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## The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans<sup>1,2</sup>

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**ABSTRACT** The inhibiting effect of phytate on nonheme-iron absorption from different protein sources was examined in human subjects using extrinsic radioiron labeling. A drink containing maltodextrose and corn oil was used as a control meal to which was added sufficient sodium phytate to provide 300 mg phytic acid and/or various protein sources. The proteins were selected to cover a broad range of effects on bioavailability and included egg white, meat, and phytate-free soy protein. When sodium phytate alone was added, there was a pronounced 83–90% reduction in mean absorption in separate studies with a composite average decline of 86%. Despite a wide range in absorption from meals containing the three protein sources, a remarkably similar relative inhibition was observed when sodium phytate was added. No significant difference in the inhibiting effect of phytate could be detected with additions ranging from the equivalent of 50–300 mg phytic acid to a meal containing egg white as the protein source. Our studies found no evidence that the inhibiting effect of phytate depends on the protein composition of the meal. *Am J Clin Nutr* 1996;63:203–7.

**KEY WORDS** Phytate, iron, absorption, proteins

### INTRODUCTION

The development of the extrinsic radioiron tag for measuring the absorption of nonheme iron in complex meals in human subjects has led to a marked increase in our knowledge of factors affecting the bioavailability of dietary iron and an expanding list of specific biochemical determinants of nonheme-iron absorption. The most important facilitating factors are ascorbic acid and animal tissue foods whereas a larger list of inhibitory factors includes polyphenols, phytate, phosphate, calcium, and fiber (1–3). To date, most studies using an extrinsic radioiron tag have focused on the effects of a specific determinant separate from other factors. Consequently, it is difficult to extrapolate the results to a complete diet that contains a variety of enhancers and inhibitors of nonheme-iron absorption.

The present investigation was undertaken to evaluate the interaction between two key determinants of nonheme-iron bioavailability: the dietary protein source and the phytate content. The type of dietary protein is believed to account in part for the marked differences in the prevalence of iron deficiency in different countries. Populations consuming ample quantities of meat and other animal foods generally have favorable iron status whereas those in countries where the diet comprises

mainly cereals or legumes are much more likely to be iron-deficient (4). The high prevalence of iron deficiency in these countries could be due to the nature of the protein in plant foods, the high content of dietary phytate, or perhaps an interaction between these two determinants of nonheme-iron absorption. Because phytate forms highly insoluble complexes with both protein and iron, the inhibiting effect of phytate on iron absorption may depend on the type of dietary protein. In the present study, we compared the inhibiting effect of phytate on three protein sources that differ markedly in their influence on nonheme-iron absorption.

### SUBJECTS AND METHODS

#### Subjects

Iron absorption tests were performed in 39 volunteer subjects between the ages of 19 and 37 y. Twenty-one women and 18 men were divided into four separate study groups containing 8–12 subjects each. All participants were in good health and denied a history of disorders that are known to affect iron absorption. Three subjects had depleted iron stores as defined by a serum ferritin concentration < 12 µg/L, but none of the subjects were anemic. Written informed consent was obtained from each volunteer before the investigation. All experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

#### Iron absorption measurements

Iron absorption was measured in each subject from four separate test meals. On the day before the first test, blood was drawn for measurement of background radioactivity, packed cell volume, and serum ferritin concentration. The first two meals, labeled with either <sup>55</sup>Fe or <sup>59</sup>Fe, were fed on the next 2 d between 0700 and 0900 after a 10-h fast. Subjects were instructed to take nothing further by mouth for 3 h after the test meal. Fourteen days after the second test dose, blood was

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obtained for measurement of incorporated red cell radioactivity. The second pair of separately labeled test meals was then fed on 2 successive days and a final blood sample was obtained 2 wk later to measure the increase in red cell radioactivity. Measurements of blood radioactivity were performed on duplicate 10-mL samples of whole blood by using a modification of the method of Eakins and Brown (5). Percentage absorption was determined from the blood volume, which was estimated from height and weight (6, 7). The red cell incorporation of absorbed radioactivity was assumed to be 80% (8).

### Preparation of labeled meals

The design and meal composition of the first three studies were similar. Meal A was a control meal containing maltodextrin and corn oil (MDO meal) but no protein or phytate. This liquid meal contained 67 g hydrolyzed corn starch (Amaizo; American Maize Products Company, Hammond, IN), 36 g corn oil (Mazola, Englewood Cliffs, NJ), and 12 mL vanilla extract. Meal B was the MDO meal to which was added 300 mg phytic acid (84.6 mg phytate-P) as sodium phytate (Fisher Scientific Company, Fair Lawn, NJ) immediately before serving. Meal C was the MDO meal with 30 g added protein; the protein source in study 1 was egg white (Monark Egg Corporation, Kansas City, MO), in study 2 it was 39.4 g cooked freeze-dried beef (Freeze-dry Products, USA, Inc, San Jose, CA), and in study 3 it was phytate-free soy protein isolate (Nestec, Lausanne, Switzerland). The phytate content of the soy protein was reduced to < 0.01 mg/g by enzymatic digestion as described previously (9). The protein sources were added as dry ingredients and mixed thoroughly with the liquid MDO before serving. Meal D was the MDO meal to which was added both sodium phytate and protein in the same amounts added to meals B and C, respectively. The total iron content of each meal was determined by atomic absorption spectrophotometry and the protein content (nitrogen  $\times$  6.25) was measured by the Kjeldahl method (Doty Laboratories, Kansas City, MO). The iron content of the meals in studies 1 and 4 was 4.1 mg and in studies 2 and 3 was 6.2 and 5.2 mg, respectively, because of the higher native iron content of meat and soy protein.

Study 4 was performed to examine the inhibiting effect of amounts of phytic acid smaller than the 300 mg tested in studies 1–3. The first pair of meals in study 4 contained 50 mg phytic acid (14.1 mg phytate-P) added as sodium phytate to the MDO meal without protein (meal A) or with 30 g protein as egg white (meal B). The second pair of meals contained 100 mg phytic acid (28.2 mg phytate-P) added as sodium phytate to the MDO meal without protein (meal C) or with 30 g protein as egg white (meal D). When compared with the results in study 1, study 4 permitted a comparison of the effect of 50, 100, and 300 mg phytic acid with and without egg white.

All meals were tagged extrinsically (10) by adding the required radioactivity to 1.0 mL 0.01 mol HCl/L containing sufficient ferric chloride to bring the iron content to 4.1 mg unless otherwise stated. The radioactive dose was 37 kBq  $^{59}\text{FeCl}_3$  or 74 kBq  $^{55}\text{FeCl}_3$  (Dupont, De Nemours and Co, Inc, NEN Products, Wilmington, DE).

### Statistical analysis

The absorption data were converted to logarithms for calculating geometric means and SEs and also for statistical analy-

sis. Then the results were reconverted to percentage absorption as antilogarithms (11). Comparison of iron absorption from any given pair of test meals within each study was made by paired *t* test to determine whether the log absorption ratio differed significantly from zero. Because of the marked effect of iron status as measured by serum ferritin on percentage absorption values, absorption data from different studies were compared by analysis of covariance using the log serum ferritin as a covariant. When the absorption ratios were compared for added protein and/or phytate obtained in different studies, the variances for these ratios were found to differ significantly based on Bartlett's test ( $P = 0.03$ ). Consequently, these ratios were compared by the Kruskal-Wallis test and Duncan's multiple-range test (SAS Institute Inc, Cary, NC).

### RESULTS

The addition of phytate equivalent to 300 mg phytic acid to the MDO meal in study 1 produced a dramatic reduction in iron absorption from 21.69% to 2.15%, giving an absorption ratio with to without phytate of 0.10 (Table 1). The inhibiting effect of egg white alone (meal C) was less pronounced, absorption from the control meal decreasing by 55% to 9.67%. The lowest mean absorption (1.41%) was observed when both egg white and phytate were added. The absorption ratio relative to the MDO meal of 0.07 was therefore not different from the ratio of 0.10 observed when phytate alone was added to the MDO meal.

Absorption from the MDO meal in study 2 was somewhat lower than in study 1 but a similar inhibiting effect of 300 mg phytic acid was observed; the reduction from 10.77% to 1.83% represented an inhibition of 83% compared with 90% in study 1. In contrast with the inhibiting effect of egg white observed in study 1, a significant increase in the percentage absorption was observed when meat was added to the MDO meal; absorption increased to 26.73%, a relative increase of 2.5-fold. However, a relative decrease of 78% in absorption was seen when phytate was added to the meal containing meat, similar to the 85% reduction observed with egg white in study 1.

In study 3, which examined the interaction between phytate-free soy protein and phytate, the inhibiting effect of 300 mg phytic acid on iron absorption from the MDO meal was similar to that observed in the first two studies as reflected by an absorption ratio with to without phytic acid of 0.16. The inhibiting effect of soy protein alone was greater than that of egg white, absorption falling from 11.44% to 2.6%, corresponding to a relative decrease of 77%. When phytate was added to the soy-containing meal, the 53% reduction in iron absorption from 2.60% to 1.22% was not significant. The absorption ratios in the first three studies with and without phytate added to the MDO meal in the absence of protein (meal B:meal A) were similar, ranging from 0.10 to 0.17. The differences were not significant when examined by analysis of variance ( $F = 2.02$ ,  $P = 0.15$ ); the pooled geometric mean ratio with and without phytate was  $0.14 \pm 1\text{SE}$  (range: 0.12–0.15). This pooled ratio was then compared with ratios observed when phytate was added to the three protein-containing meals (meal D:meal C). When the four absorption ratios were analyzed by the Kruskal-Wallis test, the differences were significant ( $F = 6.19$ ,  $P < 0.001$ ; Figure 1). The ratio of 0.47

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TABLE 1

Iron absorption from a maltodextrose and corn oil drink without and with added phytate and protein<sup>1</sup>

Study and meal additions	Serum ferritin μg/L	Iron absorption % of dose	Absorption ratios					
			B:A	C:A	D:B	D:C	D:A	
1 (n = 7F, 5M)								
A, none	34 (28-41)	21.69 (16.98-27.71)	(0.08-0.12)	(0.35-0.57)	(0.49-0.88)	(0.11-0.194)	(0.05-0.08)	
B, P (300 mg)		2.15 (1.77-2.60)	0.10 <sup>2</sup>	0.45 <sup>3</sup>	0.65	0.15 <sup>2</sup>	0.07 <sup>2</sup>	
C, E		9.67 (7.40-12.61)						
D, E + P (300 mg)		1.41 (1.03-1.90)						
2 (n = 4F, 6M)								
A, none	30 (21-43)	10.77 (7.45-15.56)	(0.14-0.21)	(2.17-2.84)	(2.46-4.00)	(0.17-0.27)	(0.41-0.70)	
B, P (300 mg)		1.83 (1.29-2.61)	0.17 <sup>2</sup>	2.5 <sup>2</sup>	3.14 <sup>3</sup>	0.22 <sup>4</sup>	0.53 <sup>5</sup>	
C, M		26.73 (19.77-36.14)						
D, M + P (300 mg)		5.76 (3.82-8.67)						
3 (n = 4F, 5M)								
A, none	49 (38-64)	11.44 (9.46-13.83)	(0.13-0.20)	(0.18-0.29)	(0.43-1.01)	(0.23-0.78)	0.07-0.16)	
B, P (300 mg)		1.82 (1.36-2.43)	0.16 <sup>2</sup>	0.23 <sup>4</sup>	0.67	0.47	0.11 <sup>4</sup>	
C, S		2.60 (2.01-3.36)						
D, S + P (300 mg)		1.22 (0.92-1.64)						
4 (n = 6F, 2M)								
A, P (50 mg)	41 (31-54)	1.70 (1.29-2.24)	(0.94-1.69)	(0.44-0.68)	(0.71-0.92)	(1.32-2.62)	—	
B, P (50 mg) + E		2.14 (1.66-2.76)	1.26	0.55 <sup>5</sup>	0.81	1.86		
C, P (100 mg)		0.93 (0.66-1.31)						
D, P + E (100 mg)		1.73 (1.33-2.25)						

<sup>1</sup> Geometric mean (±SE). Subjects aged 23-25 y and had packed cell volumes 43-45%. P, phytate; E, egg white; M, meat; S, soy protein.

<sup>2-5</sup> Significantly different from 1.0 (paired *t* test): <sup>2</sup> *P* < 0.0001, <sup>3</sup> *P* < 0.01, <sup>4</sup> *P* < 0.001, <sup>5</sup> *P* < 0.05.

obtained with soy protein was shown to be different from no protein, egg white, and meat when compared by Duncan's multiple-range test. However, the differences among the ratios obtained with the latter three were not statistically significant.

In study 4, the effect of adding smaller quantities of phytate to the MDO meal with and without egg white was tested. With meals containing either 50 or 100 mg phytic acid, the addition of egg white produced a slight increase in iron absorption, from 1.7% to 2.1% and from 0.9% to 1.7%, respectively, but in neither case was the difference significant. The findings were similar to those observed in study 1 in which the addition of egg white to a meal containing 300 mg phytic acid resulted in a slight but insignificant reduction in iron absorption (from 2.15% to 1.41%). Thus, with all three amounts of phytate, no significant effect of egg white on the inhibition by phytate was seen.

The effect of phytate dose on absorption for meals with and without added protein was examined by comparing the results in studies 1 and 4. Because there was no common meal in the two studies, the comparison was performed with analysis of covariance using log serum ferritin as a covariant (Table 2). With meals containing no protein, the reduction with all amounts of phytate was significant compared with the control meal containing no phytate. There was also a significant difference between 100 and 300 mg phytic acid but in the opposite direction than was expected. When different amounts of phytate were added to the MDO meal containing egg white, the reduction in iron absorption was significant for all amounts when compared with absorption from the MDO meal containing no phytate. Although we have observed some effect of phytate dose on iron absorption, no differences were significant among three amounts of added phytate. A 57% difference in

absorption from the meal containing 50 mg phytic acid could have been detected at the 5% significance level.

DISCUSSION

The present study was designed to examine the possible interaction between the protein moiety of a meal and its phytate content with respect to the effect on nonheme-iron absorption. There is abundant published evidence to indicate that protein per se has an important but highly varied influence on iron absorption (12-15). In the present study, we chose egg white to represent a protein with an intermediate influence on nonheme-iron absorption. This protein source has been used extensively in prior studies in this laboratory because of the high consistency of the product, its relatively neutral effect on iron absorption, and the ease with which it can be substituted serially

TABLE 2

Dose effect of phytate on iron absorption<sup>1</sup>

Phytate (mg)	Absorption			
	Uncorrected		Corrected <sup>2</sup>	
	No protein	Egg white	No protein	Egg white
	%			
0	21.69	9.67	21.19 <sup>a</sup>	9.44 <sup>a</sup>
50	1.70	2.14	1.76 <sup>b,c</sup>	2.22 <sup>b</sup>
100	0.93	1.74	0.97 <sup>b</sup>	1.80 <sup>b</sup>
300	2.15	1.41	2.10 <sup>c</sup>	1.38 <sup>b</sup>

<sup>1</sup> Values with different superscript letters are significantly different, *P* < 0.05.

<sup>2</sup> Corrected means using log serum ferritin as a covariant.

for other proteins in our control MDO meal. Egg white has a mild inhibitory effect on iron absorption based on earlier studies showing that doubling the egg white added to a meal results in a significant decrease in iron absorption (16). Nevertheless, the effect of egg white is comparable with other sources of protein including milk, cheese, and whole egg (17). Meat was the obvious choice for a protein that facilitates iron absorption, a finding that has been observed by many investigators since it was originally described by Layrisse et al (18). At the other extreme, isolated soy protein has been repeatedly shown to be one of the most potent inhibitors of nonheme-iron absorption (19–22). The striking inhibitory effect of soy protein was attenuated in the present study by removing its phytate content, one of the variables under study. Although the inhibitory effect of soy protein was reduced four- to fivefold by removing its phytate (9), it was still inhibitory compared with egg white. Thus, based on the ratios with and without protein (meal C:meal A) of 0.23 for soy protein and 2.5 for meat, there was a 10-fold difference in the effect on nonheme-iron absorption of the protein sources studied in this report.

The second variable tested in this study was the phytate content of the meal. As for protein, there is now ample evidence that dietary phytate has a potent inhibitory effect on nonheme-iron absorption. Hallberg (23) showed that the strong inhibitory influence of bran is due primarily to its phytate content although an additional unexplained component of bran may inhibit iron absorption independently of its phytate content. A recent study in which phytate was reduced in various soy protein products demonstrated that nearly complete removal of phytate to  $< 10$  mg/meal must occur before a significant increase in iron absorption occurs; no consistent relation between phytate content and iron absorption was observed at higher phytate concentrations (9). However, a recent study by Hallberg et al (24) showed a modest dose-related inhibitory effect when increasing amounts of sodium phytate were added to wheat rolls containing a low amount of phytate. The amount of added phytate in the latter study covered a broad range from 10 to 900 mg phytic acid compared with the more limited range from 50 to 300 mg phytic acid in the present study. In our study, we did not show progressive inhibition of iron absorption when phytate was added to the meal containing egg white although a slight downward trend in absorption was observed (Table 2). These studies indicate that small amounts of phytate can produce maximal inhibition of iron absorption. The fact that phytate must be almost totally removed to eliminate its inhibiting effect undoubtedly explains why in certain reports, a moderate decrease in phytate content was found to have no effect on iron absorption (19, 25).

There is general consensus that the wide range in bioavailability of dietary nonheme iron is due to the nature of the iron complexes formed in the lumen of the duodenum where iron is maximally assimilated. For protein, differences in iron bioavailability are best explained by the nature of the peptides released from protein during digestion. With meat, it is likely that soluble iron complexes are formed that prevent the precipitation of iron in the lumen and thereby facilitate its uptake by the mucosal cells. The striking inhibiting effect of phytate on iron absorption whether it is contained in the diet or added as sodium phytate is also best explained by the formation of insoluble iron complexes. Because of its strong negative charge at acidic pH, phytic acid has a tendency to bind both cations

and proteins. For example, DeRham and Jost (26) reported that at pH 7.5,  $> 40\%$  of the phytate in defatted soy flour is bound to protein based on the inability to dialyze it. At a pH of  $\approx 7$  in the lumen of the upper small intestine, there is evidence for the formation of a protein-cation-phytic acid complex in which iron would play a significant role (27). Because the nature of complexes formed between protein, phytate, and iron may depend largely on the nature of the peptides released in the gastrointestinal tract, we anticipated a variable effect of added phytic acid depending on the type of protein in the meal.

Surprisingly, the proportional reduction in iron absorption with added phytate was remarkably similar even in the complete absence of protein (Figure 1). The one exception was soy protein, with which the relative decrease was somewhat less than that observed with either egg white or meat. It is difficult to place much significance on this finding because percentage absorption was almost identical with meals that contained either egg white or soy protein. The lesser proportional inhibition observed when phytate was added to the soy protein meal may reflect the lower baseline absorption of the meal containing soy protein (2.6%) compared with a much higher basal absorption with the meal containing egg white (9.7%). There are many examples in which the relative effect of an enhancer or an inhibitor varies with the baseline availability of the meal. For example, EDTA has little or no effect on iron absorption when added to a meal of high bioavailability whereas a pronounced facilitating effect is observed when it is added to meals from which iron is poorly absorbed (28, 29). Similarly, the iron absorption-enhancing effect of vitamin C is less pronounced in a highly available meal containing meat

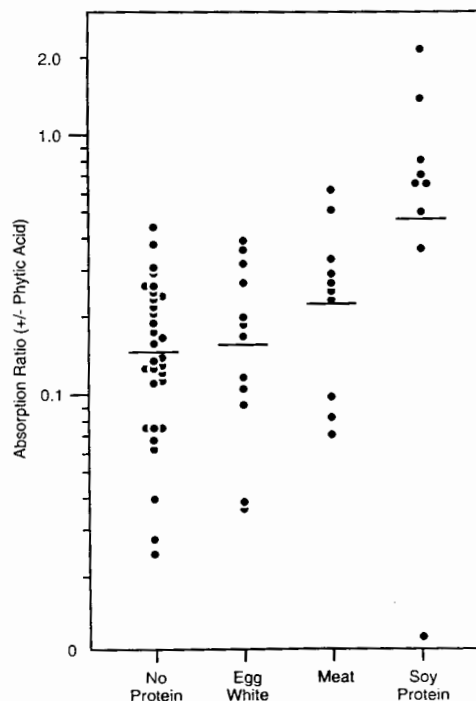


FIGURE 1. Ratio of iron absorption from the MDO meal (control meal containing maltodextrose and corn oil) with to without added phytate in the absence and in the presence of egg white, meat, and soy protein. The mean value of 0.14 for the protein-free meal represents the pooled mean ratio of the first three studies.

than it is with a meal of low bioavailability (30). Thus, it is likely that the less pronounced relative inhibition in iron absorption observed when phytate was added to soy protein reflects only a low baseline availability of the meal.

In the amounts of phytate and protein that we examined in the present study, the lack of a clear interaction between them in regard to their inhibiting effect on iron absorption is encouraging when extrapolating the effects of either of these determinants of iron absorption to complex meals. For example, the facilitating effect of meat on iron absorption compared with other proteins such as egg white and soy protein persisted in the present study despite a marked reduction in absorption with the addition of 300 mg phytic acid. Indeed, the relative facilitating effect of meat on iron absorption was even more pronounced in the presence of phytate than in its absence. At least for these two key determinants of food iron availability, it is possible to estimate their effect on iron bioavailability in a complex meal independently of one another. Much more work will be needed before this concept can be applied to a larger number of factors that effect nonheme-iron bioavailability. ■

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