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Zinc Absorption from *low phytic acid* Genotypes of Maize (*Zea mays* L.), Barley (*Hordeum vulgare* L.), and Rice (*Oryza sativa* L.) Assessed in a Suckling Rat Pup Model

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ABSTRACT: Dietary phytic acid is a major causative factor for low Zn bioavailability in many cereal- and legume-based diets. The bioavailability of Zn in seed of *low phytic acid* (*lpa*) variants of maize (*Zea mays* L.), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) was evaluated using a suckling rat pup model. Suckling rat pups (14 days old, $n = 6-8$/treatment) were fasted for 6 h and intubated with ^{65}Zn-radiolabeled suspensions prepared using seed produced by either wild-type (normal phytic acid) or *lpa* genotypes of each cereal. Test solutions were radiolabeled overnight (all genotypes) or immediately prior to intubation (barley genotypes). Pups were killed 6 h postintubation and tissues removed and counted in a gamma counter. Zn absorption was low from wild-type genotypes of maize (21, 33%) and rice (26%), and phytic acid reduction resulted in significantly higher Zn absorption, 47−52 and 35−52%, respectively. Zn absorption from wild-type barley incubated overnight was high (86−91%), and phytate reduction did not improve Zn absorption (84−90%), which is likely due to endogenous phytase activity. When the wild-type barley solutions were prepared immediately before intubation, Zn absorption was significantly lower (63, 78%) than from the *lpa* cultivars (92, 96%). Variation in seed or flour phenolic acid levels did not affect Zn absorption. Differences in seed Zn levels did not substantially affect Zn absorption. Thus, when phytic acid is abundant in a diet, it has a larger effect on Zn absorption than the level of Zn. Therefore, reducing the phytic acid content of staple cereal grains may contribute to enhancing Zn nutrition of populations consuming these staple foods.

KEYWORDS: phytic acid, *myo*-inositol hexaphosphate, zinc, phenolic acid, bioavailability, maize, barley, rice

INTRODUCTION

It is increasingly recognized that zinc deficiency or suboptimal zinc status is a worldwide nutritional problem, primarily affecting infants, children, and women of child-bearing age.[1] Although zinc intake from the diet often fails to meet recommendations in these populations, it is generally believed that a high intake of dietary components inhibiting the absorption of zinc is the major cause of impaired zinc status.[1,2] Phytic acid (*myo*-inositol hexaphosphate, $InsP_6$, or phytate) is known to have a strong inhibitory effect on the absorption of zinc.[3,4] The negatively charged phosphate groups on the phytic acid molecule can form strong chelates with divalent cations and, as humans have no phytase activity in the gastrointestinal tract, the phytate−metal complexes will pass the intestine unabsorbed and lead to lower net absorption.[3] Phytate is an integral part of cereals and legumes,[5,6] staple foods in many parts of the world. It has therefore been concluded that high phytate intake is a major causative factor for zinc deficiency in such populations.[1]

Various attempts have therefore been made to reduce the phytate content of foods. Fermentation, malting, soaking, and germination are some food preparation methods that can be used to reduce the phytate contents of foods, even in rural settings, and these approaches have been used successfully in various locations.[7−10] Treatment of animal feeds with food grade phytase, an enzyme that can cleave the phosphate groups of hexa- and pentaphosphate forms of phytic acid, is used extensively in animal husbandry,[11] but has been used only in some research trials in humans.[12,13] The acceptability, practicality, and sustainability of these methods in various areas may be limited.

Conventional plant-breeding techniques can be used to reduce the phytic acid content of seeds produced by crops.[6,14] By selecting *low phytic acid* (*lpa*) genotypes, the phytate content of cereals may be reduced considerably, resulting in enhanced zinc absorption.[15] This would provide a sustainable way to improve zinc nutrition of human populations. We therefore evaluated the effects of several novel low *lpa* genotypes of maize (*Zea mays* L.),[16] barley (*Hordeum vulgare* L.),[17,18] and rice (*Oryza sativa* L.)[19] on zinc absorption. Seeds produced by most of these genotypes have phytate concentrations that range from 30 to 60% of the wild-type (normal phytate) genotypes, but one, the barley M 955 genotype, has <10% of wild-type seed phytate.

For the studies reported here, we used a suckling rat pup model that we have previously developed for studies of zinc absorption.[20,21] This model is very sensitive to the phytate content of the diet. We have shown that infant formulas containing phytate have a negative effect on zinc absorption

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in suckling rat pups and that results from this model are correlated with results from the same diets in human adults.[22] We have also clearly demonstrated that this effect is due to phytate, as addition of sodium hexaphosphate to the low-phytate formula lowered zinc absorption to the same extent as phytate naturally present in soy formula.[23] Furthermore, increasing the level of phytate added resulted in a further decrease in zinc absorption. Thus, this is a quick and sensitive assay to screen multiple samples from different genotypes/cultivars containing various concentrations of phytate.

Use here of the suckling rat put model allowed the first side-by-side comparison of the nutritional value of normal-phytate and low-phytate variants of three different cereal species, and the studies reported here represent the first evaluation of the nutritional value of a low-phytate rice. In addition to phytate, other dietary ligands may affect zinc absorption, including organic acids such as ascorbic acid, amino acids, and phenolic acids.[6,24,25] Using the Caco-2 cell model, Sreenivasulu et al.[25] demonstrated that phenolic acids can postively affect zinc absorption. Because seeds can be a rich but variable source of phenolic acids, these were quantitated in cereal samples studied here, and the relationship or lack thereof of phenolic acid content and zinc absorption was also evaluated.

MATERIALS AND METHODS

Plant Genotypes and Grain Sources. Four barley *lpa* genotypes and one wild-type (WT) control were included in this study. All five barley lines represent sibling, near-isogenic lines isolated from the cultivar 'Harrington'. The four barley *lpa* genotypes were homozygous for the following recessive alleles at one of four *lpa* loci: *lpa*1-1, formerly referred to as M422; *lpa*2-1, formerly referred to as M1070; *lpa*3-1, formerly referred to as M635; and M955. The WT barley control was homozygous for the nonmutant alleles at all of these loci. Barley seed used in this study was produced at two locations; seeds of the WT control and *lpa*1-1 were produced in Tetonia, ID, and seeds of four genotypes, WT, *lpa*2-1, *lpa*3-1, and M955, were produced in Aberdeen, ID. Two maize *lpa* genotypes, *lpa*1-1 and *lpa*2-1, and two WT controls were included in the study. Maize *lpa*1-1 and *lpa*2-1 were originally isolated in a flint-maize-like synthetic population referred to as "Early ACR".[16] Seeds of these genotypes and a sibling WT flint line, representing a set of three near-isogenic lines derived from Early ACR, were produced in Chile. In addition, an Early ACR source of the maize *lpa*1-1 allele was used in breeding sibling, near-isogenic dent corn inbred lines that were either homozygous *lpa*1-1 or homozygous WT, and these were in turn used to produce either WT or *lpa*1-1 dent maize isohybrids.[26] Seed was produced by these hybrids near Johnston, IA. The rice *lpa*1-1 was originally identified in the M2-2045 line obtained from the cultivar 'Kaybonnet'.[19] Four progeny lines homozygous for rice *lpa*1-1, 2045-1-1, 2045-1-2, 2045-2-1, and 2045-2-2, and 'Kaybonnet' as WT control (produced near Stuttgart, AR), were tested.

Test Diets. Sixteen different batches of cereal grains were tested: 6 of barley, 5 of maize, and 5 of rice (Table 1). To use materials similar to those usually consumed by humans, the whole grain cereals were processed in different ways to remove hulls to commonly used levels. Whole rice, barley, and maize grains were premilled using a Retsch mill (0.5 mesh). Hull and partially hull-free fractions were manually separated using a no. 40 screen. The partially hull-free fraction was then milled again using a Wiley mill (60 mesh for maize and rice; no. 40 screen for barley), and the final hull and hull-free fractions were collected. Fractions were weighed and recorded.

Suckling Rat Pup Assay. Lactating Sprague−Dawley rats with 10-day-old pups (12 per dam) were purchased from a commercial breeder (Simonsen, Gilroy, CA). Following acclimatization, at 14 days of age, the pups were separated from their dams and fasted for 6 h. The diets were liquid form and consisted of a 5% w/v homogenate of the cultivar/genotype in 0.9% saline. In experiment 1, diets were prepared and radiolabeled overnight (∼16 h) at 4 °C, and in experiment 2, diets were prepared and radiolabeled ∼1 h prior to intubation. Pups (n = 6−8/diet and treatment) were intubated with 0.5 mL of radiolabeled diet (0.1 μCi of ^{65}Zn/pup) via a ball-point needle into the stomach and killed 6 h after intubation. Stomach, small intestine, cecum/colon, kidney, and liver were removed. The small intestines were perfused with ice-cold saline. Tissues, perfusate, and remaining carcass were counted separately in a gamma counter (Beckman 3600, Beckman, Fullerton, CA). Tissue and whole body absorption were calculated as percentage of administered dose. Infant formula diet was used as internal control. The protocol was approved by the Animal Use Committee at the University of California at Davis.

Phosphorus and Phytic Acid Phosphorus Assay. Total P, phytic acid P, and inorganic P of the whole grain and milled products were measured using methods as described.[16,17] Briefly, samples of mature grain or milled products were dried for 48 h at 60 °C. These were then milled to pass through a 20 mm screen and stored in a desiccator until analysis. Total P was determined following wet-ashing of aliquots of tissue (150 mg) and colorimetric assay of digest P.[27] Inorganic P was determined colorimetrically following extraction of tissue samples (0.5 g) in 12.5% (w/v) TCA/25 mM $MgCl_2$. The ferric precipitation method was used to determine phytate P.[17] Aliquots of tissue (0.5 g) were extracted in 0.4 M HCl/0.7 M Na_2SO_4. Phytic acid P was then obtained as a ferric precipitate, wet-ashed, and assayed for P as in the total P analysis. All P-containing fractions are expressed as their P (atomic weight 31) content to facilitate comparisons. Phytic acid P can be converted to units of phytic acid (inositol hexaphosphate, MW 660) by multiplying by the conversion factor 3.548.

Zinc Analysis. Zinc concentrations were determined by the Analytical Sciences Laboratory, University of Idaho, Moscow, ID, using a Perkin-Elmer Optima 3200 inductively coupled plasma-optical emission spectrometer (ICP-OES) to quantify constituents in an aqueous solution following nitric acid digestion of the samples. Standard quality control measures, including blanks, check standards, reference materials, and duplicates, were used for all analyses.

Phenolic Acid Analysis. "Free", "bound", and "total" phenolic acids were determined in whole seeds and dehulled seeds using a modification[28] of the method as originally described.[29] Samples of seeds were dehulled by removing the seed coat manually either using dry seed (rice) or following soaking overnight (4 °C) in the minimal amount of ddH_2O necessary to soften the seed coat. Seeds were subsequently dried (60 °C, 48 h) and milled (to pass a 20 mesh). Aliquots (40 mg) of milled flour were extracted in 2.0 mL of 80% methanol/1% HCl (16 h with stirring, 4 °C). Following centrifugation (3000g, 10 min, 20 °C), supernatants were decanted and assayed for "free phenolic acids" using a modification[30] of the Folin−Ciocalteu colorimetric assay as originally described.[31] Extract phenolic acid was determined via use of a gallic acid standard curve (0.0−80 nM gallic acid) and expressed in units of "gallic acid equivalents" (GAE). To determine "bound phenolic acids", precipitates obtained from the 80% methanol/1% HCl extraction were resuspended in 2.0 mL of 2 M NaOH and extracted with stirring at 8 °C for 2 h. Following centrifugation (3000g, 10 min, 20 °C), 0.5 mL aliquots of supernatant were neutralized with 0.175 mL of 6 M HCl and assayed for "bound phenolic acids" using the Folin−Ciocalteu colorimetric assay.[30] Values for total phenolic acids were obtained via summing "free" and "bound phenolic acid" values.

Statistical Analysis. One-way ANOVA using the General Linear Models (GLM) program of SAS (Statistical Analysis Software Inc., Cary, NC) was performed to test significant differences among normal and

Table 1. Seed Phosphorus and Zinc (±SD) in Barley, Maize, and Rice *low phytic acid* Genotypes and Zinc Absorption (±SD) As Assayed by the Suckling Rat Pup Model[a]

species	genotype and line or hybrid[b]	field site	seed total P (mg g^{-1})	seed phytic acid P (mg g^{-1})	seed inorganic P (mg g^{-1})	seed zinc (μg g^{-1})	phytic acid/Zn molar ratio	rat pup zinc absorption[c] (%)
barley	WT line	Tetonia, ID	3.47 ± 0.25	2.40 ± 0.04	0.36 ± 0.02	19.0 ± 1.4	44	85.6 ± 4.64a
barley	*lpa*1-1 line	Tetonia, ID	3.47 ± 0.13	1.40 ± 0.03	1.29 ± 0.04	23.5 ± 2.1	21	90.4 ± 2.62b
barley	WT line	Aberdeen, ID	3.76 ± 0.09	2.39 ± 0.04	0.38 ± 0.02	30.0 ± 2.8	28	90.5 ± 3.37b
barley	*lpa*2-1 line	Aberdeen, ID	4.35 ± 0.18	1.41 ± 0.02	1.65 ± 0.06	36.5 ± 4.9	14	83.5 ± 6.25a
barley	*lpa*3-1 line	Aberdeen, ID	3.85 ± 0.07	0.71 ± 0.02	1.94 ± 0.06	30.0 ± 1.4	8	84.4 ± 5.35a
barley	M955 line	Aberdeen, ID	4.40 ± 0.01	0.06 ± 0.01	2.95 ± 0.19	38.0 ± 4.2	0.6	85.5 ± 3.70a
maize	WT flint-like line	Chile	3.15 ± 0.03	2.37 ± 0.11	0.21 ± 0.01	35.5 ± 4.9	24	21.3 ± 7.12a
maize	*lpa*1-1 flint-like line	Chile	3.88 ± 0.31	1.11 ± 0.05	2.13 ± 0.06	48.0 ± 8.5	8	52.1 ± 16.73b
maize	*lpa*2-1 flint-like line	Chile	4.07 ± 0.04	2.62 ± 0.11	0.51 ± 0.01	40.0 ± 8.5	23	28.8 ± 7.34a
maize	WT dent hybrid	Iowa	2.42 ± 0.20	1.96 ± 0.19	0.16 ± 0.01	17.0 ± 1.4	40	33.2 ± 10.79a
maize	*lpa*1-1 dent hybrid	Iowa	2.89 ± 0.21	0.92 ± 0.12	1.53 ± 0.10	17.5 ± 0.7	19	47.2 ± 9.73b
rice	WT line	Arkansas	3.08 ± 0.01	2.17 ± 0.02	0.09 ± 0.01	24.7 ± 2.3	31	26.1 ± 5.90a
rice	*lpa*1-1 2045-1-1 line	Arkansas	3.14 ± 0.03	1.25 ± 0.08	1.01 ± 0.02	27.0 ± 2.6	16	49.1 ± 14.98b
rice	*lpa*1-1 2045-1-2 line	Arkansas	2.80 ± 0.04	1.29 ± 0.02	0.90 ± 0.01	26.3 ± 1.7	17	34.6 ± 14.04b
rice	*lpa* 1-1 2045-9-1 line	Arkansas	2.95 ± 0.15	1.24 ± 0.01	0.99 ± 0.04	27.5 ± 1.3	16	41.6 ± 10.88b
rice	*lpa*1-1 2045-9-2 line	Arkansas	2.98 ± 0.11	1.26 ± 0.01	0.95 ± 0.01	25.3 ± 2.1	17	51.9 ± 23.50b

[a] All fractions are expressed as their phosphorus (P, atomic weight 31) content on a dry weight basis. Data represent the mean of two samples for each seed lot. Liquid test diets were incubated ~16 h prior to intubation. [b] WT, wild type. [c] For each species of grain, means followed by a different letter are significantly different (P = 0.05).

genetically modified materials for each type of cereal. Chemical analysis of grain samples was conducted in duplicate unless otherwise indicated.

RESULTS

With one possible exception (maize *lpa*2-1, see below), the seed-derived flours evaluated in this study had total phosphorus, phytic acid phosphorus, and inorganic phosphorus levels similar to those reported in previous studies of these genotypes.[6,14,16,17,19,26] The total phytate phosphorus of dehulled WT maize, rice, and barley was very similar, about 2.0−2.4 mg g^{-1}, which corresponds to from 7.1 to 8.5 mg of phytate g^{-1} (Table 1). WT cultivars contained about 60−80% of total phosphorus as phytate phosphorus, with maize containing 75 and 81%, rice 70%, and barley 64 and 69%, respectively. With the exception of maize *lpa*2-1, in *lpa* genotypes phytate phosphorus represented a considerably smaller fraction of seed total phosphorus than in WT lines: 29 and 32% in maize *lpa*1-1, 40−46% in rice *lpa*1-1, and 1.0−40% in barley *lpa* genotypes, respectively. An exception is maize *lpa*2-1. In this case the percent of seed total phosphorus found as phytic acid does appear reduced as compared with WT (64% compared with 75%), but, because seed total phosphorus was high in this sample (4.07 mg/g as compared with 3.15 mg g^{-1} for WT), the absolute level of phytate phosphorus is higher than that found in the WT sample. For all genotypes other than maize *lpa*2-1, the levels of phytate phosphorus correspond to a decrease in phytate content of 61% in maize, 42% in rice, and from 41% to >95% in barley. When the phytate content was decreased, as in the *lpa* genotypes, inorganic phosphorus increased so that total phosphorus was largely unaffected. Whereas zinc levels ranged from a low of 17 μg g^{-1} in WT dent maize to 48 μg g^{-1} in *lpa*1-1 flint maize, they were similar within each group of lines from a given production site. Therefore, within each production site, phytic acid varied greatly but zinc levels were similar.

As assessed in suckling rat pups following overnight incubation, zinc absorption was low from the WT cultivars of maize, 21.3 and 33.2%, and from rice, 26.1%, whereas it was considerably higher from the WT cultivars of barley, 85.6 and 90.5% (Table 1). The *lpa* genotypes of maize resulted in significantly higher zinc absorption than from the WT, 47.2 and 52.1%, as did the rice *lpa* genotypes, 41.6, 49.1, and 51.9%, respectively. For those animals intubated with maize or rice diets, zinc absorption was highly and significantly correlated with both dietary phytic acid and dietary phytic acid/zinc molar ratio; however, zinc absorption was more highly correlated with dietary phytic acid (r = −0.85, $P > r$ = 0.0012) than dietary phytic acid/zinc molar ratio (r = −0.65, $P > r$ = 0.04). Even though there were relatively large differences in grain zinc levels, zinc absorption was not correlated with seed zinc (r = −0.008, $P > r$ = 0.98).

In the first experiment, diets prepared with barley *lpa* genotypes resulted in high zinc absorption, 83.5−90.4%, which was similar to that found for the wild-type cultivars. This possibly could be due to considerably higher endogenous phytase activity in barley homogenates versus those of maize or rice. For example, barely grain products have been reported to have endogenous phytase levels of 500−1000 U kg^{-1}, whereas whole grain or bran fractions from maize or rice contained from 15 to 45 U kg^{-1}.[32,33] To minimize the potential effect of endogenous phytase activity in barley, another experiment was conducted in which the solutions were prepared either immediately (~1 h) or 16 h prior to the intubation of the rat pups (Figure 1). Zinc absorption from solutions of the two WT cultivars of barley prepared 1 h before intubation was 63 and 78%, respectively, whereas it was 95 and 91% when they were prepared 16 h beforehand. Zinc absorption from the *lpa* genotypes, however,

was also high from the freshly prepared solutions, 91 and 96%, respectively, which was similar to what was found for the solutions prepared 16 h before intubation (94 and 94%, respectively).

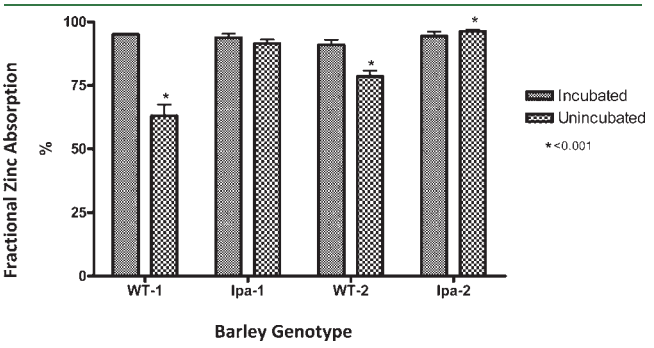

Figure 1. Fractional zinc absorption by rat pups from either wild-type (WT) or *low phytic acid* (*lpa*) barley diets prepared either immediately ("unincubated") or 16 h ("incubated") before intubation. The WT-1 and *lpa*1 barleys were those produced in Tetonia, ID, and contained 2.40 ± 0.04 and 1.40 ± 0.03 mg of phytic acid phosphorus g^{-1}, respectively. The WT-2 and *lpa*2 barley were those produced in Aberdeen, ID, and contained 2.39 ± 0.04 and 1.41 ± 0.02 mg of phytic acid phosphorus g^{-1}, respectively.

Analyses of both whole seed and dehulled seed flours for phenolic acid fractions revealed statistically significant differences both between species and among genotypes/lines/hybrids within species, but the greatest differences by far were between species (Table 2). This is illustrated in part via the analysis of variance (Table 2, bottom): the magnitude of the *F* values for "species" as a source of variation was far greater than those for lines within species (line (species)). These differences between species were largely in the free phenolic acid fraction of dehulled flours, and the most dramatic difference was between the free phenolic acid fraction in dehulled rice flour versus the free phenolic acid fraction in dehulled flours of the other species. Dehulled rice flour had 5−10-fold lower free phenolic acid (species mean = 0.21 mg of GAE g^{-1}) as compared with barley (species mean = 2.57 mg of GAE g^{-1}) or maize (means were 2.02 mg of GAE g^{-1} for flint-like maize lines and 1.35 mg of GAE gm^{1} for the dent hybrids). Large differences in bound phenolic acids across species were not observed, and the overall differences in total phenolic acids between species were largely due to the observed differences in free phenolic acids. In two cases individual lines or genotypes within a species differed substantially from the other lines or WT. These were barley *lpa*2-1, which had 40−60% higher bound phenolic acid in its dehulled flour than did its WT control or sibling lines, and the dehulled flint-like

Table 2. Free, Bound, and Total Phenolic Acid in Flours Obtained from Whole Seed and Dehulled Seed Fractions Prepared from Seed Batches Used in Study[a]

			mg of GAE g^{-1} ± SD					
			free phenolic acid		bound phenolic acid		total phenolic acid	
species	genotype and line or hybrid	field site	whole seed	dehulled	whole seed	dehulled	whole seed	dehulled
barley	WT line	Tetonia, ID	2.56[b]	2.52[b]	4.23[b]	3.11[b]	6.79[b]	5.63[b]
barley	*lpa*1-1 line	Tetonia, ID	2.70 ± 0.02	2.68 ± 0.05	4.54 ± 0.10	3.25 ± 0.27	7.24 ± 0.08	5.93 ± 0.32
barley	WT line	Aberdeen, ID	2.60 ± 0.05	2.50 ± 0.02	4.25 ± 0.57	2.94 ± 0.21	6.85 ± 0.52	5.44 ± 0.18
barley	*lpa*2-1 line	Aberdeen, ID	2.79 ± 0.07	2.79 ± 0.01	3.52 ± 0.38	4.70 ± 0.42	6.30 ± 0.31	7.49 ± 0.42
barley	*lpa*3-1 line	Aberdeen, ID	2.80 ± 0.22	2.38 ± 0.01	4.56 ± 0.44	3.16 ± 0.12	7.36 ± 0.22	5.54 ± 0.13
barley	M955 line	Aberdeen, ID	2.66 ± 0.07	2.52 ± 0.03	5.14 ± 0.29	3.44 ± 0.04	7.80 ± 0.36	5.97 ± 0.01
maize	WT flint-like line	Chile	2.11 ± 0.25	1.91 ± 0.09	5.43 ± 0.38	2.67 ± 0.36	7.55 ± 0.14	4.58 ± 0.45
maize	*lpa*1-1 flint-like line	Chile	2.30 ± 0.15	2.15 ± 0.13	5.26 ± 0.10	3.41 ± 0.09	7.56 ± 0.25	5.55 ± 0.05
maize	*lpa*2-1 flint-like line	Chile	2.29 ± 0.01	1.99 ± 0.06	4.53 ± 0.59	2.69 ± 0.45	6.82 ± 0.59	4.68 ± 0.39
maize	WT dent hybrid	Iowa	1.78 ± 0.23	1.32 ± 0.01	4.54 ± 0.07	1.89 ± 0.08	6.32 ± 0.16	3.21 ± 0.08
maize	*lpa*1-1 dent hybrid	Iowa	2.33 ± 0.41	1.38 ± 0.01	4.99 ± 0.30	2.07 ± 0.42	7.32 ± 0.10	3.44 ± 0.42
rice	WT line	Arkansas	0.77 ± 0.11	0.28 ± 0.12	5.61 ± 0.22	2.92 ± 0.17	6.39 ± 0.10	3.19 ± 0.06
rice	*lpa*1-1 2045-1-1 line	Arkansas	0.88 ± 0.01	0.20 ± 0.14	5.57 ± 0.11	2.51 ± 0.41	6.45 ± 0.12	2.71 ± 0.55
rice	*lpa*1-1 2045-1-2 line	Arkansas	0.76 ± 0.23	0.09 ± 0.06	5.43 ± 0.26	3.22 ± 0.21	6.19 ± 0.49	3.31 ± 0.27
rice	*lpa*1-1 2045-9-1 line	Arkansas	1.21 ± 0.13	0.10[b]	5.79 ± 0.61	1.23[b]	7.00 ± 0.48	1.33[b]
rice	*lpa*1-1 2045-9-2 line	Arkansas	0.84 ± 0.11	0.36 ± 0.19	5.43 ± 0.60	3.10 ± 0.21	6.27 ± 0.50	3.46 ± 0.40
			analysis of variance					
source of variation		df	*F* value and significance[c]					
species		2	289.6 ***	1802.4 ***	24.6 ***	30.9 ***	11.8 ***	237.3 ***
line (species)		13	1.9 ns	14.2**	2.3 ns	9.7 ***	3.8 **	12.8 ***

[a] Meals evaluated using the suckling rat pup model were prepared using only dehulled seed. Data are the mean of duplicate assays unless otherwise indicated, and phenolic acids are expressed in units of gallic acid equivalents (GAE). [b] Data for only one sample were obtained. [c] Significance, defined as the probability (*P*) of a greater *F*: ns, not significant, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

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maize *lpa*2-1, which had 27% more bound phenolic acid than did its WT control of sibling line. However, none of the observed variation in flour phenolic acid fractions, whether between species or within a given species, was correlated with rat pup zinc absorption. The correlations between rat pup zinc absorption (Table 1) and free phenolic acid, bound phenolic acid, and total phenolic acid in the dehulled flours (Table 2) were $r = -0.177$, $r = 0.047$, and $r = -0.103$, respectively, and none were statistically significant.

DISCUSSION

These results clearly indicate an inverse relationship between grain-derived dietary phytic acid and zinc absorption in the suckling rat pup model. Because the different genotypes within each species (or in the case of maize within either the dent or flint-like groups), represent near-isogenic lines that differ only in their alleles at a given *lpa* gene, the observed differences in rat pup zinc absorption can be attributed to these crop plant single-gene differences that affect seed phytic acid. This illustrates the importance of phytic acid to zinc bioavailability but also clearly illustrates that a single-gene allelic difference in a food crop can nearly double fractional zinc absorption. Whereas differences in grain characteristics between species, such as the high endogenous phytase levels in barley, clearly are important to the impact of phytate on P and mineral nutrition, the studies reported here demonstrate a negative relationship between seed phytate and zinc absorption in all three crop species tested. The efficacy of the rat pup assay used here makes possible the simultaneous, side-by-side evaluation, in an animal model, of several grain samples from multiple crop species.

Of the many other constituents in flours or foods that can affect zinc absorption, one class of ligands, the phenolic acids, can vary greatly in seed-derived foods. Using the Caco-2 cell model, Sreenivasulu et al.[25] compared zinc absorption from three flours: (1) "high-extraction" wheat flour, where relatively little of the outer bran fraction is removed, resulting in relatively high zinc (45.8 $\mu g\ g^{-1}$), phytic acid (5.81 mg g^{-1}), and phenolic acid (0.12 mg of catechin units g^{-1}); (2) "low-extraction" wheat flour, where more of the bran fraction is removed, resulting in reduced zinc (12.6 $\mu g\ g^{-1}$) and phytic acid (1.09 mg g^{-1}) accompanied by relatively low phenolic acid (0.03 mg of catechin units g^{-1}); (3) milled white rice, which contains relatively low zinc (13 $\mu g\ g^{-1}$) and phytic acid (1.60 mg g^{-1}) but which had intermediate levels of phenolic acid (0.08 mg of catechin units g^{-1}). The fractional uptake of zinc (expressed as a percent of control) was highest from white rice (59.6%), intermediate from low-extraction wheat flour (40.4%), and lowest from high-extraction wheat flour (21.9%). The authors observed that whereas phytic acid probably played a major role in determining zinc uptake, the marginally greater uptake of zinc from rice as compared to low-extraction wheat flour may be due to its relatively higher phenolic acid content. Supplementation with high levels of tannic acid (to achieve a 1:50 molar ratio of zinc to tannic acid) resulted in substantial increases in zinc absorption (25−50%), but only in the case of milled rice and low-extraction wheat flour. Similarly high levels of tannic acid supplementation had little positive effect on zinc absorption in the high-phytate, high-extraction wheat flour. The phenolic acid assay used in ref 25 most closely parallels the "free phenolic acid" assay used here. Therefore, comparison of the results in the present study with those of Sreenivasulu et al.[25] indicates that the levels of phenolic acid in milled cereal grains and the differences that may be observed between different species or products probably will have relatively little impact on zinc absorption as compared with that of phytic acid.

The present study represents the first evaluation of the nutritional value of an *lpa* rice in any model system, animal or in vitro. The negative relationship between grain-derived phytate and zinc absorption/utilization had been observed in previous studies, in various animal models or with human volunteers, utilizing various subsets of the maize and barley isolines studied here. Two studies with chicks documented enhanced measures of zinc utilization following consumption of *lpa* isolines as compared with WT isolines of maize and barley. Jang et al.[34] compared P and mineral availability and utilization in chicks fed diets prepared with the WT and *lpa*1-1 dent maize isohybrids and the WT and *lpa*1-1 barley isolines and reported 45% higher tibia zinc in animals consuming the *lpa* lines as compared with the WT lines. They also reported 36−45% higher P, Ca, and Mg in animals consuming *lpa* diets as compared with WT diets. In a study that focused on zinc, Linares et al.[35] evaluated the WT and M955 barley isolines. They found that tibia and toe zinc levels were 46 and 25% higher in chicks consuming an M955-based diet as compared with WT and that this increase was equal to or greater than that observed in animals consuming the WT diet supplemented with 20 mg kg^{-1} zinc.

Two studies with human subjects (involving five and ten volunteers, respectively) found that there was an inverse and highly linear relationship between dietary phytate:zinc molar ratio and fractional zinc absorption from individual meals.[15,36] For example, when meals were prepared using the WT and *lpa*1-1 dent maize, they had phytate/zinc molar ratios of 36:1 and 17:1, respectively, and the corresponding fractional zinc absorptions were 0.17 ± 0.11 and 0.30 ± 0.13, respectively, an increase of 76%.[36]

The genotypes tested in the present study were dehulled prior to making the solutions for the zinc absorption studies. This is commonly done for these staple foods in most settings and was necessary to allow the solutions to pass through the intubation needle. We analyzed total phosphorus, phytate phosphorus, and inorganic phosphorus in the dehulled fractions and found that the phytate contents were similar in wild-type varieties of maize, rice, and barley (data not shown). This is probably due to the fact that the hull contains essentially none of the phytate found in the whole grain. Most is contained within the germ (embryo and scutellum) and aleurone fractions.[6] This emphasizes that although maize is usually recognized as a "high-phytate" diet, and a concern for populations having a maize-based diet,[37] whole-grain rice is also high in phytate. As the iron and zinc contents of rice are relatively low,[38] the combination with high-phytate content will result in very low amounts of iron and zinc being available from rice. In a study on human volunteers, we previously found that fractional iron absorption was 48% greater from a meal prepared with *lpa*1-1 flint-like maize as compared with a meal prepared form the WT flint-like maize isoline.[39] This result was paralleled in an evaluation of WT and *lpa*1-1 maize isolines using the in vitro Caco-2 cell assay for iron bioavailability, which also showed that the increase in relative iron bioavailability achieved by the genetic reduction in grain phytic acid was numerically equivalent to the increase achieved by supplementation with ascorbic acid, which is known to enhance iron bioavailability[6] (R. P. Glahn, USDA, Ithaca, NY, personal communication). Thus, the potential benefit of genetic reductions in cereal or legume seed phytate must be broadly viewed as enhancement of

mineral nutrition in a global sense, for example, including enhanced zinc, iron, calcium, magnesium, and phosphorus nutrition.[40]

The reduction in phytate in the *lpa* genotypes ranged from 40 to >95% and varied among maize, barley, and rice. The yield of lines homozygous for *lpa* alleles is variable and is often reduced as compared with wild-type lines.[6,26,41] In fact, the impact on plant growth and performance of a single, homozygous phytic acid pathway allele can range from nondetectable to lethal.[42] Phytic acid may function as an antioxidant in seeds and therefore may be critically important to seed viability.[43] This role may explain in part why genetic reductions in seed phytic acid sometimes affect seed germination, such as in the case of low-phytate soybean lines.[44] However, Spear and Fehr[45] demonstrated that selection for restored germination and stand establishment in these same soybean lines can be successful. Field studies have shown that the line with the largest reduction in seed phytic acid studied here, barley M955, also has the lowest yield as compared with other sibling lines and appears to be most susceptible to reduced yield as a result of drought stress.[41] This study[41] also indicated that the extent of yield reduction was correlated with increasing reduction in seed phytic acid. Whereas it is possible that lines having adequate yield with the type of large reduction in phytic acid observed in barley M955 (before incubation) might be possible to achieve via breeding for restored yield, biotechnology might also provide a means to achieve this end.[46] However, it is also obvious that the smaller scale reductions in seed phytate found in other low-phytate lines also led to a marked increase in zinc absorption in our model. In at least one case, barley *lpa*1-1, yield is similar to wild-type in both optimal and stressful production environments,[41] and this has led to the release of the first low-phytate cultivar.[14] Cultivars developed using barley *lpa*1-1 therefore represent a proof-of-principle that adequate-yielding, low-phytate crops can be developed if sufficient effort and the right genetics and breeding technologies are utilized. Ongoing research efforts are aimed at developing high-yielding, low-phytate types for most major crops.[47]

Any discussion of the role of dietary phytic acid in human nutrition and health, and any effort to engineer altered levels of phytic acid in food crops, must also take into consideration its potential heath-beneficial roles. Dietary phytic acid may function as an antioxidant and anticancer agent,[48−52] or as an inhibitor of renal stone formation.[53] Therefore, caution must be taken in breeding for the low-phytate trait in crops destined for human consumption. However, such considerations should include the fact that the negative impacts of dietary phytic acid are mostly important in infancy and youth and during child-bearing years in populations in the developing world that rely on cereal grains and legumes as staple foods, whereas the potentially positive roles probably are most important to aging in communities in developed countries. Thus, the issue of dietary phytic acid in human nutrition and heath, and the breeding of crop phytic acid levels, must be considered on a case-by-case basis. Also, consideration must be given to the role of feed phytic acid in animal agricultural production and to the results of numerous studies that evaluated the impact of phytic acid in animal feeds. Nearly all such studies have reported positive outcomes to reduced dietary phytic acid, and none have reported negative outcomes.[54] In addition, products made from nearly all crops are used in both animal feeds and human foods. Future discussions and strategies should take into account both the varying roles of dietary phytic acid in different human populations and life stages and the common use of most crops in both animal and human foods.

In conclusion, a reduction of the phytate content of maize, rice, and barley led to a significant increase in zinc absorption in our rat pup model. This indicates that the consumption of low-phytate cultivars is likely to have a positive effect on zinc status of populations consuming these cereals as major components of their diets. Long-term intervention studies are needed to verify this. Results from the first generation of such studies revealed no clear benefit to zinc nutrition resulting from the consumption of low-phytate maize.[55,56] There are at least two possible explanations for this lack of agreement in results in clinical versus field studies. First, it is possible that the extent of phytate reduction achieved was enough to positively affect zinc absorption from single meals but inadequate to result in long-term effects on zinc status. Second, it is possible that the lack of positive effects observed in these initial field studies is due to the study design or conditions in the field setting. These results also illustrate that in the case of "high-phytate" foods, zinc biofortification (elevating zinc levels via plant breeding or agronomic practices)[57,58] may not result in enhanced zinc nutritional status in populations dependent on cereal grains as staple foods, if not accompanied by a reduction of phytate content.

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