Mineral Bioavailability in Rats from Intrinsically Labeled Whole Wheat Flour of Various Phytate Levels†

Puspa R. Saha, Connie M. Weaver, and April C. Mason*

Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907

The bioavailability of calcium, iron, zinc, and selenium from hydroponically grown wheat flour containing low (0.19%), medium (0.67%), medium-high (1.64%), or high (1.85%) phytate levels fed to rats was determined. Mineral loads in the test meals were 639 μg of calcium, 132 μg of iron, 24 μg of zinc, and 1 μg of selenium. The percent of ^{45}Ca absorption from high-phytate wheat (85.7 ± 5.2) test meals was significantly ($P < 0.01$) less than from test meals containing low (92.8 ± 4.8) and medium-high-phytate (91.9 ± 5.0) wheat. The percent of ^{59}Fe absorbed by rats was significantly different ($P < 0.01$) at 81.47 ± 6.8, 73.38 ± 6.9, and 66.05 ± 7.7 for low-, medium-high-, and high-phytate flour. The percent absorption of ^{65}Zn from medium- (91.55 ± 3.0), medium-high- (89.36 ± 1.7), and high- (88.91 ± 1.6) phytate wheat flour was significantly lower than that from low-phytate (94.55 ± 1.8) flour ($P < 0.05$). Absorption of ^{75}Se in rats from medium-high- (81.52 ± 2.0) and high- (81.08 ± 1.8) phytate wheat flour was significantly lower than that from low-phytate (84.49 ± 1.8) flour ($P < 0.05$). Absorption of all minerals decreased with increasing phytate but was high at all concentrations due to low mineral density in wheat.

Keywords: *Bioavailability; wheat; phytate; rats; mineral absorption*

INTRODUCTION

Wheat products contribute significantly to the dietary intake of minerals, particularly when the whole grain is used since 60% of the minerals reside in the aleurone cells, which are a component of bran (Betschart, 1988). Concern exists about the nutritional quality of wheat products because the bioavailability of minerals may be low due to the presence of fiber and phytate. Dietary fiber has no effect on the absorption of iron, calcium, zinc, and selenium in animal and human studies (Andersson et al., 1983; Betschart, 1988; Ellis and Morris, 1981; Morris and Ellis, 1980, 1985; Turnlund et al., 1984). Therefore, the major dietary factor affecting mineral bioavailability from whole wheat products is thought to be phytate. Whole grain wheat contains 70–75% of its total phosphorus as phytic acid; 85% of the phytate is associated with the aleurone layer, 13% in the germ, and 2% in the endosperm (O'Dell et al., 1972). The phytic acid molecule is highly charged with six phosphate groups extending from the central inositol ring and serves as an excellent chelator of mineral ions such as Ca^{2+}, Zn^{2+}, and Fe^{3+}. Bindra et al. (1986) and Ellis et al. (1987) reported that the general American population consumes about 750 mg of phytic acid per day and that amount is increased to about 1500 mg for American vegetarians. Phytic acid is not readily absorbed or digested in the small intestine due to the absence of dietary or intestinal phytases. This could decrease absorption and availability of phytate-bound minerals.

Early studies showed that foods rich in phytate (i.e., whole wheat products and wheat bran) or sodium phytate added extrinsically to diets were the main causes of decreased overall calcium, iron, and zinc balance in rats and humans (McCance and Widdowson, 1942; Oberleas, 1983; Reinhold et al., 1976; Widdowson and McCance, 1942). In contrast, Andersson et al. (1983) failed to find a decrease in calcium, iron, and zinc balance in humans with consumption of high-phytate-containing wheat bran products. Balance studies have been criticized for providing less precise estimates of calcium absorbability from test foods compared to isotopic tracer methods (Weaver et al., 1991). Using more precise isotopic tracer methodology, calcium, iron, zinc, and selenium bioavailability in humans was shown to be inversely related to dietary phytate (Brune et al., 1992; Hallberg et al., 1989; Morris and Ellis, 1985; Morris et al., 1985; Sandstrom et al., 1990; Turnlund et al., 1984). In these studies, mineral absorption was measured using extrinsically labeled radioisotopes ($^{45}CaCl_2$, $^{59}FeCl_3$, $^{65}ZnCl_2$, and $Na^{75}SeO_3$) and purified sodium phytate.

Inorganic salts may be absorbed differently compared to minerals that are an integral part of the wheat kernel or other natural food components. Purified sodium phytate is soluble and known to be very reactive with minerals. Whether the same inhibitory effect can be seen when endogenous phytate and intrinsically labeled ^{45}Ca, ^{59}Fe, ^{65}Zn, and ^{75}Se are used must be established. The present study was conducted to determine calcium, iron, zinc, and selenium bioavailability from intrinsically labeled whole wheat flour, which is a natural source of phytic acid and calcium, iron, zinc, and selenium.

MATERIALS AND METHODS

Preparation of Radiolabeled Wheat Flour. Spring wheat (*Triticum aestivum* L. cv. Wampum) was grown in hydroponic culture in 16 L pots (Weaver, 1985). Culture conditions were adjusted to 6 and 31 ppm of phosphorus to produce grain of medium-high and high phytate content, respectively. When grain began to fill with whitish liquid ("milk" stage), stems were injected with 0.93 MBq of $^{45}CaCl_2$/head, 0.44 MBq of $^{59}FeCl_3$/head, 0.22 MBq of $^{65}ZnCl_2$/head, and 0.30 MBq of $Na_2{}^{75}SeO_3$/head of wheat. The grain was harvested at maturity and ground into a flour using a Tekmar

* Author to whom correspondence should be addressed [telephone (317) 494-8539].

† Journal Paper 13989, Purdue Agricultural Experiment Station, West Layette, IN.

0021-8561/94/1442-2531$04.50/0

Table 1. Elements in Hydroponically Grown Wheat Flour[a]

wheat flour	phytate, %	phosphorus, μg/g	calcium, μg/g	iron, μg/g	zinc, μg/g	selenium, μg/g
low phytate	0.19 ± 0.11	5211 ± 32[c]	631 ± 6.0[c]	102 ± 0.2[c]	48.5 ± 0.9[c]	1.69 ± 0.5[c]
medium phytate	0.67 ± 0.11	5954 ± 35[c]	ND[b]	ND	49.9 ± 1.6[c]	ND
medium-high phytate	1.67 ± 0.06	5075 ± 40	599 ± 6.5	92 ± 1.0	48.8 ± 1.1	1.52 ± 0.6
high phytate	1.85 ± 0.05	5669 ± 47	560 ± 5.2	72 ± 2.7	45.7 ± 0.5	1.54 ± 0.4

[a] Mean ± SD, $n = 4$. [b] Not determined. [c] On a dry weight basis.

Table 2. Composition of Diets

component	diet: basal,[a] g/kg	iron deficient,[b] g/kg	marginal zinc,[c] g/kg	selenium adequate,[d] g/kg
sucrose[e]	500	500	499.5	500
casein (vitamin-free)[f]	200	200		
egg white (spray-dried)[f]			200	
torula yeast[f,g]				500
cornstarch[f]	150	150	150	150
fiber (Alphacel)[f]	50	50	50	50
corn oil[e]	50	50	50	50
AIN mineral mix[f]	35			35
AIN mineral mix (iron-free)[h]		35		
AIN mineral mix (zinc-free)[h]			35	
AIN vitamin mix[f]	10	10	10	10
DL-methionine[f]	3	3	3	3
choline bitartrate[f]	2	2	2	2
d-biotin[f]			3.5[j]	
zinc mix[i]			0.5	

[a] Contained 6840 μg/g calcium, 31.95 μg/g zinc, and 20% protein. [b] Contained 15.16 μg/g iron and 20% protein. [c] Contained 9.41 μg/g zinc and 20% protein. [d] Diet contained 0.145 μg/g selenium and 20% protein. [e] Purchased locally. [f] ICN Biochemicals, Cleveland, OH. [g] Contained 40.85% protein. [h] Difference made up with sucrose; Nutritional Biochemicals, Cleveland, OH. [i] Zinc mix 0.249 g of ZnO and 0.9751 g of sucrose/g of mix provides 20 μg of Zn/g of diet when added at 1.0 g/kg. [j] Units are mg/kg.

A-10 analytical mill (Tekmar, Cincinnati, OH). Low and medium levels of phytate were obtained by dephytinizing wheat flour containing the medium-high and high level of phytate with phytase (*myo*-inositol hexakisphosphate-6-phosphohydrolase, Sigma Chemical Co., St. Louis, MO). The efficiency of ^{45}Ca, ^{59}Fe, ^{65}Zn, and ^{75}Se incorporation into grain was 8%, 27%, 57% and 37%, respectively. Milled wheat flour contained 20% protein and 12% moisture. Phytate, total phosphorus, calcium, iron, zinc, and selenium contents of wheat flour are shown in Table 1.

Animal Protocol. Male Sprague Dawley rats (Harlan Industries, Indianapolis, IN) were individually housed in stainless steel cages on a controlled 12-h light–dark cycle. Care and use of laboratory animals was in accordance with the *Guide for the Care and Use of laboratory Animals* (NIH Publication 86-23). The animals were given free access to deionized water and experimental diets. The semisynthetic experimental diets were fed to rats according to their study group described as follows.

Calcium Absorption Experiment. Rats ($N = 40$, 5 weeks old) were fed basal diet (Table 2) for 14 days before being assigned randomly to one of four groups ($n = 10$). On day 15, rats were food-deprived for 12 h to ensure they consumed all of the test meal. Then three groups of rats were fed test meals containing intrinsically ^{45}Ca labeled wheat flour of various levels of endogenous phytate and one group of rats was given intraperitoneal (ip) injection (Table 3). The ip injection was given to rats 2 h after they had received an unlabeled 3 g meal of basal diet. The phytate level in the test meals was provided by the intrinsically labeled whole wheat flour (Table 3). Forty-eight hours after administration of test meal or injection, all rats were killed by overexposure to carbon dioxide, and left femurs were removed. Radioactivity in bone was determined using a β-counter (Beckman LS 1800 scintillation counter, Fullerton, CA), after the bone was dissolved in HNO_3 and 15 mL of liquid scintillation cocktail (Amersham, Arlington Heights, IL) was added. Percentage absorption of ^{45}Ca was calculated by comparing the percentage of oral dose in the femur and the percentage of ip dose in the femur using a procedure similar to that reported by Wein and Schwartz (1983).

Iron Absorption Experiment. Rats ($N = 41$, 3 weeks old) were fed an iron-deficient diet (Table 2) for 28 days to produce mild anemia. Rats were tail-bled to determine iron status by measuring hemoglobin (Hb) and hematocrit (PCV). Hb levels were determined according to the cyanomethemoglobin method (Crosby et al., 1954), and PCV levels were determined using heparinized microhematocrit capillary tubes. Rats having extremely high or low body weight or iron status were excluded from the study. On day 28, 30 rats were assigned to three groups ($n = 10$) with similar mean body weights, blood hemoglobin, and PCV (mean body wt = 168.7 ± 11.4 g, Hb = 10.9 ± 1.0 g/dL, PCV = 27.1 ± 1.8%). Animals were food-deprived for 12 h and fed ^{59}Fe labeled test meals (Table 3). Different amounts of ^{59}Fe labeled wheat flour were used to prepare test meals for the three treatment groups to maintain the same amount of total iron in test meals. All rats were assayed for ^{59}Fe retention by whole body counting for 10 days, during which time they were fed basal diet (Fe adequate, Table 2).

Zinc Absorption Experiment. Rats ($N = 40$, 4 weeks old) were fed a marginal zinc diet for 9 days to induce a marginal deficiency of zinc (Table 2). Then rats were randomly assigned into four groups ($n = 10$) and fed intrinsically ^{65}Zn labeled wheat flour test meals containing various levels of endogenous phytate (Table 3). All rats were assayed for ^{65}Zn retention for a period of 10 days, during which time they were fed basal diet (zinc adequate, Table 2).

Selenium Absorption Experiment. Rats ($N = 30$, 5 weeks old) were fed a torula yeast based selenium adequate diet (Table 2) throughout the experimental period. On the eighth day, animals were randomly assigned to three groups ($n = 10$) and fed test meals containing intrinsically ^{75}Se labeled wheat flour of various levels of endogenous phytate (Table 3). All rats were assayed for ^{75}Se retention by whole body counting for 10 days, during which time they were fed basal diet (Se adequate, Table 2).

Whole Body Counting of ^{59}Fe, ^{65}Zn, and ^{75}Se and Percent of Absorption Determination. After animals consumed the radioactive test meals (4–5 h) (day 0), whole body ^{59}Fe, ^{65}Zn, and ^{75}Se were determined using a large-well crystal whole body γ-counter (Harshaw Chemical Co., Cleveland, OH) equipped with a Canberra, Series 30, multichannel analyzer (Canberra Industries, Inc.). Coincidence loss of this system is less than 2% at 250 000 cpm. Activity of ^{59}Fe was measured using a window setting extending from 965 to 1490 keV, which included the two ^{59}Fe photopeaks (1099 and 1291 keV). ^{65}Zn γ emission was measured using a window setting extending from 700 to 1644 keV, which included the ^{65}Zn photopeak (1115 keV). ^{75}Se γ emission was measured using a window setting extending from 100 to 380 keV, which included the ^{75}Se photopeak (264 keV).

Whole body radioactivity was measured on days 1, 2, 4, 6, 8, and 10 to determine the retention of the nuclides. The measured radioactivity was corrected for background, nuclide decay, and daily whole body γ counter fluctuations. Percent retention of administered radioisotopes was calculated using the following formula:

$$\% \text{ retention} = \frac{\text{whole body count at time } t}{\text{whole body count at time 0}} \times 100$$

Radioactivity measured on day 0 (5 h after rats were given radioactive test meals) represents absorbed nuclides plus unabsorbed radioactivity in the gastrointestinal tract before

Table 3. Test Meals for ^{45}Ca, ^{59}Fe, ^{65}Zn, and ^{75}Se Absorption Experiment

ingredient	^{45}Ca meals,[a] g/3 g				^{59}Fe meals,[b] g/3 g			^{65}Zn meals,[c] g/3 g				^{75}Se meals,[d] g/5g		
	LP	MHP	HP	IP[f]	LP	MHP	HP	LP	MP	MHP	HP	LP	MHP	HP
whole wheat flour[e]														
low phytate (LP)	1.00				0.90			0.51				0.50		
medium phytate (MP)									0.50					
medium-high phytate (MHP)		1.00				1.09				0.51			0.50	
high phytate (HP)			1.00				1.30				0.55			0.50
sucrose	1.61	1.61	1.61		1.71	1.52	1.31	2.10	2.11	2.10	2.06	3.85	3.85	3.85
fiber (Alphacel)	0.15	0.15	0.15		0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
corn oil	0.24	0.24	0.24		0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
phytate (mg)/3 and 5 g meals	2.8	16.4	18.3		2.8	17.4	23.4	0.56	3.35	8.31	10.06	0.40	8.65	9.65

[a] Contained 0.06 MBq of ^{45}Ca and 0.64 mg of calcium by analysis. [b] Contained 0.009 MBq of ^{59}Fe and 132 μg of iron by analysis. [c] Contained 0.01 MBq of ^{65}Zn, 24 μg of zinc, and 336 μg of calcium by analysis. [d] Contained 0.007 MBq of ^{75}Se and 1.0 μg of selenium analysis. [e] Intrinsically labeled with $^{45}CaCl_2$, $^{59}FeCl_2$, $^{65}ZnCl_2$, and $Na_2{}^{75}SeO_3$. [f] IP, rats given intraperitoneal injection (0.185 MBq/0.5 mL per rat).

Table 4. Absorption of ^{45}Ca, ^{59}Fe, ^{65}Zn, and ^{75}Se from Whole Wheat Flour As Affected by Phytic Acid

	mineral											
	^{45}Ca			^{59}Fe			^{65}Zn				^{75}Se	
treatment group[a]	phytate, mg	phytate:Ca molar ratio	absorption,[b] %	phytate, mg	phytate:Fe^{2+} molar ratio	absorption,[c] %	phytate, mg	phytate:Zn^{2+} molar ratio	phytate × Ca^{2+}:Zn^{2+} molar ratio	absorption,[c] %	phytate mg	absorption,[c] %
LP	2.80	0.25	92.8 ± 4.8[a]	2.8	2.65	81.4 ± 6.8[a]	0.56	2.22	0.02	94.5 ± 1.8[a]	0.40	84.4 ± 1.7[a]
MP	ND[d]	ND	ND	ND	ND	ND	3.35	14.66	0.13	91.5 ± 3.0[b]	ND	ND
MHP	16.4	1.56	91.9 ± 5.0[a]	17.4	14.71	73.3 ± 6.9[b]	8.31	36.09	0.30	89.3 ± 1.7[c]	8.65	81.5 ± 2.0[b]
HP	18.3	1.81	85.7 ± 5.2[b]	23.4	21.14	66.0 ± 7.7[c]	10.06	41.09	0.35	88.9 ± 1.6[c]	9.65	81.0 ± 1.8[b]

[a] Group of rats fed radiolabeled wheat flour test meal containing low, medium, medium-high, and high phytate. [b] Mean ± SD, $n = 10$. Different letter superscripts within column indicate significant differences among means ($p < 0.01$), SNK multiple range test. [c] Mean ± SD, $n = 10$. Values followed by different letter superscripts within column are significantly different ($p < 0.05$), comparison of y-intercepts between groups after linear regressions. [d] ND, not determined.

excretion of any isotope. Because not all of the administered radionuclides were absorbed on day 0, percent apparent absorption of radionuclides was calculated by extrapolating the linear portion of a plot of percent retention versus time, from day 3 to day 10 after the isotope administration. Calculated y-intercepts of the linear regression analysis were the percent absorption of ^{59}Fe, ^{65}Zn, and ^{75}Se on day 0 (Mason and Weaver, 1986). The SAS Regression Procedure (SAS Institute, 1989) was used to obtain y-intercepts.

Analytical Methods. Phytate in wheat flour was analyzed colorimetrically after separation by ion-exchange chromatography (Latta and Eskin, 1980). Total phosphorus in wheat flour was quantified according to the modified method of Murphy and Riley (1962) on samples digested in HNO_3–$HClO_4$. Total calcium, iron, and zinc were determined by atomic absorption spectroscopy after sample digestion in HNO_3–$HClO_4$ (Model 5100PC, Perkin-Elmer, Norwalk, CT). A modification of Kjeldahl nitrogen determination method (AOAC, 1980) was used to estimate protein. Selenium was analyzed by gas chromatography (McCarthy et al., 1981) using a Varian 3400 gas chromatography unit (Walnut Creek, CA). National Bureau of Standards (NBS) wheat flour 1567 was analyzed to ensure accuracy. Mean values in micrograms per gram for calcium (187 ± 1.5), iron (19.4 ± 0.7), zinc (10.2 ± 1.5), selenium (0.9 ± 0.2), and phosphorus (1372 ± 8.5) were similar to those provided by the NBS (calcium 190 ± 10; iron 18.3 ± 1.0; zinc 10.6 ± 1.0; selenium 1.1 ± 0.2; and phosphorus 1390 ± 30) (National Bureau of Standards, 1978).

Statistical Methodology. Homogeneity of variance and normality of data were tested by the Bartletts homogeneity of variance test and by the Wilk–Shapiro W test, respectively. Residual and normal probability plots along with standardized residuals of data were used to detect outlier data. The absorption of ^{45}Ca among groups was compared by one-way analysis of variance (ANOVA) using Student–Newman–Keuls multiple-range test at $\alpha = 0.05$. Multiple linear regression with different levels of phytate in the test meals as the indicator variable (Neter et al., 1985) was used to determine whether the percent absorption of ^{59}Fe, ^{65}Zn, and ^{75}Se from test meals containing different levels of phytate were equal.

RESULTS

The dependence of intrinsically labeled ^{45}Ca, ^{59}Fe, ^{65}Zn, and ^{75}Se absorption on endogenous phytate concentration in wheat flour is shown in Table 4. Absorption of ^{45}Ca in the femurs of rats was significantly lowered ($P < 0.01$) by 8% with high phytate concentration in wheat flour test meals. However, the difference in ^{45}Ca absorption from wheat flour test meals containing low and medium-high phytate concentrations was not significant. Rats consuming test meals containing medium-high- and high-phytate wheat showed 10% and 19% decreases ($P < 0.01$) in the absorption of ^{59}Fe, respectively, compared to rats consuming test meals containing low phytate. Absorption of zinc was significantly lower ($P < 0.05$) from test meals containing medium-, medium-high-, and high-phytate wheat compared to the test meals containing low-phytate wheat. Rats consuming test meals containing either medium-high- or high-phytate wheat showed a significant ($P < 0.05$) decrease in ^{65}Zn absorption compared to rats consuming test meals containing medium-phytate wheat. There was no difference, however, in ^{65}Zn absorption from test meals containing medium-high- and high-phytate wheat. The decrease in absorption of ^{75}Se from medium-high- and high-phytate wheat was statistically significant ($P < 0.05$) compared to that from low-phytate wheat.

DISCUSSION

The inhibitory effects of endogenous phytate on the absorption of minerals is still a controversial issue. For calcium, absorption from intrinsically labeled soy flour in rats was not affected by increased levels of endogenous phytate in soy flour (Mason et al., 1993; Weaver, 1989). In contrast, Heaney et al. (1991) reported that human calcium absorption from intrinsically labeled

soybeans was significantly affected by endogenous soybean phytate. Mason et al. (1993) and Weaver (1989) suggested that it is possible that rats, unlike humans, have a limited amount of intestinal phytase to free calcium from phytate, especially up to the level of 10 mg of phytate, the load used in the soy study. In the present study, significant reduction of ^{45}Ca absorption was observed at the level of 18.3 mg of endogenous phytate in the test meals. Alternatively, phytate–protein–mineral interactions could be different between soy and wheat.

The phytate:Ca^{2+} molar ratio of the three test meals containing low, medium-high, and high phytate used in this study were 0.25, 1.56, and 1.81, respectively. Absorption of ^{45}Ca was decreased significantly when rats were fed high-phytate test meals with a phytate:Ca^{2+} molar ratio >1.56. In contrast to this finding, Morris and Ellis (1985) concluded that persons consuming diets with phytate:Ca^{2+} molar ratios of >0.2 may be at risk of calcium deficiency. These researchers fed muffins with added wheat bran or sodium phytate to adult men and found that the calcium balance decreased when phytate:Ca^{2+} molar ratios reached 0.24. In another study on human calcium absorption from whole wheat products, Weaver et al. (1991) concluded that total phytate content of food may be the predictor of calcium bioavailability rather than the molar ratio. In the present study, phytate:Ca^{2+} molar ratios were not found to be a better predictor of calcium absorption than the total phytate content of test meals. However, both total phytate and phytate:Ca^{2+} molar ratios predicted less than 20% of the total variation in calcium absorption.

The inhibitory effect of phytate on iron absorption observed in this study is consistent with recent findings by Brune et al. (1992). These authors reported that phytate present after fermentation of wheat bread was responsible for poor absorption of extrinsically labeled ^{59}Fe in humans. The inhibitory effect of whole wheat products on iron absorption was first described by Widdowson and McCance (1942), who determined by chemical iron balance that normal subjects retained less iron from a diet containing brown bread than from one containing white bread. The bran effect was studied by Bjorn-Rasmussen (1974), who demonstrated, by a dual isotope method, that the addition of 7% bran to white flour decreased iron absorption from baked rolls by a factor of 2 due to the addition of phytate from bran. The phytate:Fe^{2+} molar ratios of the three test meals containing low, medium-high, and high phytate in this study were 2.65, 14.71, and 21.14, respectively. Absorption of ^{59}Fe was decreased significantly when rats were fed test meals with a phytate:Fe^{2+} molar ratio of 14.17 and higher.

The inhibitory effect of phytate on zinc absorption observed in this study was also reported by other researchers (Kivisto et al., 1989; Oberleas, 1983; Sandstrom et al., 1987, 1990; Turnlund et al., 1984). They observed the absorption of extrinsically labeled ^{65}Zn was negatively correlated to the phytic acid content of the meal. The source of phytate in experimental diets was either purified sodium phytate (Turnlund et al., 1984) or endogenous phytate from whole-wheat bread (Sandstrom et al., 1987).

The present study shows an inverse relationship between increasing phytate:Zn^{2+} and phytate × Ca^{2+}:Zn^{2+} molar ratios and mean absorption of ^{65}Zn (Table 4). The molar ratio between phytic acid and zinc was suggested as an index of zinc bioavailability. Davies and Olpin (1979), Gibson et al. (1991), Oberleas (1983), and Morris and Ellis (1980) reported that with moderate calcium intake, phytate:Zn^{2+} molar ratios <10 are likely to provide adequate available zinc and molar ratios >10 are associated with symptoms of zinc deficiency such as growth rate depression. The phytate:Zn^{2+} molar ratios of the four test meals containing low, medium, medium-high, and high phytate used in this study were 2.22, 14.66, 36.09, and 41.09, respectively. A significant reduction of ^{65}Zn absorption resulted with phytate:Zn^{2+} molar ratios of 14.7 and higher (Table 4).

Phytate × Ca^{2+}:Zn^{2+} molar ratios are also suggested as a predictor of zinc absorption in rats and humans from a high-phytate-containing diet (Bindra et al., 1986; Ellis et al., 1987; Fordyce et al., 1987; Gibson et al., 1991). Fordyce et al. (1987) observed poor zinc availability in rats when the phytate × Ca^{2+}:Zn^{2+} molar ratio exceeded 3.5. These researchers also claimed that the phytate:Zn^{2+} molar ratio was a poor indicator of zinc absorption from a high-phytate diet as it did not take into account the aggravating effects of calcium on zinc absorption. The phytate × Ca^{2+}:Zn^{2+} molar ratio of the four test meals containing low, medium, medium-high, and high phytate used in this study were 0.02, 0.13, 0.3, and 0.35, respectively. A significant reduction of zinc absorption was observed at phytate × Ca^{2+}:Zn^{2+} molar ratios of 0.13 and higher (Table 4). Although the results of the present study are consistent with those of previous studies, total phytate content in the diets should be the predictor of zinc bioavailability rather than phytate:Zn^{2+} and phytate × Ca^{2+}:Zn^{2+} molar ratios since saliva and pancreatic fluid secrete large quantities of zinc, which would lower the phytate:Zn^{2+} molar ratios, and the amount of endogenous zinc secretion varies from person to person. In this study, phytate:Zn^{2+} and phytate × Ca^{2+}:Zn^{2+} molar ratios did not improve the variation in zinc retention over total phytate since R^2 = 62.5–63.6% for all predictors.

The inhibitory effect of phytate on selenium absorption found in this study is consistent with findings of Morris et al. (1985). These authors studied the effect of sodium phytate and α-cellulose on selenium balance in young men. They observed that sodium phytate increased fecal selenium excretion and thereby caused negative selenium balance. However, selenium balance was not affected by dietary fiber.

Selenium in wheat appears to be present to the extent of 50% as protein-bound selenomethionine, but the chemical nature and quantitative distribution of the other half are unknown (Olson et al., 1970). The decline of wheat selenium absorbability with increasing levels of endogenous phytate from wheat is more difficult to interpret because the selenomethionine present in wheat compared to other multivalent cations, in theory, should not interact with phytate. The possible interactions between protein containing selenoamino acids and phytate may have caused the decreased ^{75}Se absorption and retention from wheat flour test meals.

Although whole wheat products are not concentrated sources of dietary calcium, iron, zinc, and selenium in diets, mineral bioavailability from whole wheat was high regardless of the endogenous phytate content (Table 4). Weaver et al. (1991) reported that absorption of calcium (over 80%) in humans from whole wheat products (except for wheat bran cereal) was better than that of calcium from milk at a comparable load (15 mg) in adult women.

In conclusion, phytate from whole wheat flour has a significant inhibitory effect on calcium, iron, zinc, and selenium absorption. The extent of inhibition of mineral bioavailability depends on the total amount of phytate

present in the diet. This finding has wide nutritional implications among populations who are marginally deficient or have borderline body stores of those minerals. The intake of high-phytate-containing foods is one of the main determinants of iron, zinc, calcium, and selenium nutrition in groups with a regular high-phytate intake.

LITERATURE CITED

Andersson, H.; Navert, B.; Bingham, S. A.; Englyst, H. N.; Cummings, J. H. The effect of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balance in man. *J. Nutr.* **1983**, *50*, 503–510.

AOAC. *Official methods of analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980; Sections 7.015 and 14.063.

Betschart, A. A. Nutritional quality of wheat and wheat foods. In *Wheat: Chemistry and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1988; Vol. ii, pp 1–480.

Bindra, G. S.; Gibson, R. S.; Thompson, L. [Phytate][Calcium]/[Zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. *Nutr. Res.* **1986**, *6*, 475–483.

Bjorn-Rasmussen, E. Iron absorption from wheat bread. *Nutr. Metabol.* **1974**, *16*, 101–110.

Brune, M.; Rossander-Hulten, L.; Hallberg, L.; Gleerup, A.; Sandberg, A. Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *J. Nutr.* **1992**, *122*, 442–449.

Crosby, W. H.; Munn, J. I.; Furth, F. W. Standardizing a method for hemoglobinometry. *U.S. Armed Forces Med. J.* **1954**, *5*, 693.

Davies, N. T.; Olpin, S. E. Studies on the phytate: zinc molar contents in diets as a determinant of Zn availability to young rats. *Br. J. Nutr.* **1979**, *41*, 590–603.

Ellis, R.; Morris, E. R. Phytate-zinc molar ratio of breakfast cereals and bioavailability of zinc to rats. *Cereal Chem.* **1981**, *58*, 363–366.

Ellis, R.; Kelsay, J. L.; Reynolds, R. D.; Morris, E. R.; Moser, R. B.; Frazier, C. W. Phytate:Zinc and Phytate X Calcium: Zinc millimolar ratios in self-selected diets of Americans, Asian Indians and Nepalese. *J. Am. Diet. Assoc.* **1987**, *87*, 1043–1047.

Fordyce, E. J.; Forbes, R. M.; Robbins, K. K.; Erdman, J. W., Jr. Phytate X calcium/zinc molar ratios: are they predictive of zinc bioavailability? *J. Food Sci.* **1987**, *52*, 440–444.

Gibson, R. S.; Vanderkooy, P. D. S.; Thompson, L. Dietary phytate X Calcium/Zinc milimolar ratios and zinc nutriture in some Ontario preschool childern. *Biol. Trace Elem. Res.* **1991**, *30*, 87–94.

Hallberg, L.; Brune, M.; Rossander, L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *Am. J. Clin. Nutr.* **1989**, *49*, 140–144.

Heaney, R. P.; Weaver, C. M.; Fitzsimmons, M. L. Soybean phytate content: effect on calcium absorption. *Am. J. Clin. Nutr.* **1991**, *53*, 745–747.

Kivisto, B.; Cederblad, A.; Davidson, L.; Sandberg, A.; Sandstrom, B. Effect of meal composition and phytate content of zinc absorption in humans from extruded bran product. *J. Cereal Sci.* **1989**, *10*, 189–197.

Latta, M.; Eskin, M. A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.* **1980**, *28*, 1313–1315.

Mason, A. C.; Weaver, C. M. Metabolism in rats of selenium from intrinsically and extrinsically labeled isolated soy protein. *J. Nutr.* **1986**, *116*, 1883–1888.

Mason, A. C.; Weaver, C. M.; Kimmel, S. E.; Brown, R. K. The effect of soybean phytate content on calcium bioavailability to mature and immature rats. *J. Agric. Food Chem.* **1993**, *41*, 246–249.

McCance, R. A.; Widdowson, E. M. Mineral metabolism of healthy adults on white and brown bread dietaries. *J. Physiol.* **1942**, *101*, 44–85.

McCarthy, T. P.; Brodie, B.; Milner, J. A.; Bevill, R. F. Improved method for selenium determination in biological samples by gas chromatography. *J. Chromatogr.* **1981**, *225*, 9–16.

Morris, E. R.; Ellis, R. Bioavailability to rats of iron and zinc in wheat bran: response to low-phytate bran and effect of the phytate/zinc molar ratio. *J. Nutr.* **1980**, *110*, 2000–2010.

Morris, E. R.; Ellis, R. Bioavailability of dietary calcium—effect of phytate on adult men consuming nonvegetarian diets. In *Nutritinal Bioavailability of Calcium*; Kies, C., Ed.; ACS Symposium Series 275; American Chemical Society: Washington, DC, 1985; pp 63.

Morris, V. C.; Turnlund, J. R.; King, J. C.; Levander, O. A. Effect of sodium phytate and alpha-cellulose on selenium balance in young men. *Fed. Proc.* **1985**, *44*, 1670.

Murphy, J.; Riley, J. R. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta* **1962**, *27*, 31–36.

National Bureau of Standards. Standard Reference Material 1567 (Wheat Flour), Washington, DC, 1978.

Neter, J.; Wasserman, W.; Kutner, M. H. Indicator Variables. In *Applied Linear Statistical Models*, 2nd ed.; Richard D. Irwin: Homewood, IL, 1985; pp 297–338.

Oberleas, D. The role of phytate in zinc bioavailability and homeostasis. In *Nutritional Bioavailability of Zinc*; Inglett, G. E., Ed.; American Chemical Society: Washington, DC, 1983; p 145.

O'Dell, B. L.; Deboland, A. R.; Koirtyohann. Distribution of phytate and nutritionally important elements among morphological components of cereal grains. *J. Agric. Food Chem.* **1972**, *20*, 718–720.

Olson, O. E.; Novacek, E. J.; White, E. I.; Palmer, I. S. Investigation of selenium in wheat. *Phytochemistry* **1970**, *9*, 1181–1188.

Reinhold, J. G.; Faradji, B.; Abadi, P.; Ismail-Beigi, F. Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption as wheat bread. *J. Nutr.* **1976**, *106*, 493–503.

Sandstrom, B.; Almgren, A.; Kivisto, B.; Cederblad, A. Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. *J. Nutr.* **1987**, *117*, 1898–1902.

Sandstrom, B.; Cederblad, A.; Stenqreist, B.; Andersson, H. Effect of inositol hexaphosphate on retention of zinc and calcium from the human colon. *Eur. J. Clin. Nutr.* **1990**, *44*, 705–708.

SAS Institute Inc. *SAS User's Guide: Statistics*, 4th ed.; SAS Institute: Cary, NC, 1989; Vol. 1 and 2.

Turnlund, J. R.; King, J. C.; Keyes, W. R.; Gong, B.; Michael, M. A stable isotope study of zinc absorption in young men: effect of phytate and alpha-cellulose. *Am. J. Clin. Nutr.* **1984**, *40*, 1071–1077.

Weaver, C. M. Intrinsic mineral labeling of edible plants: methods and uses. *Crit. Rev. Food Sci. Nutr.* **1985**, *23*, 75–101.

Weaver, C. M. Effect of phytate on mineral bioavailability. *Symposium proceedings*, Protein and co-products division, AOCS; American Oil Chemists' Society: Champaign, IL, 1989.

Weaver, C. M.; Heany, R. P.; Martin, B. R.; Fitzsimmons, M. L. Human calcium absorption from whole wheat products. *J. Nutr.* **1991**, *121*, 1769–1775.

Widdowson, E. M.; McCance, R. A. Iron exchange of adults on white and brown bread diets. *Lancet* **1942**, *1*, 588–591.

Wien, E. M.; Schwartz, R. Comparison of in vitro and in vivo measurements of dietary Ca exchangeability and bioavailability. *J. Nutr.* **1983**, *113*, 388–393.

Received for review August 11, 1994. Accepted August 22, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1994.