Phytate-Zinc Molar Ratio of Breakfast Cereals and Bioavailability of Zinc to Rats

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ABSTRACT

The concept of the phytate-zinc molar ratio as a predictor of zinc bioavailability to rats was tested with breakfast cereals. Seven ready-to-eat and two quick-cooking breakfast cereals with phytate-zinc molar ratios from 2 to 43 served as dietary sources of zinc. Growth and bone zinc responses of rats were bioassay criteria, and $ZnSO_4 \cdot 7H_2O$ was the reference zinc compound. Breakfast cereals with phytate-zinc molar ratios of 15 or less supported growth as well as the reference source did, but cereals with higher ratios depressed growth. The bone zinc response was slightly greater to the cereal with a phytate-zinc molar ratio of 3 than to the reference $ZnSO_4$, but the response to all other cereals, including a cereal with a phytate-zinc molar ratio of 2, was less than to the reference compound. The biological response of rats was not directly correlated with dietary fiber in the cereals. The phytate-zinc molar ratio was a major factor affecting bioavailability of zinc in breakfast cereals.

Phytate affects the bioavailability of dietary zinc (Davies and Nightingale 1975, Oberleas and Harland 1977, O'Dell and Savage 1960). The dietary zinc requirement for growth generally is higher if the protein source is a seed protein, which contains phytate, rather than an animal protein (O'Dell 1969). O'Dell et al (1972a) found that zinc in wheat was 38% as effective as zinc carbonate in supporting growth of rats. High dietary calcium accentuates the effect of phytate on bioavailability of zinc (Likuski and Forbes 1965, O'Dell 1969).

After identifying monoferric phytate in extracts of wheat bran (Morris and Ellis 1976), we began to study the nutritive value of wheat bran as a source of other trace inorganic nutrients, particularly zinc. We found that dephytinized wheat bran is equal to zinc sulfate in supporting biological response of rats, and we agreed with Davies et al (1977) that the fiber component is not a major factor in determining the bioavailability to rats of zinc in wheat bran.

Morris and Ellis (1980b) and Davies and Olpin (1979) determined that growth of rats is not adversely affected when dietary phytate-zinc molar ratios are about 12 or less and dietary zinc is near the minimum requirement for growth, 12 ppm (NAS 1978). In the present study, we tested the influence of the phytate-zinc molar ratio on the bioavailability of zinc in breakfast cereals.

As dietary zinc sources, we fed to rats commercially available breakfast cereals that provided products of similar composition but varied in phytate-zinc molar ratios. We tested the cereals with and without the extra calcium that would have been supplied by the consumption of milk with the cereal. The results generally agreed with our expectation based on published studies that used zinc sulfate and sodium phytate.

MATERIALS AND METHODS

Material

Breakfast cereals were purchased from a local grocery, ground in a Wiley mill to pass the 1-mm sieve and then incorporated into the test diets. Description and composition of the cereals is given in Table I. The cereals are listed in order of increasing magnitude of the phytate-zinc molar ratio and are identified by the ratio. Corn oil (Mazola) was purchased at a local grocery, dextrose (cerelose) from Capital Bakers Supply (Alexandria, VA), and egg white solids (type P-20) from Henningsen Foods, Inc. (White Plains, NY). Other diet ingredients were from Teklad Test Diets (ARS Sprague-Dawley, Madison, WI). Chemicals were reagent grade.

[1]Mention of a trade name or supplier is for information only and does not imply endorsement by the USDA to the exclusion of other suitable products or suppliers.

Rat Feeding Trials

Two hundred twenty male weanling rats of the Sprague-Dawley CD strain weighing between 40 and 50 g from Charles River Breeding Colonies (North Wilmington, MA) were allocated at random to the 22 dietary treatments. Animals were caged individually in suspended stainless steel cages placed in quarters with controlled environment. Food and distilled water were supplied ad libitum. After a four-week growth period rats were killed by carbon dioxide asphyxiation. Femurs were excised, cleaned of adhering tissue, and dried overnight in a vacuum oven at 65° C before analysis. Femurs were analyzed from the five rats of each dietary group that had a gain nearest the mean gain of that group.

Composition of the semipurified basal diet, in grams per kilogram, was: egg white solids, 200; dextrose, 675; mineral mix, 40 (Briggs and Williams 1963, with zinc salt omitted); non-nutritive fiber, 20; biotin mix (2 mg of biotin per 5 g of mix), 5; vitamin mix, 10; and corn oil, 50. Details of mineral and vitamin mixes were published by Morris and Ellis (1980b). The basal diet as formulated contained less than 1 μg of zinc per gram but met the recommended requirement for other nutrients (NAS 1978). Additions to the diets were at the expense of dextrose. Each cereal diet was formulated to contain 12 μg of zinc per gram from the cereal; required amounts of the respective cereals are given in Table II. The phytate-zinc molar ratio of each diet is identified here by the ratio of the cereal used as zinc source. Each cereal was tested with and without added calcium. Change in phytate-zinc molar ratio was precluded by addition of calcium as $CaHPO_4$, rather than as nonfat milk solids, in amounts proportional to the amount that would be supplied if milk were used in the amount per serving suggested from the nutrition information panel of each cereal. The amounts of added calcium for the respective cereals are also shown in Table II. The reference zinc compound was $ZnSO_4 \cdot 7H_2O$, fed at three dietary levels of zinc: 6, 9, and 12 μg/g. Table II also shows the amount of fiber contributed by the cereal, calculated from the analysis given in Table I and the amount of cereal in each diet.

Analytical Methods

Femur or diet ingredient was dry ashed, the ash dissolved in 1 *N* HCl, and the zinc determined by flame atomic absorption spectroscopy as previously described (Morris and Ellis 1980b). Phytate was determined as described by Ellis et al (1977). Fiber analysis was by the AACC neutral detergent fiber method (1977). All analyses reported are means of duplicate determinations. Data were treated by analysis of variance and Duncan's multiple range test.

RESULTS

The cereals are listed in Tables I and II in order of increasing phytate-zinc molar ratio, which ranged from 2.0 to 42.9. The phytate-zinc molar ratio was calculated from the results of analysis

TABLE I
Description and Analysis of Breakfast Cereals

Cereal Grain Component(s)[a]	Zinc Fortification Compound[a]	Analysis[b] Phytate (%)	Zinc μg/g	Fiber[c] (%)	Phytate-Zinc Molar Ratio
Corn flour, oat flour	ZnO	0.22	111	1.2	2.0
Rice, wheat gluten, defatted wheat germ	ZnO	0.39	151	0.8	2.6
Wheat bran	ZnO	2.85	193	25.9	14.6
Whole wheat	None	0.96	39.9	8.5	23.8
Oat flour, soy protein concentrate	None	0.95	33.9	2.8	27.7
Whole wheat	None	0.82	27.5	9.2	29.6
Oats	None	1.16	37.9	5.2	30.3
Oat flour, wheat starch	None	1.07	32.2	4.0	32.9
Wheat bran	None	3.40	78.6	27.6	42.9

[a] Information from ingredients lists on packages of cereals purchased at local grocery store.
[b] As purchased, moisture was 3.1, 2.7, 2.9, 10.0, 4.6, 6.6, 7.2, 4.8, and 3.5% for ratios 2.0–42.9, respectively.
[c] Neutral detergent fiber.

for phytate and zinc given in Table I. The data are for the actual lot of cereal incorporated into the test diets and do not represent a statistical sampling of the cereals available to consumers. Cereals with the two lowest phytate-zinc molar ratios contained less than 0.5% phytate and had ZnO fortification to give zinc concentrations greater than 100 μg/g. The bran-based cereals, 14.6 and 42.9, contained the highest levels of phytate, 2.85 and 3.4%, respectively. Cereal 14.6 was fortified with ZnO to a zinc level of 193 μg/g, which explained its low phytate-zinc molar ratio. Phytate level was about 1% in the two whole wheat cereals, 23.8 and 29.6. The ratios for these two cereals approximates the calculated ratio for whole wheat kernels reported by O'Dell et al (1972b). Oats was the major ingredient in cereals 27.7, 30.3, and 32.9. Their phytate content was about 1% and zinc content about 35 μg/g.

Cereals 2.0 and 2.6, with the lowest ratios, were lowest in neutral detergent fiber, about 1%, and the wheat bran cereals, 14.6 and 42.9, were the highest, about 24%. The other five cereals ranged from 3–9% neutral detergent fiber.

The four-week growth response (gain) of rats is shown in Fig. 1. Rats fed the basal diet gained very little, significantly less than rats fed any other diet. Gain did not differ significantly between diets with zinc of 12 and 9 μg/g as $ZnSO_4 \cdot 7H_2O$ but was significantly lower for those with 6 μg/g. Growth did not differ significantly between rats fed a cereal with a ratio of 14.6 or less and rats fed the reference salt at 12 μg/g. Growth was significantly depressed, however, if the ratio of the cereal was 23.8 or greater. Cereals with ratios of 23.8–32.9 supported less growth than did 9 μg of $ZnSO_4$ per gram, but the differences were not significant. Added calcium restricted growth; the growth deficit was statistically significant for cereals 27.7–42.9 but not for cereals 2.6–23.8. The analysis of variance (Table III) indicated that an overall highly significant effect on growth was made by the phytate-zinc molar ratio, added calcium, and an interaction of phytate-zinc molar ratio with added

TABLE II
Test Diet Composition of Breakfast Cereal, Fiber from Cereal, and Added Calcium

Phytate-Zinc Molar Ratio	Cereal[a] (g/kg)	Cereal[b] (g/kg)	Calcium[c] (g/kg)
2.0	108	1.2	0.59
2.6	80	0.6	0.81
14.6	62	16.1	0.29
23.8	301	25.6	0.30
27.7	354	9.9	1.76
29.6	436	40.0	2.64
30.3	358	18.6	1.61
32.9	373	14.9	1.91
42.9	153	42.0	0.81

[a] Amount of cereal to supply 12 mg of zinc per kilogram of diet.
[b] All diets contained 20 g of cellulose type nonnutritive fiber per kilogram of diet in addition to that supplied by the cereal.
[c] Each cereal was tested with and without added calcium. The amount of calcium added as $CaHPO_4$ was proportional to that supplied by the suggested serving of milk and cereal from the nutrition information panel on the cereal package.

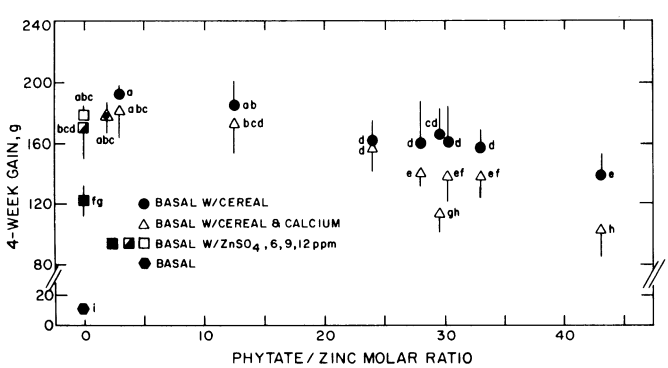

Fig. 1. Growth response of rats. Each data point is mean ± SD for 10 rats. Data points that do not share common superscripts are significantly different at $P < 0.05$.

TABLE III
Analysis of Variance[a]

Error Source	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Four-Week Gain, g					
± Ca[b]	1	18,080.1	18,080.1	62.367	0.001
Phy/Zn[c]	8	73,521.2	9,190.2	31.701	0.001
Ca × Phy/Zn	8	10,832	1,354.0	4.671	0.001
Residual	162	46,960	289.9		
Total	179				
Femur Zinc Concentration, μg/g					
± Ca	1	3.21	3.21	0.2359	ns[d]
Phy/Zn	8	13,764.65	1,720.58	126.4203	0.001
Ca × Phy/Zn	8	219.94	27.49	2.0198	ns
Residual	72	980.10	13.61		
Total	89	14,967.9			
Femur Zinc, μg/femur					
± Ca	1	187.78	187.78	2.4981	0.05
Phy/Zn	8	109,010.22	13,626.28	181.2728	0.001
Ca × Phy/Zn	8	1,300.62	162.58	2.1628	0.05
Residual	72	5,412	75.17		
Total	89	115,916.62			

[a] For diet treatments for which a cereal was the zinc source.
[b] − = calcium supplied by basal diet ingredients, + = added $CaHPO_4$.
[c] Phytate-zinc molar ratio.
[d] Not significant.

calcium.

Femur zinc concentration is shown in Fig. 2 and total zinc per femur in Fig. 3. The pattern of response was similar for zinc concentration and total zinc per femur. Each increment of femur zinc response between the three dietary zinc concentrations of $ZnSO_4$ was statistically significant. Femur zinc concentration did not differ significantly between the basal and 6 μg/g-reference diet groups, but the total zinc per femur was greater for the latter group. Cereal 2.6 elicited a significantly greater femur zinc response than did any other cereal; without added calcium, the response exceeded that to the 12 μg/g-level of zinc as $ZnSO_4$. The response was significantly greater to cereals 2.0, 2.6, and 14.6 than to cereals with ratios of 23.8 and greater. The femur zinc accumulation was slightly less for cereal 14.6 than for 9 μg of $ZnSO_4$ per gram. The femur zinc response to cereals with ratios 23.8–42.9 was essentially the same throughout and equivalent to or slightly better than that with only 6 μg of zinc per gram as $ZnSO_4$. Except for cereal 2.6, added calcium did not significantly depress the femur zinc response. The overall effect of phytate-zinc molar ratio on femur zinc was highly significant (Table III). The calcium effect on concentration was not significant and just significant for total zinc per femur.

DISCUSSION

In our present study, growth of rats was not depressed when cereals with phytate-zinc molar ratios of 15 or lower were the dietary zinc source, but growth was significantly depressed when cereals with ratios of 24 and greater were the zinc source. Overall, the results of this study correspond with previous reports on phytate-zinc ratio and zinc bioavailability to rats. Rats grew well on semipurified diets with phytate-zinc molar ratios of 12–15 when egg white was the protein source (Davies and Olpin 1979, Morris and Ellis 1980b). When the protein source is soy, the critical ratio may be lower than 12–15 (Oberleas and Prasad 1976), or it may be higher if the dietary zinc is several times the minimum requirement for growth (Morris and Ellis 1980a).

Davies and Olpin (1979) found that plasma zinc values were depressed when phytate-zinc molar ratios were less than the ratios permitting maximum growth, and we found a similar effect on bone zinc (Morris and Ellis 1980a, 1980b). In our present study only the cereal with molar ratio of 2.6 supported bone zinc accumulation equal to that supported by the same dietary zinc concentration of $ZnSO_4$. The low bone zinc values for rats fed cereal 2.0 as zinc source indicate that a factor other than the phytate-zinc molar ratio influenced bioavailability of zinc from this cereal. When the phytate-zinc molar ratio was 14.6, bone zinc values were much lower than the values for the reference diet with 12 μg of zinc per gram as $ZnSO_4$. The concentration of zinc in the femurs of rats fed cereals with ratios of 23.8 and greater tended to plateau at a concentration only 10–20 μg/g more than the concentration for rats fed the basal diet that contained less than 1 μg of zinc per gram. The requirement for growth evidently takes precedence over deposition in bone in competing for the bioavailable zinc.

The effect of added calcium was more marked on growth than on bone zinc values. In many studies on the interaction of calcium and phytate, levels of dietary calcium were high, often 1% above that provided by the basal diet . The calcium level in our basal diet was about 0.75%, which is 1.5 times the recommended requirement. When calcium was added to cereal diets, the total calculated dietary calcium, 0.75% plus the addition, was 1.6–1.7 times the National Research Council's calcium requirement (NAS 1978) for cereals with phytate-zinc molar ratios of 2.0–23.8 and 29.6 and was 1.85–2 times the requirement for the remaining cereals. Work in our laboratory showed that when the phytate-zinc molar ratio was 25, a dietary calcium level 1.75 times the requirement significantly depressed growth of rats compared to growth achieved at a calcium level 1.5 times the requirement. However, when the phytate-zinc molar ratio was 3.0, a calcium level 3.5 times the requirement did not depress growth (Morris and Ellis 1980b). In the present rat study, the added calcium resulted in significantly restricted growth of rats fed cereal diets with phytate-zinc molar ratios greater than 28, diets in which the added calcium resulted in a level at least 1.8 times the requirement and in which the phytate-zinc molar ratio was high. Except at ratios 2.0–14.6, additional calcium had little effect on femur zinc values—possibly because the femur zinc concentration approached the physiological minimum, as can be seen by a comparison of the femur zinc concentration of cereal groups 27.7–42.9 with that of the basal diet group.

O'Dell et al (1972a) established a standard rat growth response curve for bioassay of zinc in various foods. They found that the zinc in wheat was 38% as available for growth as the zinc from $ZnCO_3$. The growth response to the reference $ZnSO_4$ in our study (Fig. 1) was linear from 0 through 9 μg of added dietary zinc per gram. With this standard curve, relative biological value (RBV) could be calculated for test cereals that supported four-week gains of less than 170 g. As an example, the gain for cereal 42.9, a wheat bran cereal, without added calcium was 138 ± 5 g (mean ± standard error). This gain was equal to the gain expected from 7.1 μg of $ZnSO_4$ per gram or an RBV of 59 (7.1/12 × 100). A 95% confidence interval can be estimated in like manner from the mean ± 2.26 times the standard error for limits of gain. The calculated RBV for cereal 42.9 would be 59 with a 95% confidence interval of 54–64. This method of estimation does not, however, account for variation in the standard response curve. The above calculations for cereal 23.8, a whole wheat cereal, gives an RBV of 70 (65–74). This value is somewhat greater than O'Dell et al (1972a) reported for wheat. They used unprocessed whole grain, however, and the processing into the breakfast cereal may have improved bioavailability. Growth and bone zinc responses were slightly greater for autoclaved than for native bran, even though the phytate concentration did not change (Morris and Ellis 1980a).

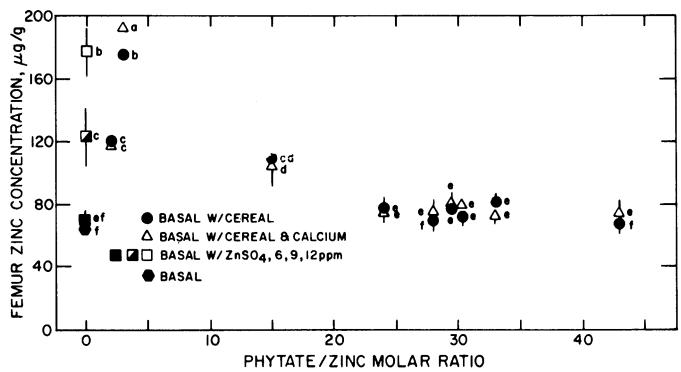

Fig. 2. Femur zinc concentration of rats. Each data point is mean ± SD for five rats. Data points that do not share common superscripts are significantly different at $P < 0.05$.

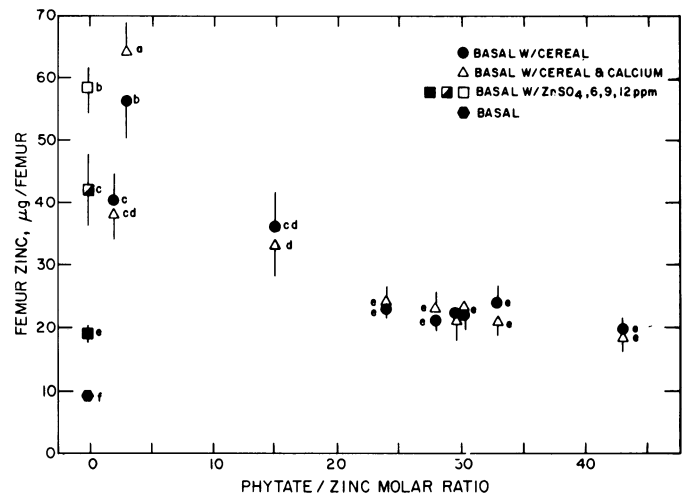

Fig. 3. Total femur zinc of rats. Each data point is mean ± SD for five rats. Data points that do not share common superscripts are significantly different at $P < 0.05$.

Momcilovic et al (1975) suggested that total femur zinc after three weeks is a suitable bioassay criterion for zinc bioavailability to rats. Our values for bone zinc, both microgram per gram and microgram per femur, from the $ZnSO_4$ reference groups were linear over the range of 6–12 μg of dietary zinc per gram, but linearity did not continue through the values obtained for the basal diet, possibly because our experiment spanned four rather than three weeks. Even though the bone zinc response was linear through 6 and 9 μg of dietary zinc per gram for $ZnSO_4$ an estimation of RBV may not be valid because the response at 6 μg/g may be approaching a physiological minimum. Only cereal 2.6 with ZnO fortification supported bone zinc values equivalent to the values supported by an equal dietary zinc concentration supplied as $ZnSO_4$. The bone zinc response to cereal 2.0 was deficient; possibly a component of oats or corn other than phytate inhibits bioutilization of zinc. This possibility cannot be supported on the basis of the response to the other oat-containing cereals (phytate-zinc molar ratios of 27.7, 30.3, and 32.9) because the low bone zinc values could be attributed to the high phytate-zinc molar ratio. None of the other cereals contained a corn fraction. O'Dell et al (1972a) found the zinc in corn to be more bioavailable than the zinc in rice or wheat. The bone zinc response to cereal 14.6 agrees with our study in which we fed Na phytate at a phytate-zinc molar ratio of 15 (Morris and Ellis 1980b); values were lower for bone zinc but equal for growth in comparison to reference values.

Cereal 27.7 contained soy protein concentrate, and its phytate-zinc molar ratio predicted that its zinc bioavailability would be low. Because cereal 27.7 also contained oat flour, no conclusion may be made about the effect of the soy protein concentrate per se. Dietary zinc requirements of rats and chicks are higher when soy protein rather than casein is used in semisynthetic diets (O'Dell et al 1972a). Ranhotra et al (1979) found that soy flour or protein concentrate tended to reduce zinc bioavailability in cookies, but not in bread, possibly because the phytate was hydrolyzed in bread (Ranhotra et al 1978). Forbes and Parker (1977) concluded that whole fat soy flour may not greatly reduce the bioavailability of an exogenous inorganic zinc salt when fed to rats. Soy fractions are used in infant formulas and cereals, and the bioavailability of zinc in these products is important (Momcilovic et al 1976, Shah et al 1979).

The effects of fiber, a component of cereal grains, on trace element bioavailability has been studied (Harland and Prosky 1979, Oberleas and Harland 1977, Reinhold et al 1976). In the present study, the breakfast cereals that contributed the greatest amounts of fiber to the test diets also had high phytate-zinc molar ratios and the zinc was of low bioavailability. Cereals 27.7 and 29.6 supported similar growth and bone zinc responses, but cereal 29.6 contributed four times as much fiber to the diet as did cereal 27.7. No uncomplicated correlation between fiber and bioavailbility of zinc was developed in the present study. Davies et al (1977) concluded that fiber of wheat bran was not the determinant of bioavailability of zinc in wheat bran, and, in studies with dephytinized wheat bran, we could not show that wheat bran fiber per se depressed bioavailability of zinc to rats (Morris and Ellis 1980a).

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