Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate[1-3]

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ABSTRACT The dose-dependent inhibitory effect of sodium phytate on iron absorption was studied in man by serving wheat rolls containing no phytates and rolls to which various amounts (seven dose levels between 2 and 250 mg expressed as phytate phosphorus) were added just before serving. Fe in the two kinds of rolls was labeled with two radioisotopes of Fe (^{55}Fe, ^{59}Fe) and the rolls were served on alternate days. The inhibition of Fe absorption was strongly related to the amount of phytate added; 2 mg inhibited absorption by 18%, ($p < 0.001$), 25 mg by 64% ($p < 0.001$), and 250 mg by 82% ($p < 0.001$). The addition of ascorbic acid significantly counteracted the inhibition whereas the corresponding effect of meat was less well defined and only seen at the highest phytate level. The marked inhibition of Fe absorption by phytates and the significant counteracting effect of ascorbic acid have wide nutritional implications. *Am J Clin Nutr* 1989;49:140–4.

KEY WORDS Iron absorption, man, phytates, ascorbic acid, meat

Introduction

The inhibiting effect of bran on iron absorption in man is well established and the high content of phytates in bran has long been considered to be the main causative factor (1). Recently, however, the role of phytates in explaining inhibition by bran has been questioned because of two important observations in an extensive study in man (2): a marked reduction of the phytate content of bran by autoenzymatic digestion did not increase the absorption of Fe to a statistically significant degree and Fe in monoferric phytate, the most common form of Fe in wheat, was as absorbable when it was given alone as a simple Fe salt. No explanation, however, was found for the marked inhibitory effect of bran.

Similar studies made more recently in our laboratory to find the cause of the inhibitory effect of bran on Fe absorption showed that phytates are the main cause of inhibition (3). Removal of the phytates in bran by endogenous phytase significantly increased the absorption of Fe especially if the phosphates formed from phytates during the enzymatic dephytinization were removed by washing with water. Moreover, inhibition could be restored to a marked extent by restituting the phytate content.

With reference to the second observation, < 5% of the phytates in bran is in the form of monoferric phytate. Adding the other 95% of phytates (in the same amounts as present in bran) to white wheat flour inhibited Fe absorption to the same extent as an addition of bran.

Thus there is strong support for the prevailing opinion that phytates do in fact inhibit Fe absorption in man. The dietary intake of phytates is often high especially in developing countries. Efforts in industrialized countries to increase fiber intake can be expected to increase the content of phytates in the diet. Little is known about the relationship between the amount of phytates in a meal and the extent of inhibition of Fe absorption. This study addresses this relationship and attempts to counteract the inhibitory effect of phytates with ascorbic acid and meat.

Subjects and methods

Experimental design

The effect of phytates was studied by comparing in the same subject Fe absorption from wheat rolls containing no phytates

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with Fe absorption from wheat rolls to which known amounts of sodium phytate were added just before serving. The Fe content of the rolls was adjusted to 4.1 mg by adding ferrous sulphate to the dough. The two types of rolls (A and B) were given on alternate mornings after an overnight fast on four consecutive days in the order ABBA or BAAB. Water (150 mL) was drunk with the rolls. The A and B rolls were labeled with two different Fe isotopes, ^{55}Fe and ^{59}Fe. A blood sample was drawn 2 wk after the last roll was served to determine the content of ^{55}Fe and ^{59}Fe. The total retention of ^{59}Fe was measured by whole-body counting at the same time and the total retention of ^{55}Fe was calculated from the ratio of ^{55}Fe and ^{59}Fe in red cells.

Subjects

One hundred twenty-four subjects, 34 men and 90 women, volunteered for the present 14 studies. All subjects were healthy volunteers aged 19–47 y and each group included both men and women. Some of the subjects in each group were regular blood donors, which provided a reasonable range of intersubject variation in Fe absorption. Subjects were given written information about the aims and procedure of the study. The project was approved by the Ethical Committee of the Medical Faculty of the University of Göteborg.

Oral reference doses

A solution of 10 mL of 0.01 mol hydrochloric acid/L containing 3 mg of Fe as $FeSO_4$ and 30 mg of ascorbic acid labeled with ^{59}Fe was used as a reference in all studies. The 10-mL vials containing the Fe solution were rinsed twice with water and this was also consumed. Each subject received two reference doses on two consecutive mornings after overnight fasts. No food or drink was allowed for 3 h after the reference dose. Each subject received a total of 55.5 kBq ^{59}Fe.

Meal composition and iron isotope labeling of meals

All meals contained two wheat buns, each prepared from 40 g unfortified white wheat flour, (60% extraction) yeast, sugar, table salt, and water. The dough was fermented for 30 min at 23 °C. It was then kneaded and weighed amounts were transferred to small aluminum forms, which were left standing for 10 min for further fermentation. The bread was baked at 250 °C for 15 min. The flour was fortified with 3.7 mg Fe as $FeSO_4$ per 80 g flour. Native Fe content was 0.4 mg. Two buns were served with 20 g margarine and 150 mL water. In series 13 and 14, 50 g of grilled beef (round) seasoned with salt was served with the buns. The dough for the wheat rolls was labeled by mixing Fe isotope with water and yeast before adding the flour. The Fe isotope was added as ferric chloride in 0.01 mol HCl/L. Each meal was labeled with 46.3 kBq ^{59}Fe or 55.5 kBq ^{55}Fe. Sodium phytate (sodium inositol hexaphosphate, Sigma Chemical Co, St Louis, MO) was added in various amounts (2–250 mg expressed as phytate phosphorus) to the rolls before the margarine was spread. Ascorbic acid (AA) was added in the same way in some of the studies.

Chemical measurements

Aliquots of buns and hamburgers were freeze dried and ground to a powder in a porcelain mortar. Weighed amounts of this powder were analyzed for total Fe (4) and phytate P (5).

Iron absorption measurements

Relative absorption of ^{55}Fe and ^{59}Fe was calculated from analyses of blood samples. Absolute absorption of the two tracers was calculated from whole-body counting of ^{59}Fe and the relative absorption of the two tracers. Analysis of ^{55}Fe and ^{59}Fe in blood was made with a modification of the method described by Eakins and Brown (6). All procedures and methods of calculation were described previously (7, 8).

Expressing results of absorption measurements and statistical analyses

The ratio (A:R) of absorption of nonheme Fe from a meal (A) and from reference doses (R) is an expression of the bioavailability of nonheme Fe in the meal. There is a normal distribution of these ratio values, and mean and SDs of the ratio values are calculated in the usual way. The mean values of these ratios and their SEMs were multiplied by 40 to obtain the percentage absorption of Fe that corresponds to a 40% reference-dose absorption ($A_{40\%}$). Absorption values adjusted to a 40% absorption from reference doses were chosen because they correspond to the absorption expected in subjects who are borderline Fe deficient (9).

Results

Relationship between amount of sodium phytate added and inhibition of iron absorption

As shown in **Table 1** and **Figure 1**, there was a marked decrease in the absorption of Fe the more phytate P was added. The decrease was already significant when 2 mg phytate P was added and the rate of decrease was more marked at the lowest dose levels (from 2 to 10 mg phytate P). From 10 to 250 mg of phytate P, the rate of decrease of Fe absorption was lower but strongly significant. The 10-mg level corresponds to a ratio of 1 mol phytate P to 1 mol Fe. Visually, there seems to be a rectilinear, exponential relationship from this 10-mg point. The correlation coefficient for the range (10–250 mg) was very high ($r = 0.99$).

Effect of ascorbic acid on the inhibition of iron absorption by sodium phytate

The effect of AA was studied at three dose levels of phytates: 0, 25, and 250 mg of phytate P (Table 1). At all three levels 50 mg AA induced a marked increase of Fe absorption ($p < 0.01$, $p < 0.005$, and $p < 0.001$, respectively). The increase was most marked when the wheat rolls contained no phytate. The relative increase of the absorption was more marked the more the absorption was inhibited by phytate P. The increases at the levels 250 mg, 25 mg, and 0 mg phytate P were 184, 117, and 75%, respectively. The effect of adding 100 mg AA was also studied at the dose levels of 25 and 250 mg phytate P and showed a similar pattern.

Effect of meat on the inhibition of iron absorption by sodium phytate

At the dose level of 25 mg phytate P, there was no effect from adding 50 mg meat served as a hamburger with the wheat roll (Table 1). There was a significant in-

TABLE I
Iron absorption from different meals*

Study meals	Subjects†	Percent absorption: Meal A	Percent absorption: Reference dose R	A:R	$A_{40\%}$	$A_{40\ mg}$	Absorption ratio with and without Phy P (and/or AA)
1. W	3 M	17.6	28.2	0.54 ± 0.09‡	21.6 ± 3.6	0.89 ± 0.15	0.82 ± 0.04
W + 2 mg Phy P	6 F [3]	13.6	28.2	0.42 ± 0.06	16.8 ± 2.4	0.69 ± 0.10	
2. W		22.2	37.9	0.57 ± 0.08	22.7 ± 3.0	0.93 ± 0.12	0.61 ± 0.04
W + 5 mg Phy P	9 F [3]	13.7	37.9	0.35 ± 0.06	14.0 ± 2.4	0.57 ± 0.10	
3. W		17.4	39.0	0.47 ± 0.12	18.8 ± 4.8	0.77 ± 0.20	0.41 ± 0.04
W + 10 mg Phy P	6 F	7.7	39.0	0.21 ± 0.06	8.4 ± 2.4	0.34 ± 0.10	
4. W	4 M [1]	16.8	35.2	0.49 ± 0.07	19.6 ± 2.8	0.80 ± 0.11	0.36 ± 0.04
W + 25 mg Phy P	5 F [1]	6.1	35.2	0.16 ± 0.02	6.4 ± 0.8	0.26 ± 0.03	
5. W	5 M [2]	17.9	42.6	0.43 ± 0.06	17.2 ± 2.4	0.71 ± 0.10	0.31 ± 0.03
W + 50 mg Phy P	5 F [1]	5.1	42.6	0.13 ± 0.02	5.2 ± 0.8	0.21 ± 0.03	
6. W	4 M [1]	16.7	31.2	0.53 ± 0.09	21.2 ± 3.6	0.87 ± 0.15	0.29 ± 0.04
W + 100 mg Phy P	6 F [1]	5.4	31.2	0.17 ± 0.04	6.8 ± 1.6	0.28 ± 0.07	
7. W	2 M [2]	14.0	30.0	0.47 ± 0.05	18.8 ± 2.0	0.77 ± 0.08	0.18 ± 0.03
W + 250 mg Phy P	8 F	2.3	30.0	0.07 ± 0.01	2.8 ± 0.4	0.11 ± 0.02	
8. W	1 M [1]	16.7	36.6	0.56 ± 0.15	22.4 ± 6.0	0.92 ± 0.25	1.75 ± 0.17
W + 50 mg AA	6 F [1]	27.3	36.6	0.94 ± 0.27	37.6 ± 10.8	1.54 ± 0.44	
9. W + 25 mg Phy P	4 M [2]	7.1	36.3	0.18 ± 0.04	7.2 ± 1.6	0.30 ± 0.07	
W + 25 mg Phy P + 50 mg AA	6 F	14.2	36.3	0.36 ± 0.08	14.4 ± 3.2	0.59 ± 0.13	2.17 ± 0.20
10. W + 25 mg Phy P		6.5	40.6	0.17 ± 0.04	6.8 ± 1.6	0.28 ± 0.07	
W + 25 mg Phy P + 100 mg AA	8 F [2]	19.5	40.6	0.49 ± 0.09	19.6 ± 3.6	0.80 ± 0.15	3.56 ± 0.53
11. W + 250 mg Phy P	3 M [2]	6.2	36.4	0.14 ± 0.04	5.6 ± 1.6	0.23 ± 0.07	
W + 250 mg Phy P + 50 mg AA	5 F	15.0	36.4	0.38 ± 0.10	15.2 ± 4.0	0.62 ± 0.16	2.84 ± 0.48
12. W + 250 mg Phy P	3 M [1]	3.2	30.7	0.10 ± 0.02	4.0 ± 0.8	0.16 ± 0.03	
W + 250 mg Phy P + 100 mg AA	7 F [2]	10.0	30.7	0.31 ± 0.04	12.4 ± 1.6	0.51 ± 0.07	3.43 ± 0.53
13. W + 25 mg Phy P	4 M [2]	4.8	28.5	0.20 ± 0.04	8.0 ± 1.6	0.33 ± 0.07	
W + 25 mg Phy P + 50 g meat	4 F [1]	5.1	28.5	0.21 ± 0.03	8.4 ± 1.2	0.34 ± 0.05	1.12 ± 0.08
14. W + 250 mg Ph P	1 M [1]	4.0	35.9	0.09 ± 0.02	3.6 ± 0.80	0.15 ± 0.03	
W + 250 mg Phy P + 50 g meat	9 F [3]	6.9	35.9	0.17 ± 0.03	6.8 ± 1.20	0.28 ± 0.05	1.86 ± 0.22

* W, buns from white wheat flour; Phy P, phytate P; AA, ascorbic acid.
† Number of regular blood donors in brackets.
‡ $\bar{x}$ ± SEM.

crease (86%, $p < 0.001$), however, when the meat was given with rolls containing 250 mg phytate P.

Discussion

This is a model study on the effect of phytates on the absorption of dietary nonheme Fe. A very simple meal with a high bioavailability of Fe was chosen to increase the accuracy in measuring the inhibitory effect of the phytates and to avoid the influence of confounding dietary factors.

From comparisons of the inhibiting effect of bran, Na phytate, and mixtures of potassium and magnesium phytates, there is no reason to believe that the Na phytate used in this study has properties other than those of naturally occurring inositol hexaphosphates (3).

Fermentation and baking cause a partial degradation of phytates to simple phosphates and to inositol phosphates with fewer phosphate groups (10, 11). It is possible that these latter phosphates may have other Fe binding properties than those of hexaphosphates. The purpose of the present study, however, is not to address this question

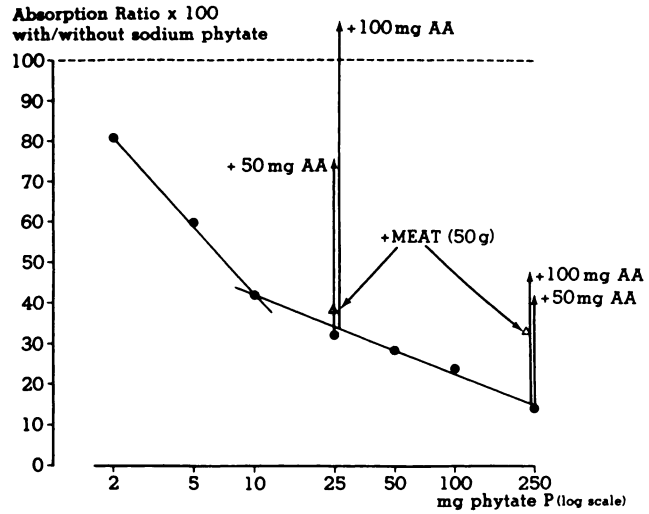

FIG 1. Effect on the absorption of iron from wheat rolls of adding different amounts of sodium phytate and effect of adding 50 and 100 mg ascorbic acid and 50 g meat.

but to investigate the effect of inositol hexaphosphate on Fe absorption, which is the main form of phytates in seeds, tubers, and roots.

The inhibition of inositol hexaphosphates is probably of the same magnitude as the inhibition obtained by the mixture of inositol phosphates remaining in the bread after baking. This opinion is based on a comparison of the Fe absorption from wheat rolls to which 10 mg of inositol hexaphosphate was added after baking (study 3) and from rolls made with another wheat flour that initially contained more phytates but had almost an identical content of phytate P (11 mg) after fermentation and baking (study 7 in a previous paper [3]). The phytate P content in the wheat rolls in the latter study was reexamined in stored freeze-dried samples with the method used in the present study. The Fe absorption from the rolls containing phytate in the previous study was 10% compared with the average absorption of 26.5% from rolls containing no phytates (mean value of 11 series: series 1, 4, and 8 in the previous study [3] and 1–8 in the present study). Thus the reduction in absorption was 57% from the phytate mixture remaining after fermentation and baking (3) and 60% from the added inositol hexaphosphates in the present study. This strongly suggests that the different inositol phosphates have a similar inhibitory effect on Fe absorption when the amount of phytate is expressed as phytate P.

The main finding in the present study is the increasing inhibition of Fe absorption with increasing amounts of phytate. As shown in Figure 1, this relationship seems to be composed of two regression lines with a point of intersection at 10 mg phytate P. It is possible that the relationship could also be described by a single equation. The reasons for describing the relationship as two regression lines are *1*) the very high correlation coefficient (r = 0.99) for the high range of phytate P (10–25 mg) and *2*) that the point of intersection (10 mg) corresponds to 1 Fe per molecule of inositol hexaphosphate. Thus there is a probable explanation for a two-phase relationship. The meal in this study contained 4.1 mg Fe. It is reasonable to assume that the relationship between inhibition of Fe absorption and amount of phytate P varies with the Fe content of the meals.

An unexpected finding in this study is the marked inhibitory effect of even small amounts of phytates. An important implication of this observation is that in studies on the effect of phytates on Fe absorption it is necessary to have a control meal containing no phytates. Otherwise the true inhibitory effect of phytates will be considerably underestimated. Most meals in Western countries contain phytates and many meals have a phytate-P content in the 10–100 mg range. In vegetarian diets and diets in developing countries, levels in the 250 mg range are not uncommon. A wheat roll made from 80 g of 70% extraction flour has a phytate-P content of 30 mg and a roll made from 80 g of 80% extraction flour has a content of 60 mg (12). The phytate content of breakfast cereals varies very much and may be in the range of 25–150 mg phytate P in a portion.

The inhibition by phytates observed in this study may not be the same in composite meals, which may contain factors that interact with the phytates. However, because inhibition of phytate (25 mg phytate P) was the same whether or not meat was present, the inhibition may be of about the same magnitude in composite meals as in wheat rolls. We have no explanation why meat had only a weak antiphytate effect. The present results are not consistent with early findings from our laboratory suggesting that the meat effect was an antiphytate effect (13).

The inhibitory effect of phytates was markedly counteracted by AA. This neutralizing effect of AA was related to the amount of AA given and the amount of phytates present. The effect of AA on increasing absolute absorption was greatest when no phytates were present in the meal and was smallest at the highest level of phytate. The relative effect of AA, however, was more marked the more the absorption was inhibited by phytates. About 80 mg AA would be needed to fully counteract the inhibition of 25 mg phytate P and that probably hundreds of milligrams of AA would be needed to fully counteract the inhibition of the 250 mg phytate P in the rolls.

The interaction observed between AA and phytates has wide nutritional implications. In diets with a high phytate content, the desired levels of AA should also be high. The most feasible way to improve Fe nutrition in populations where the traditional diet has a high phytate content would probably be to increase the AA content.

More studies are needed on the interaction of factors inhibiting and enhancing Fe absorption. These may lead to a better understanding of the causes of the variation of the bioavailability of Fe from different meals and diets, to a better ability to predict the absorption from various meals and diets, and to the development of new, effec-

tive, and realistic ways to improve Fe nutrition in different populations.

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