Effect of Dietary Phytic Acid on the Availability of Iron and Phosphorus

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ABSTRACT

In bread-based diets fed to rats for 6 weeks, increasing the level of phytate in diets otherwise identical in protein, calcium, iron, phosphorus, and vitamin D content did not appear to interfere substantially with the availability of iron naturally occurring in wheat. The availability of phytate phosphorus was also not affected appreciably. No relationship, in a quantitative sense, was observed between intestinal phytase activity and iron or phytate availability.

It is generally recognized that phytates are poorly available to man and monogastric animals and that the presence of undegraded phytates in the intestine decreases the absorption of calcium, iron, magnesium, and zinc. Wheat protein concentrate (WPC), a high-protein. high-lysine, low-fiber flour prepared from fibrous mill-fractions, is quite high in phytic acid (1,2). Some of this phytic acid undergoes hydrolysis during breadmaking resulting in an increase in the amount of inorganic or available phosphorus. However, a substantial amount still remains unhydrolyzed (1-3). In view of possible contribution of cereals and their products and by-products like WPC in human dietary (4,5), and because of certain recent reports (6,7) indicating that phytates perhaps do not interfere with the availability of iron, the present studies, using rats, were undertaken. The effect of naturally occurring and added dietary phytic acid on the availability of iron and phosphorus and its relationship to intestinal phytase activity was thus examined.

MATERIALS AND METHODS

Table I lists the composition of test diets. Bread used to formulate diets was made with blend A flour (70% wheat flour; 30% WPC) using the sponge-dough

procedure (2). Each diet contained 44.8 mg. phytic acid phosphorus per 100 g. diet furnished by bread. Sodium phytate (General Biochemicals), sodium phosphate, and calcium carbonate were added in the amounts indicated in Table I to raise the phytate phosphorus content of diets A, B, C, and D to levels of 1:4:8:12, respectively, and of total phosphorus and calcium to 650 mg. per 100 g. each, a level close to that recommended (8). Each diet contained 15 p.p.m. of iron furnished entirely by bread.

Weanling, male, Sprague-Dawley rats, average weight about 50 g., were housed individually in stainless-steel cages and offered diets and distilled water ad libitum. Thirty rats were used per diet, 10 each being sacrificed at the end of 2, 4, and 6 weeks of feeding. Body-weight gain and diet-intake records were kept for individual rats. In the final week, feces were collected quantitatively in order to determine the amount of ingested phosphorus, calcium, and iron that was retained (digested) and of phytate that had undergone hydrolysis.

Pound loaves, sliced immediately after baking, were air-dried and ground to fine crumbs. Calcium, phosphorus, iron, and protein were determined in these crumbs, in resultant diets and in air-dried feces by standard AACC methods (9). Phytic acid phosphorus was determined by the method of Makower (10), based on the content of iron in extracted phytate after its precipitation as ferric phytate. A 4:6 iron:phosphorus molecular ratio was used to calculate phosphorus content. Inorganic phosphorus in the test diets and in feces was determined by the method of Pons and Guthrie (11).

In balance studies, the feces of 10 remaining rats were pooled for analyses. Hemoglobin (Hb) was determined on tail blood by the Cyanomethemoglobin method (12) and hematocrit (Hct) by microcapillary centrifugation. Inorganic

TABLE I. COMPOSITION OF TEST DIETS

	Diet (Protein, 10%)			
	A	B	C	D
Sodium phytate, g./100 g.	----	0.62	1.45	2.27
$NaH_2PO_4 \cdot H_2O$, g./100 g.	2.22	1.62	0.82	0.02
Others[a], g./100 g.	97.78	97.76	97.73	97.71
Calcium[b], mg./100 g.	650.0	650.0	650.0	650.0
Phosphorus[c], mg./100 g.	650.0	650.0	650.0	650.0
From sodium phytate	----	134.4	313.6	492.8
From $NaH_2PO_4 \cdot H_2O$	498.3	363.9	184.6	5.4
Phytic acid phosphorus[d], mg./100 g.	44.8 (1)	179.2 (4)	358.4 (8)	537.6 (12)
Iron, p.p.m.	15.0	15.0	15.0	15.0

[a] Bread, 27.8 g.; casein, 7.4 g.; $CaCO_3$, 1.42 g.; Vitamin Diet Fortification Mixture (Nutritional Biochemicals), 2 g. (vitamin D, 200 units); corn oil, 4 g.; sodium chloride, 1 g.; trace minerals ($MgSO_4 \cdot 7H_2O$, 414 mg.; $MnSO_4 \cdot H_2O$, 15 mg.; $CuSO_4$, 1.3 mg.; $ZnCl_2$, 2.5 mg.; and KI, 0.024 mg.), 0.43 g.; and sucrose to make 100.

[b] From bread, 75.8 mg./100 g.; from $CaCO_3$, 564.2 mg./100 g.; from other diet ingredients, 10 mg./100 g.

[c] From bread, 107.1 mg./100 g. (44.8 mg./100 g. as phytic acid P); from salts as indicated; from other diet ingredients, 44.6 mg./100 g.

[d] Values within parentheses indicate levels of phytic acid phosphorus.

phosphorus in blood serum (blood collected by heart puncture) was determined by the method of Fiske and Subbarow (13). To determine the percentage bone ash, the right humerus of each rat was removed, cleared of adhering tissues, dried overnight at 100°C., extracted with a mixture (1:1) of petroleum ether and ethanol for 8 hr., dried, weighed, and ashed at 650°C. for 3 hr.

For the determination of intestinal phytase activity, rats were starved overnight and then sacrificed. The duodenal segment of their intestines was removed, thoroughly flushed with cold normal saline, blotted dry, weighed, cut into small pieces and homogenized in Potter-Elvehjem homogenizer using normal saline. All steps were carried out in the cold using crushed ice. After the volume was recorded (tissue weight and volume about constant), a suitable aliquot of the homogenate was taken to determine phytase activity by the method of Pileggi (14). The reaction mixture consisted of 1 ml. of 0.0067M sodium phytate, 2 ml. tris-HCl buffer (pH 7.8), 1 ml. tissue homogenate, and 1 ml. 0.01M $MgSO_4$. A blank was run simultaneously using water instead of phytate. A 2-ml. portion of the suspension was removed at zero hour, added to 8 ml. of 10% trichloroacetic acid, well shaken, and filtered; and inorganic phosphorus in the filtrate was determined immediately by the method of Fiske and Subbarow (13). Inorganic phosphorus was similarly determined again after 2 hr. incubation at 37.5°C. One unit of phytase activity is defined as the increase (mg.) in inorganic phosphorus per gram of wet tissue in 2 hr.

RESULTS AND DISCUSSION

A great deal of controversy exists regarding the effect of phytates on the availability of dietary iron. Of the many factors involved, calcium, iron, and phosphorus, as well as phytate itself, appear to be mutually interacting and affecting the availability of iron. In most such studies, however, iron availability has been examined in relation to administered iron salts and rarely to naturally occurring iron. In the present studies, the availability of iron in wheat was examined, keeping the dietary levels of calcium, phosphorus, iron, and vitamin D constant and varying only the level of dietary phytate.

The growth of rats and diet:gain ratios were little affected by the level of dietary phytate, although the efficiency of diet utilization decreased somewhat with continued feeding (Table II). Since iron was provided at a submarginal level (15 p.p.m.), Hb and Hct values failed to reach normal levels in 6 weeks. This level was chosen deliberately to enable the distinction of inhibition of iron absorption by phytates. At 2 and 4 weeks, some inhibition of iron absorption was noted as the Hb and Hct levels on diets B through D were lower compared to diet A. This inhibition, however, appeared unrelated either to the level of dietary phytate (Table I) or to intestinal phytase activity (Fig. 1) and was not sustained through the sixth week when Hb, Hct levels on diets A through C equalized and were only slightly lower in the case of diet D. On all diets a substantial amount of ingested iron was retained by rats (Table III), reflecting a very efficient assimilability of iron under these conditions of submarginal supplies and high physiological needs. While Widdowson and McCance (15), Elwood et al. (16), and Sathe and Krishnamurthy (17) have reported inhibition, due to phytates, of the availability of iron in wheat and rice, Walker et al. (18) found that consumption of brown bread did not affect the amount of iron retained and Collumbine et al. (19) reported only an inconsistent effect. Recently Callender and Warner (7) showed an even better assimilability of

TABLE II. EFFECT OF DIETARY PHYTIC ACID ON THE AVAILABILITY OF IRON AND PHOSPHORUS TO RATS[a]

	Week	Diets[b] A (44.8)	B (179.2)	C (358.4)	D (537.6)
Body weight gain, g.	2	40.1 ± 8.4	41.3 ± 9.6	41.5 ± 5.1	41.8 ± 6.1
	4	85.8 ± 12.5	86.6 ± 12.8	81.2 ± 11.2	72.6 ± 10.6
	6	121.0 ± 18.3	132.7 ± 18.2	115.3 ± 21.2	119.2 ± 13.3
Diet:gain ratio[c]	2	2.37 ± 0.16	2.41 ± 0.16	2.42 ± 0.08	2.43 ± 0.11
	4	2.42 ± 0.29	2.32 ± 0.13	2.50 ± 0.23	2.65 ± 0.40
	6	2.87 ± 0.12	2.83 ± 0.10	2.97 ± 0.14	2.99 ± 0.20
Hemoglobin, g./100 ml.	2	9.0 ± 0.9	7.8 ± 0.7	8.3 ± 1.0	8.2 ± 1.1
	4	9.2 ± 0.9	7.6 ± 0.7	8.8 ± 1.1	8.7 ± 1.4
	6	9.4 ± 1.3	9.4 ± 1.5	9.6 ± 1.9	8.9 ± 1.9
Hematocrit, %	2	34.9 ± 2.0	32.7 ± 3.1	33.3 ± 2.6	31.7 ± 1.9
	4	34.5 ± 2.7	30.8 ± 3.6	33.8 ± 3.0	33.8 ± 4.2
	6	38.0 ± 3.3	38.8 ± 4.4	38.3 ± 5.7	35.9 ± 4.7
Inorganic serum P, mg./100 ml.	2	6.1 ± 0.9	7.2 ± 0.6	6.7 ± 0.5	7.3 ± 1.0
	4	8.4 ± 0.6	8.7 ± 0.5	8.7 ± 0.8	8.7 ± 1.4
	6	8.5 ± 0.2	8.7 ± 0.2	9.1 ± 0.2	9.1 ± 0.2
Bone ash, %	2	51.7 ± 1.9	52.1 ± 2.3	54.5 ± 1.5	53.4 ± 1.9
	4	50.1 ± 2.1	49.1 ± 2.0	49.7 ± 2.1	51.4 ± 2.8
	6	50.7 ± 2.8	47.0 ± 1.9	48.8 ± 1.6	49.9 ± 2.1

[a] All values represent average of 10 rats ± standard deviation.

[b] Values within parentheses indicate the level (mg./100 g.) of dietary phytic acid phosphorus.

[c] Diet consumed (g.):body weight gain (g.)

iron from brown bread than from enriched white bread. It is suggested that the lack of inhibition due to phytates in studies in which rats were used may be explained by the presence of intestinal phytase activity in rats. While phytate-splitting activity has not clearly been shown in man, Subrahmanyan et al. (20) found that 85% of phytate phosphorus in rice was hydrolyzed during digestion. It could be that the calcium-phytate complex is hydrolyzed lower in the gut and that the released calcium and phosphorus is then poorly absorbed. Such, however, may not be the case, since Bitar and Reinhold (21) have recently reported direct evidence on the presence of phytase-specific activity, apparently an enzyme system separate from alkaline phosphatase, in the intestinal mucosa of man and of the same order as in the rat, chicken, and calf. The prevalence of anemia in population groups subsisting on cereals thus may not necessarily be due to high phytate content of the diet but more to a low calcium intake or high bulk which impairs iron absorption (22,23) or to a failure to adapt to such a diet.

The availability of phytate phosphorus as measured in terms of growth rate, serum inorganic phosphorus levels, bone ash content, phytate hydrolysis, and phosphorus retention likewise did not appear to be affected by dietary phytate levels. Serum inorganic phosphorus levels increased with time but did not differ appreciably among diets (Table II). The percentage bone ash was even higher, though only slightly, on high phytate levels when measured at 2 weeks; during the subsequent 4 weeks it changed very little for rats on diet A, although some inhibition of bone calcification was observed on other diets, particularly diet B. All diets had a calcium:phosphorus ratio well within the range considered optimal for calcification and, in agreement with the results on Nicolaysen and Njaa (24) but

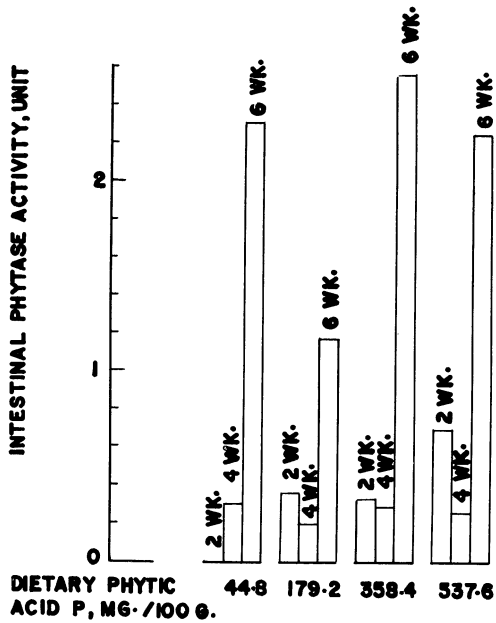

Fig. 1. The effect of dietary phytic acid on intestinal phytase activity at 2, 4, and 6 weeks.

TABLE III. EFFECT OF PHYTIC ACID ON THE RETENTION OF INGESTED PHOSPHORUS, CALCIUM, AND IRON

Diet[a]	Phytic Acid Phosphorus Hydrolyzed		Total Phosphorus Retained		Calcium Retained		Iron Retained	
	mg.	%	mg.[b]	%	mg.	%	γ	%
A (44.8)	78.6	21.8	4,152.5 (4,104.9)	79.4	3,001.5	57.4	6,650.5	53.3
B (179.2)	1,220.0	75.3	4,414.0 (3,181.7)	75.1	3,247.0	55.3	6,539.4	47.1
C (358.4)	2,391.7	83.8	3,845.3 (1,395.6)	74.3	2,779.3	53.7	5,368.4	44.0
D (537.6)	3,351.1	81.1	3,545.5 (----------)	71.0	2,751.5	55.0	5,513.5	46.0

[a]Values within parentheses refer to the amount (mg./100 g.) of dietary phytic acid phosphorus.
[b]Values within parentheses refer to the amount (mg.) of inorganic phosphorus retained.

contrary to those of Maddaiah et al. (25), retention of calcium remained unaffected by dietary phytate levels (Table III). Normal calcification apparently resulted from hydrolysis of phytates to yield available phosphorus. The amount of phytate hydrolyzed increased with dietary phytate levels (Table III). Probably not all of the phytate hydrolyzed underwent complete cleavage to inositol and inorganic phosphorus since, of the total phosphorus that was retained, progressively less was represented by inorganic phosphorus as the dietary phytate level was increased.

The influence of the duration of feeding and of the levels of dietary phytates on intestinal phytase activity did not appear to suggest that the enzyme is inducible by its substrate (Fig. 1). At 2 weeks, enzyme activity was highest on diet D. No appreciable change occurred by the fourth week; however, enzyme activity rose sharply by the sixth week except that the increase on diet B was only about half as much as on the other three diets. Bone-ash content and Hb, Hct levels were lowest also on diet B, suggesting a relationship between enzyme activity and iron and phytate availability. On the whole, the failure to obtain a consistent quantitative correlation suggests that the enzyme was not adaptable presuming that the in vitro measurement of enzyme activity represents the in vivo activity prevalent during digestion. Roberts and Yudkin (26) reported that administration of bran or sodium phytate usually decreased rather than increased the enzyme activity. It may be inferred that the availability of iron may not be affected by a somewhat increased consumption of natural phytate in the form of cereals on diet otherwise low in bulk and adequate in calcium, vitamin D, and iron.

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