diet is low in phytic acid.

Design: Healthy adults served as their own control subjects in a crossover design. All meals on 1 d consisted of polenta prepared from a low–phytic acid maize homozygous for the recessive low phytic acid 1–1 (lpa1-1). On the preceding or following day, all meals consisted of polenta prepared from a sibling isohybrid homozgyous wild-type maize with a “normal” phytic acid content. The low–phytic acid maize contained ~60% less phytic acid than did the wild-type maize. All test meals were extrinsically labeled with zinc stable-isotope tracers. The fractional absorption of zinc was determined on the basis of fecal enrichment.

Results: The molar ratios of phytic acid to zinc in the polenta prepared from lpa1-1 maize and the wild-type maize were 17:1 and 36:1, respectively. The corresponding fractional absorptions of zinc were 0.30 ± 0.13 and 0.17 ± 0.11, respectively (P < 0.005).


KEY WORDS Plant breeding, maize, phytic acid, zinc, ratio of phytic acid to zinc, zinc absorption

INTRODUCTION

Zinc deficiency in humans is recognized as a public health problem of global proportions (1, 2). Correction of zinc deficiency in young children has been effective in reducing major causes of morbidity and mortality (1) and in improving growth and development (3, 4). In many populations, zinc deficiency has been attributed to an impaired bioavailability of dietary zinc (5). Phytic acid (myo-inositol-1,2,3,4,5,6-hexa-kis-phosphate) is widely regarded as the principal dietary factor that impairs zinc bioavailability (5–9). Indeed, current World Health Organization calculations of dietary zinc requirements vary 3-fold depending on the phytic acid content of the diet (10). Phytic acid is the storage form of phosphorus in seeds (11) and typically represents ~1–2% of seed dry weight. Cereal grains, including maize (11), are among those foods of vegetable origin that have the highest content of phytic acid.

Hence, persons whose diets are dependent on maize as a major food staple appear likely to be at risk of zinc deficiency. In accord with this conclusion is the finding that zinc deficiency has been documented in both Guatemala (12, 13) and Malawi (6), 2 countries that depend on maize as the principle dietary staple. Maize contains amounts of total zinc and other minerals that are adequate for human nutritional needs, and zinc intake from a maize-based diet is likely to be adequate if bioavailability could be improved without concurrent reduction of minerals. Therefore, the breeding of low–phytic acid mutations (14–17) offers potential for improving mineral, including iron (18) and zinc, bioavailability from grain-based diets. Low–phytic acid cereal grains bred with the use of these mutants produce grain containing large reductions in phytic acid phosphorus but normal amounts of total phosphorus and other constituents and that are phenotypically similar to grain produced by normal, wild-type lines.

The goal of this phase 1 study was to determine the short-term effects of consuming low–phytic acid maize on zinc absorption. The hypothesis to be tested was that the fractional absorption of zinc is higher from meals prepared from low–phytic acid maize than from meals prepared from its matched wild-type hybrid with a “normal” phytic acid content.

SUBJECTS AND METHODS

Subjects

Three women and 2 men aged 24–39 y were enrolled in the study. These healthy volunteers, recruited from the Denver metropolitan area, had a mean (±SD) daily zinc intake of 9.2 ± 5.6 mg determined with the use of a 7-d diet record. Only one subject took...
a daily zinc supplement (30 mg/d), which was discontinued 10 d before the study. This study was approved by the Colorado Multiple Institutional Review Board of the University of Colorado Health Sciences Center. Signed consent was obtained from all participants according to the stringent rules of this university’s ethics committee.

**Study design and source of maize**

Healthy adult volunteers served as their own control subjects in a crossover design. Each subject received maize polenta as their only food for 2 d. On one of these days, the polenta was prepared from maize homozygous for the recessive low phytic acid 1-1 (lpa1-1; 17) and on the other day from a sibling isohybrid homozygous wild-type maize (control) with a “normal” phytic acid content. Both grains were developed by Pioneer Hi-Bred, Inc (Dupont Corp, Des Moines, IA), in cooperation with the US Department of Agriculture, Agricultural Research Service (14). The phenotypic appearance and palatability of these matched hybrids are similar. The total phosphorus content of both maize is similar, but the phytic acid phosphorous content of the *lpa1-1* seeds is 60% lower than that of the wild-type seeds. The polenta fed on both days was extrinsically labeled with an accurately weighed quantity of either $^{67}$Zn or $^{70}$Zn ($\approx 700 \mu g$).

**Preparation and administration of test meals**

In this initial pilot study, maize tortillas were not used because of the possibility that their high calcium content would augment the inhibitory effect of phytic acid on zinc absorption. Initial testing of the acceptability by volunteers of other maize-based meals indicated that polenta was preferred; therefore, polenta was used in all test meals. Subsequent experience during the study, however, was that polenta produced satiety at levels of consumption below those indicated by pilot testing. Hence, maize intake during the test days was lower than anticipated.

The meals for an entire day were prepared in one batch from 390 ± 80 g dry ground-maize kernels. The ground maize was added to boiling water and allowed to simmer. The mixture was stirred until stiffened. The maize was then transferred to a mold and baked in an oven at 150°C until firm. The meals for each day were weighed in specific containers before and after the polenta was consumed to obtain an accurate measure of intake. The total wet weights of polenta prepared each day were 1504 ± 219 and 1509 ± 227 g for the low-phytic acid and wild-type maize, respectively.

The subjects were randomly assigned into 2 groups: control maize on day 1 and low–phytic acid maize on day 2 or low–phytic acid maize on day 2, regardless of which maize was fed on these days. The mean quantity of isotope added was 0.72 ± 0.13 mg for both types of meals. The isotope accounted for 12.6% and 11.4% of the total zinc intake from the low–phytic acid and control meals, respectively.

**Sample collection**

**Feces**

Fecal samples were collected and analyzed with the use of a fecal enrichment method (19). Complete fecal samples were collected once before the test meals were administered to obtain a baseline measurement and then beginning as soon as the first labeled meal was consumed and continuing for a minimum of 8 d and until 8 samples were collected. Fecal samples were collected for a mean of 9 d (range: 8–11 d). Individual fecal samples were stored in plastic bags at −20°C until processed.

**Diet**

At the time each meal was fed, 3 (≈10 g each) aliquots of the polenta (≈30 g/meal, 90 g/d) were collected. These aliquots were used to measure the total zinc and phytic acid contents of the meals and to measure the isotope ratios to verify the homogeneity of mixing.

**Sample processing and analyses**

**Feces**

The fecal samples were homogenized with milli-Q water, and 2 accurately weighed aliquots (≈150 g each) were analyzed. These fecal aliquots were dried, wet and dry ashed in a muffle furnace, and then reconstituted in 6 mol HCl/L. The total zinc content was determined by atomic absorption spectrophotometry. Zinc was isolated by ion-exchange chromatography, and the isotope ratios were measured by fast atom bombardment mass spectrometry on a double focusing mass spectrometer (model VG7070E HF; Fisons-VG Analytic, Manchester, United Kingdom) equipped with an Ion Tech (London) atom gun (20). The precision for the entire technique, including sample preparation, had a CV of 1.5%.

**Diet**

The aliquots of polenta from each meal were dried, ashed, and reconstituted in 6 mol HCl/L before analysis of zinc by atomic absorption spectrophotometry. The wet and dry weights of the aliquots were recorded, and the isotope ratios in each aliquot were between the last meal on day 1 and the first meal on day 2 ranged from 11 to 13.5 h.
FIGURE 1. Comparison of the fractional absorption of zinc from polenta prepared with a low–phytic acid (lpa1-1) or a matched wild-type isohybrid (control) maize in individual subjects.

determined as described for the fecal samples. Aliquots of both types of maize kernels were analyzed to obtain zinc and phytic acid concentrations of the maize before cooking.

Phytic acid analysis

Phytic acid and other inositol phosphates were precipitated from acid extracts of polenta as ferric salts. These salts and individual samples were digested by wet ashing, and the phosphorous content was determined colorimetrically (21). Anion-exchange HPLC was used to directly measure phytic acid and related inositol phosphates after a modified version of the method described by Rounds and Nielson (22).

Data processing and statistical analysis

The data were analyzed by using paired-comparisons t tests. All results are presented as means ± SDs.

RESULTS

The analyses confirmed that the isotope tracers were mixed homogeneously with the polenta while cooking. The relative mean (±SD) quantity of isotope in 12 dietary aliquots taken from each subject was 2.8 ± 1.1%.

The mean intakes of the low–phytic acid and control polenta were 1127 ± 386 and 1189 ± 371 g wet wt/d, respectively; the corresponding zinc concentrations were 3.8 ± 0.22 and 4.2 ± 0.22 μg Zn/g wet wt polenta. The mean zinc intakes from the low–phytic acid and control polenta were 4.3 ± 1.5 and 5.0 ± 1.4 mg Zn/d, respectively. No correlation between the quantity of polenta consumed (P = 0.56) or total zinc intake (P = 0.66) and the fractional absorption of zinc was observed.

The phytic acid contents of the low–phytic acid and control polenta were 2.7 and 5.8 mg/g dry wt, respectively. The corresponding zinc concentrations were 15.4 ± 0.7 and 15.9 ± 1.0 μg Zn/g dried polenta. The resulting molar ratios of phytic acid to zinc were 17:1 and 36:1 for the low–phytic acid and the control polenta, respectively.

As shown in Figure 1, each subject had a consistently greater fractional absorption of zinc from the polenta prepared with the low–phytic acid maize than from the control maize. The mean fractional absorptions of zinc from the low–phytic acid and control maize were 0.30 ± 0.13 and 0.17 ± 0.11, respectively. The mean difference between the low–phytic acid and control maize was 0.13 ± 0.05 (P < 0.005). On average, the fractional absorption of zinc from polenta prepared from the low–phytic acid maize was 78% greater than that from the polenta prepared with the control maize.

DISCUSSION

The initial motivation for the isolation of lpa 1-1 mutants of maize was to improve the nutritional quality of maize as a food for humans. However, the initial motivation for commercial planting of low–phytic acid maize in the United States with these mutants concerns issues of phosphorous management in livestock production (14). Phosphate is primarily present as phytic acid in grain and in legume-based animal feeds and is therefore largely unavailable for absorption and utilization. In feeds prepared with low–phytic acid grains, a much larger fraction of total phosphate from grain or feed is available, absorbed, and utilized by animals. This negates the need for phosphate supplements and, more importantly, minimizes environmental phosphate pollution from animal waste. The potential for improving human mineral nutrition has, however, also been recognized from the outset and was previously shown in studies of iron absorption (18).

The literature reporting the effects of phytic acid on zinc absorption is to some extent conflicting. Some investigators have reported a threshold effect at a molar ratio of phytic acid to zinc of 15:1 or 20:1 (23). Others, especially in human studies, concluded that no threshold value of this ratio has to be reached before an inhibitory effect of phytic acid is detectable and that the magnitude of this effect is dose or ratio dependent (9, 24). We are not aware of any previous human studies of this effect, in which high molar ratios of phytic acid to zinc are reduced but yet the reduced ratio is still > 15:1. The results of this study indicate that even a relatively modest reduction in the ratio to a value that is still > 15:1 can have a notable effect on zinc bioavailability. In human nutrition, these low–phytic acid grains offer the potential for J) long-term studies of the effects of phytic acid reduction on mineral metabolism in populations or in persons whose habitual diet is high in phytic acid; 2) simultaneous improvements in nutrition with respect to multiple minerals, especially divalent cations. [Of special note are iron and zinc deficiencies, which pose a public health challenge of global proportions (1–3, 25)]; 3) sustainable community-wide interventions, which are equally applicable to families that grow their own food or who purchase grains or flour; and 4) improved bioavailability and utilization of essential minerals already present in grains or legumes, often in substantial quantities.

Improving zinc and iron nutrition in communities that depend on grains as staple food via the breeding of low–phytic acid grains has some additional advantages over other approaches, such as supplementation. This approach is advantageous because it uses existing technology that is based on traditional maize-breeding techniques. Transferring the lpa1-1 trait to locally produced maize is straightforward and inexpensive and, once accomplished, is heritable and sustainable.

Phytic acid in the diet may not only interfere with the absorption of exogenous dietary zinc but also with the reabsorption of the substantial quantities of endogenous zinc that are secreted into the gut lumen postprandially (26). This concern enhances the apparent importance of dietary phytic acid for zinc nutrition. In this phase 1 study, the relatively complex task of measuring the excretion of endogenous zinc was not undertaken. Rather, this
study measured short-term (1 d) fractional absorption of zinc. Moreover, the study was conducted in apparently healthy omnivorous subjects who were not previously challenged to adapt to a diet low in bioavailable zinc. Hence, the immediate inhibitory effect of a high–phytic acid (control) diet on the fractional absorption of zinc is likely to be maximal.

In conclusion, the daylong consumption of a maize diet with a typical high–phytic acid content and a high molar ratio of phytic acid to zinc is associated with a low fractional absorption of zinc. A low phytic acid intake and a low molar ratio of phytic acid to zinc is associated with a substantially and significantly greater fractional absorption of this micronutrient.

REFERENCES