

Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption^{1,2}

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ABSTRACT The effects of maize-bran phytate and of a polyphenol (tannic acid) on iron absorption from a white-bread meal were tested in 199 subjects. The phytate content was varied by adding different concentrations of phytate-free and ordinary maize bran. Iron absorption decreased progressively when maize bran containing increasing amounts of phytate phosphorous (phytate P) (from 10 to 58 mg) was given. The inhibitory effect was overcome by 30 mg ascorbic acid. The inhibitory effects of tannic acid (from 12 to 55 mg) were also dose dependent. Studies suggested that ≥ 50 mg ascorbic acid would be required to overcome the inhibitory effects on iron absorption of any meal containing > 100 mg tannic acid. Our findings indicate that it may be possible to predict the bioavailability of iron in a diet if due account is taken of the relative content in the diet of the major promoters and inhibitors of iron absorption. *Am J Clin Nutr* 1991;53:537-41.

KEY WORDS Polyphenols, phytates, ascorbic acid, iron absorption

Introduction

The bioavailability of nonheme iron from any diet is dependent on the interaction of the promoters and the inhibitors of iron absorption contained within that diet (1). Two potent promoters of nonheme-iron absorption are meat and ascorbic acid (2, 3), and a number of studies documented the relative quantities of these two substances to be the most important determinants of iron bioavailability from Western-type meals (3, 4). In contrast, the diets of people in developing countries are often poor in meat- and ascorbic acid-containing foodstuffs (5-7) and, in addition, the staple cereals contain potent inhibitors of iron absorption. As a result, the prevalence of nutritional iron-deficiency anemia in many developing countries is high (8).

In planning strategies for the prevention of iron-deficiency anemia, it is therefore essential to have information on the bioavailability of iron in the diets of vulnerable populations (9). Although such information can be obtained by testing iron absorption from individual components or from the whole diet, such studies are extremely expensive and labor intensive. Alternative more cost-effective methods therefore warrant investigation. In this context the two most common inhibitors of nonheme-iron absorption are polyphenols and phytates, and the presence of either of them in foods such as cereals and legumes

has been shown to decrease the bioavailability of dietary iron markedly (10-13).

The prediction of iron bioavailability from different foodstuffs might therefore be facilitated if the dose-dependent inhibitory effects of phytates and polyphenols were systematically studied. This was done in the present study by use of bread as the food vehicle. In addition, the quantity of ascorbic acid needed to overcome the maximal effect of each inhibitor was documented.

Subjects and methods

Subjects

A total of 199 parous Indian housewives participated in these studies. The salient characteristics of this population group were described previously (13). Approval for the studies was obtained from the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg. Written informed consent was obtained from all subjects before the study. Each subject participated in one experiment only.

Preparation and administration of meals

Refined, unfortified wheat cake flour (Snowflake) was obtained from Premier Milling, Isando, South Africa. It contained 0.25 mg phytate phosphorus (phytate P)/g flour (14). Phytate-free maize bran was kindly supplied by AE Staley Manufacturing Company, Decatur, IL, and phytate-containing maize bran was purchased from Maize Corp, Silverton, Pretoria, South Africa. The phytate-free maize bran was produced by a procedure involving the soaking of shelled maize for 30-50 h in water (49-54 °C) containing 0.1-0.2% sulfur dioxide, followed by wet milling and the separation of starch and protein from the final fiber product. Tannic acid was obtained from E Merck, Darmstadt, FRG, and ascorbic acid from BDH Chemicals, Poole, UK.

The basic constituents of the bread prepared in all the studies were 1 kg wheat flour, 5 g salt, 5 g white granulated sugar, and

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TABLE 1

Iron absorption from a basic bread meal containing 10 mg phytate P (meal A) and from a bread meal containing different proportions of phytate-free and phytate-containing maize bran (meal B)

	Hemoglobin*	Transferrin saturation*	Serum ferritin†	Iron absorption†			Absorption ratio‡
				Meal A	Meal B	Reference	
	g/L	%	µg/L		%		
Meal B, 14 mg phytate P (n = 16)	117 ± 23	19 ± 11	6.7 (1.3-35.2)	14.3 (5.9-34.8)	12.6 (5.6-28.4)	78.3 (56.3-109.1)	1.21
Meal B, 22 mg phytate P (n = 14)	123 ± 17	23 ± 12	11.6 (2.2-61.4)	14.5 (5.4-39.4)	11.1 (4.2-29.0)	60.2 (35.0-103.6)	0.77
Meal B, 34 mg phytate P (n = 16)	118 ± 12	22 ± 9	10.8 (4.6-25.5)	14.4 (6.0-34.2)	7.7 (2.9-20.0)	57.1 (31.3-104.2)	0.61
Meal B, 58 mg phytate P (n = 11)	129 ± 13	25 ± 5	6.9 (2.0-23.4)	9.5 (4.7-19.3)	4.5 (1.8-11.6)	57.3 (35.7-91.8)	0.54

* $\bar{x} \pm SD$.

† Geometric mean and SD range in parentheses.

‡ Mean of individual ratios. Meal B/Meal A.

20 g rapid rising yeast (Anchor Yeast Pty Ltd, Industria, South Africa). A further 250 g bran was then added in certain studies (see below); the type of additive varied according to the aims of the particular study. The resultant mix was made into a dough with the addition of 10 mL sunflower oil and 800 mL water, and was baked for 1 h at 400 °C. Each person was then served 80 g bread with margarine.

Phytate studies. The bread used in one limb of each of the studies reported in Table 1 was prepared by the addition of 250 g phytate-free maize bran to the basic constituents of the bread. The comparison meals consisted of bread, to which 250 g of various mixtures of phytate-free and phytate-containing maize brans had been added. In this way it was possible to prepare breads with the same bran content but which had various phytate contents. The bread used in all the studies detailed in Table 2 consisted of the basic ingredients plus 250 g phytate-containing maize bran.

Tannic acid studies. White tannic acid-free bread was prepared from the basic ingredients alone, as described above. Bread

with various concentrations of tannin was prepared by adding tannic acid to the flour, mixing it thoroughly, and then baking it in the usual fashion (Table 3). In the studies documented in Table 4, a constant amount of tannic acid (420 mg/portion) was used in all the bread meals.

Additional elemental iron was given in all the studies. In the studies reported in Table 1, iron as $FeSO_4 \cdot 7H_2O$ was baked into the bread so that each individual received a total of 5-6 mg Fe. In the remaining studies it was considered desirable to keep the iron separate from the ascorbic acid and/or tannic acid for both technical and cosmetic reasons. To achieve this the bread meal was served with 15 g mashed potato/person on both days of the study. A solution of 3 mg Fe as $FeSO_4 \cdot 7H_2O$ was then added to each portion of mashed potato. When ascorbic acid was used, it was dissolved in water and added directly to each individual's bread serving. In this way it was separated from the iron by margarine and potato.

On one morning the meal was labeled with 111 kBq ^{55}Fe and on the other with 74-111 kBq ^{59}Fe (Amersham International).

TABLE 2

Iron absorption from a bread meal containing 58 mg phytate P (meal C) and from a bread meal containing 58 mg phytate P and increasing doses of ascorbate (meal D)

	Hemoglobin*	Transferrin saturation*	Serum ferritin†	Iron absorption†			Absorption ratio‡
				Meal C	Meal D	Reference	
	g/L	%	µg/L		%		
Meal D, 30 mg ascorbate (n = 11)	127 ± 17	23 ± 7	6.6 (1.6-27.0)	6.7 (2.4-18.4)	12.6 (3.5-45.7)	46.7 (22.7-96.4)	2.08
Meal D, 50 mg ascorbate (n = 12)	121 ± 17	23 ± 12	20.7 (5.0-85.2)	3.8 (1.3-11.4)	10.4 (3.5-30.6)	52.7 (29.0-95.7)	2.97
Meal D, 150 mg ascorbate (n = 14)	120 ± 9	19 ± 8	6.2 (1.4-28.2)	10.4 (4.9-22.2)	27.4 (12.1-62.2)	76.3 (41.9-118.0)	3.21

* $\bar{x} \pm SD$.

† Geometric mean and SD range in parentheses.

‡ Mean of individual ratios. Meal C/meal D.

TABLE 3
Iron absorption from a bread meal (meal E) and from a bread meal with increasing doses of tannic acid (meal F)

	Hemoglobin*	Transferrin saturation*	Serum ferritin†	Iron absorption†			Absorption ratio‡
				Meal E	Meal F	Reference	
	g/L	%	µg/L	%			
Meal F, 12 mg tannic acid (<i>n</i> = 12)	115 ± 14	17 ± 12	11.5 (3.2–41.4)	9.1 (2.6–32.1)	6.0 (1.7–20.6)	66.8 (38.6–115.8)	0.70
Meal F, 26 mg tannic acid (<i>n</i> = 12)	128 ± 15	20 ± 10	10.4 (2.0–54.7)	7.4 (2.4–22.9)	3.3 (1.0–10.8)	59.7 (38.6–92.3)	0.48
Meal F, 55 mg tannic acid (<i>n</i> = 15)	126 ± 15	20 ± 10	8.3 (1.8–37.5)	18.4 (8.5–39.7)	5.0 (1.8–13.9)	62.5 (40.0–97.6)	0.33
Meal F, 263 mg tannic acid (<i>n</i> = 14)	120 ± 13	25 ± 14	24.8 (4.0–151.9)	8.5 (3.0–24.0)	1.5 (0.5–4.7)	40.8 (18.7–89.3)	0.22
Meal F, 833 mg tannic acid (<i>n</i> = 11)	128 ± 16	24 ± 10	24.0 (10.0–57.3)	13.9 (6.4–30.1)	2.6 (1.2–5.7)	50.0 (24.3–102.8)	0.21

* $\bar{x} \pm$ SD.

† Geometric mean and SD range in parentheses.

‡ Mean of individual ratios. Meal F/meal E.

Amersham, Buckinghamshire, UK). The solutions of radiolabeled iron were either added directly onto the bread (studies listed in Table 1) or to the mashed potato studies (listed in Tables 2, 3, and 4). The absorption of iron from the two meals was measured and compared for each group of subjects. The meals were consumed on consecutive mornings after an overnight fast, and no food or drink other than water was allowed for 4 h thereafter.

Two weeks after the administration of the meals, blood samples were obtained by peripheral venipuncture after an overnight fast for the measurements of ^{55}Fe , ^{59}Fe , hemoglobin concentration, serum iron, unsaturated iron-binding capacity, and serum ferritin concentration. The capacity of each individual to absorb iron was then measured by feeding a standard dose (3 mg Fe) of radiolabeled ferrous ascorbate (13) and taking a further blood sample 2 wk later. The calculations used to determine the absorption of iron from the two meals and the reference iron salt were identical to those described previously (13).

Radioisotopic, chemical, and statistical methods

The methods used to determine the radioactivity in the blood and food samples, as well as those used to measure the hemoglobin and serum iron and ferritin concentrations and the unsaturated iron-binding capacity, were as described previously (13). The concentration of phytate P in foods was measured by a modification (13) of the method of Wheeler and Ferrel (14), with a minimum limit of detection of 0.3 mg phytate P and a CV of 2.4%. The maximum potential whole-body-irradiation dosage if all the radiolabeled iron was absorbed fell well within the permissible annual dose (13).

Because the percentage iron absorptions and serum ferritin concentrations were positively skewed, results were expressed as geometric means and SD ranges. The significance of differences between the absorption of the two isotopes in each study was calculated by use of Student's *t* test for paired observations.

Results

The individual studies demonstrating the dose-dependent inhibitory effect of phytate are shown in Table 1. The phytate P

content of the wheat bread (10 mg) was serially increased to 58 mg by the addition of appropriate mixtures of phytate-free and phytate-containing maize bran. In each instance iron absorption was compared with that from the wheat bread prepared with the addition of phytate-free maize bran. The fiber content in both meals was thus kept constant in all the studies. The geometric-mean iron absorptions from the two breads were very similar when the phytate P contents were 10 and 14 mg, respectively ($t = 0.9$, $P = 0.4$). However, when the phytate P was raised from 10 to 22 mg, the geometric-mean iron absorption was significantly inhibited ($t = 3.9$, $P = 0.002$). With greater increases to 34 and 58 mg, respectively, there was a further reduction to between 40% and 50% in iron bioavailability ($t = 3.2$, $P = 0.006$; and $t = 4.6$, $P = 0.001$, respectively).

The effect of increasing doses of ascorbic acid on iron absorption from bread prepared with phytate-containing maize bran (58 mg phytate P/portion served) is shown in Table 2. All three doses (30, 50, and 150 mg) of ascorbic acid resulted in significant improvements in the geometric-mean absorption of iron from the phytate-containing bread meal ($t = 3.2$, $P = 0.01$; $t = 3.5$, $P = 0.005$; and $t = 6.2$, $P = 0.0001$, respectively). Calculation of the absorption ratio showed that a dose of 30 mg ascorbic acid doubled iron absorption (absorption ratio 2.08), which suggests that this dose was able to overcome the effect of 58 mg phytate P (Table 1). A further increase in the absorption ratio from 2.08 to 2.97 was obtained when the dose of ascorbic acid was increased from 30 to 50 mg. Raising the dose of ascorbic acid to 150 mg caused only a modest further incremental effect (absorption ratio 3.21).

The effects of increasing doses of tannic acid on iron absorption from a wheat-bread meal are shown in Table 3. A significant inhibitory effect was noted with as little as 12 mg tannic acid ($t = 2.2$, $P = 0.05$). The inhibitory effect was greater with 26 and 55 mg tannic acid ($t = 4.1$, $P = 0.002$; and $t = 5.0$, $P = 0.0002$, respectively) and was profound with 263 and 833 mg ($t = 4.6$, $P = 0.0005$; and $t = 7.7$, $P = 0.0001$, respectively). Absorption ratios indicated that the maximal inhibitory effect was achieved between 12, 26, and 55 mg tannic acid, with the ratio dropping to 0.7, 0.48, and 0.33, respectively. The dose-response curve

TABLE 4

Iron absorption from a bread meal containing 420 mg tannic acid (meal G) and from a bread meal containing 420 mg tannic acid and increasing doses of ascorbate (meal H)

	Hemoglobin*	Transferrin saturation*	Serum ferritin†	Iron absorption†			Absorption ratio‡
				Meal G	Meal H	Reference	
	g/L	%	µg/L		%		
Meal H, 25 mg ascorbate (<i>n</i> = 12)	128 ± 5	32 ± 9	21.0 (6.3–70.8)	1.5 (0.8–3.0)	3.9 (1.8–8.3)	61.7 (46.5–81.8)	2.90
Meal H, 100 mg ascorbate (<i>n</i> = 16)	126 ± 13	25 ± 12	18.9 (6.0–59.0)	2.0 (0.6–6.7)	7.9 (3.1–20.0)	63.1 (36.9–107.9)	5.51
Meal H, 500 mg ascorbate (<i>n</i> = 13)	114 ± 12	19 ± 8	8.1 (3.4–19.3)	3.6 (1.0–13.3)	18.0 (8.1–39.9)	44.7 (29.2–68.2)	7.11

* $\bar{x} \pm SD$.

† Geometric mean and SD range in parentheses.

‡ Mean of individual ratios. Meal H/meal G.

then leveled off, even with very large doses (263 and 833 mg) of tannic acid, which reduced absorption to about one-fifth (absorption ratio 0.22 and 0.21, respectively). The effect of ascorbic acid on iron absorption from a bread meal containing 420 mg tannic acid is shown in Table 4. All three doses (25, 100, and 500 mg) of ascorbic acid significantly improved iron absorption from the inhibitory bread meal ($t = 4.1$, $P = 0.002$; $t = 5.0$, $P = 0.0002$; and $t = 5.0$, $P = 0.0003$, respectively). A dose of 25 mg ascorbic acid restored the absorption ratio to only 0.5 of that expected from tannin-free bread (calculated as fivefold that of the bread containing 420 mg tannic acid). Corresponding ratios with 100 and 500 mg ascorbic acid were 0.79 and 1.0, respectively (Table 4).

Discussion

The effect of phytate on nonheme-iron absorption has been extensively investigated. The *in vitro* solubility characteristics of phytate suggest that it would have an inhibitory effect on iron bioavailability (15, 16). This was confirmed in a number of animal studies (17, 18), and, despite a few reports to the contrary (19, 20), the bulk of the evidence indicates that added sodium phytate diminishes iron bioavailability from a meal (12, 21–23). Although it has been questioned whether endogenous dietary phytates exert a similar effect (24–28), it has been shown that the removal of endogenous phytate from a wheat-bran meal improves iron absorption (27). When small amounts of added sodium phytate (2, 5, and 10 mg phytate P) were added to a bread meal, there was a progressive drop in the absorption ratio (0.82, 0.61, and 0.41, respectively) (23). A further reduction in the absorption ratio to 0.31 was obtained when the phytate P content was increased to 50 mg. Direct comparison with the present results is difficult because our basic bread meal contained 10 mg phytate P, which must have been exerting a significant inhibitory effect on iron absorption. In addition, the phytate that was added to the bread in the various experiments was not in the form of sodium phytate that was present in bran. However the same trends were noted, with a reduction in the absorption ratio to 0.54 when the amount of phytate P was increased from 10 to 58 mg. This reduction was reversed by a dose of 30 mg ascorbic acid, and iron absorption was increased to a geometric

mean of 27.4% when the dose of ascorbic acid was raised to 150 mg. In another study it was calculated that a dose of ~80 mg ascorbic acid would be required to counteract fully the effects of 25 mg phytate P (23).

Polyphenols have been shown to have a marked inhibitory effect on nonheme-iron absorption. This was first suggested in 1975 when tea was shown to inhibit iron absorption in humans (29), and subsequent *in vitro* and *in vivo* work provided evidence that it is the polyphenol content of tea that is the inhibitor (30). A negative correlation between the bioavailability of iron from different foods and their polyphenol contents was shown *in vitro* for a variety of legumes and spices (31) and *in vivo* for a number of vegetables (12). The inhibitory effect is probably due to the polymerization of polyphenols with iron, and to the consequent formation of insoluble complexes (30). In the present study as little as 12 mg tannic acid decreased iron absorption by one-third and 50 mg reduced absorption by almost 70%. This was close to the maximal suppression of ~80% obtained with much larger doses. Ascorbic acid was able to overcome the inhibitory effect and the results suggest that ~50 mg ascorbic acid would be necessary to restore iron bioavailability to normal values from any meal containing > 100 mg tannic acid.

The present results confirm previous observations that small amounts of phytate and polyphenols significantly inhibit iron absorption, with the effects being dose related (12, 26). At the same time it is apparent that these effects can be effectively prevented by the simultaneous administration of ascorbic acid. Although the results for phytate in maize and for tannic acid may not necessarily reflect the quantitative inhibitory effects of phytates and polyphenols in other foods, they nevertheless provide a theoretical framework for making more precise estimates of the bioavailability of iron in different diets. For example, it might be anticipated that 150 mL orange juice or 30 g papaya, both of which contain ~50 mg ascorbic acid (32), would be sufficient to largely neutralize the inhibitory effects on iron absorption of a bread meal with a bran content equivalent to 40–50 mg phytate P. However, if 100 g butter beans (350 mg polyphenols) (12) were present in a meal, then 300 mL orange juice or 60 g papaya would be necessary. Such an approach could be applied if dietary modifications were to be recommended in developing countries. What is currently needed is more quantitative information on

interactions between promoters and inhibitors of iron absorption. In this regard, it will also be important to find out whether promoting and inhibitory effects are additive. For example, there is evidence that the promoting effect of lemon juice is not only due to its ascorbic acid content but also to its citric acid content (32). Similar evidence in relation to the inhibitory effects of phytate and polyphenols is, however, not available.

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