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Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal¹⁻³

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ABSTRACT A study in 49 subjects compared different methods for increasing the absorption of iron from a simple Latin American-type meal composed of maize, rice, and black beans. The addition of meat (75 g) increased the nonheme iron absorption from 0.17 to 0.45 mg; soy protein in an amount corresponding to the protein content of the meat increased the absorption to 0.51 mg (due to the high iron content of soy flour); cauliflower as a source of ascorbic acid (65 mg) increased the absorption to 0.58 mg, pure ascorbic acid (50 mg) to 0.41 mg, and ferrous sulphate mixed into the meal in an amount (6 mg) corresponding to the iron content of the soy flour increased the absorption to 0.64 mg. The addition of citric acid (1 g) reduced the absorption to 0.06 mg (to about one-third). We conclude that several methods are available for increasing iron absorption from a Latin American meal and that the choice of method depends on several factors, particularly cost. *Am J Clin Nutr* 1984;39:577-583.

KEY WORDS Iron absorption, diet, man, meat, soy protein, ascorbic acid, citric acid, ferrous sulphate

Introduction

It has been shown that the absorption of nonheme iron from diets in developing countries is often low due to the limited content of foods stimulating the absorption of nonheme iron. Thus, the availability of meat or fish and of foods rich in ascorbic acid is often quite low. Moreover, diets in developing countries often have a high content of factors inhibiting nonheme iron absorption such as phytates and tannins. The present study was designed to compare the effect of adding various foods or compounds which could be expected to enhance nonheme iron absorption from a simple meal. A type of meal was chosen which is common in Latin America and which has a high content of phytates. The following items were added to this meal: 1) meat; 2) soy protein as a realistic alternative to meat in

order to improve protein nutrition; 3) cauliflower as a source of ascorbic acid; 4) pure ascorbic acid; 5) citric acid, which has been reported to enhance nonheme iron absorption; and 6) ferrous sulphate as a reference compound in food iron fortification.

Materials and methods

The investigation is comprised of five studies and in total 49 subjects. Hematological and other data are given in **Table 1**.

Study 1 (four men and five women; one of the women and four of the men were blood donors) com-

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TABLE 1
The material (mean and values \pm SEM)

Series	No and sex of subjects	Age	Ht	Wt	Hb	Hematocrit
		yr	cm	kg	g/l	%
1	4 M	34	178.5 \pm 3.5	74.3 \pm 7.2	151 \pm 4	45.5 \pm 1.0
	5 F	25	171.6 \pm 3.6	66.2 \pm 5.5	139 \pm 1	42.2 \pm 0.9
2	7 M	29	182.7 \pm 4.5	76.0 \pm 4.6	151 \pm 4	45.4 \pm 1.4
	3 F	21	172.6 \pm 3.2	67.7 \pm 4.1	140 \pm 5	42.3 \pm 1.9
3	9 M	29	185.5 \pm 1.2	80.3 \pm 4.1	146 \pm 3	44.9 \pm 0.6
	1 F	46	170.0	81.0	150	45
4	4 M	30	178.8 \pm 2.3	70.3 \pm 1.9	157 \pm 3	48.0 \pm 0.9
	6 F	24	166.3 \pm 1.9	56.7 \pm 1.6	132 \pm 4	40.0 \pm 1.0
5	5 M	30	179.8 \pm 1.6	71.0 \pm 5.1	149 \pm 5	46.2 \pm 1.5
	5 F	24	163.6 \pm 4.6	56.2 \pm 2.5	129 \pm 6	38.2 \pm 1.7

pared the effect of adding 75 g ground beef meat to the basal meal composed of maize, rice, and black beans.

Study 2 (seven men and three women; five of the men were blood donors) measured the effect of adding soy protein (defatted soy flour) to the basal meal in an amount corresponding to the amount of protein in the meat given in study 1. As a comparison the same amount of iron as is present in the soy protein was added to the basal meal (6 mg iron as FeSO_4).

Study 3 (nine men and one woman; the woman was a blood donor as were six of the men) measured the effect of adding 125 g boiled cauliflower containing 65 mg ascorbic acid when served.

Study 4 (four men and six women; one of the women and two of the men were blood donors) compared the effect of adding 50 mg ascorbic acid to the basal meal.

Study 5 (five men and five women; four of the men were blood donors) compared the effect of adding 1 g citric acid to the basal meal.

Experimental design

The design was similar to other recent food iron absorption studies made in our laboratory (1-3). In each series the subjects were served two kinds of meals, A and B, on four consecutive mornings (day 1 to 4) in the order ABBA or BAAB. The meals were labeled with two different radioiron isotopes, ^{55}Fe or ^{59}Fe . In all studies the A and B meals were identical except that the B meals contained the iron absorption promoting factor under investigation. On day 18 a blood sample was drawn to determine the content of ^{55}Fe and ^{59}Fe in blood and a whole body counting of ^{59}Fe was made. Immediately after this count on days 18 and 19 a reference dose of 3 mg iron as ferrous ascorbate labeled with ^{59}Fe was given in a fasting state. A new whole body counting of ^{59}Fe was made 2 wk later. The details of the procedures were the same as described in recent studies (2, 3).

Meal composition and preparation

The basal meal consisted of maize chapattis, black beans, and rice. Maize (80 g) was boiled in water with

quicklime added. When the grain had become swollen and soft, the maize was ground into a dough and tortillas were prepared and baked. Polished rice (50 g) was cooked with 125 ml water. Black beans (31 g) were cooked with water and ground to a paste when they had become soft. Vegetable oil, onion, salt, and garlic powder were added. The weight of each portion was 81 g.

In study 1 the basal meal was served with the addition of 75 g ground beef. The meat was served grilled, seasoned with salt and pepper.

In study 2 fat-free soy flour (Ralston Purina Company, St Louis, MO) was added to the basal meal. Thirty-three g soy flour were added to the chapatti dough.

In study 3 the basal meal was served with the addition of 125 g boiled cauliflower, providing 65 mg ascorbic acid.

In study 4 the basal meal was served with and without 50 mg ascorbic acid mixed in dry form with each portion of black beans just before serving.

In study 5 the basal meal was served with and without 1 g citric acid mixed in dry form with the black beans.

Each meal was labeled with 1.5 μCi ^{59}Fe or 2 μCi ^{55}Fe . The radioiron solution was added to each portion of water and rice, before boiling the rice. All meals included 150 ml water taken as a drink with the meals.

Chemical composition of meals

Aliquots of the meals were freeze-dried and then finely ground to a powder in a porcelain mortar. Weighed amounts of this powder were analyzed for total iron (4), nonheme iron (3), phosphorus (5), phytic acid (6), and ascorbic acid (7). Due to the difficulties in determining heme directly in mixtures containing other chromogens, heme iron was calculated as the difference between total iron and nonheme iron. The chemical composition of the meals is shown in Table 2.

TABLE 2
Chemical composition of the meals

Meal	Energy	Protein	Phyti. P	Ascorbic acid
	kcal	g	mg	mg
Basal meal: Maize, rice, black beans	580	16	250	0
Basal meal with meat	680	31	250	0
Basal meal with soy	680	31	400	0
Basal meal with cauliflower	615	19	250	65

Oral reference doses of iron

A solution of 10 ml 0.01 M hydrochloric acid containing 3 mg iron as ferrous sulphate and 30 mg ascorbic acid was used as a reference in all studies. The 10-ml vials containing the iron solution were rinsed twice with water and this was also consumed. Each subject received two reference doses on two consecutive mornings after an overnight fast. No food or drink was allowed after the reference dose for 3 h. Each subject received a total of 1.5 μ Ci 59 Fe.

Expressing results of absorption measurements and statistical analysis

In each subject the ratio of the absorption of nonheme iron from a certain meal and from the reference doses is an expression of the bioavailability of nonheme iron in the meal. There is a normal distribution of these ratio values, and mean and SDs of the ratio values are therefore calculated in the usual way.

These ratio values in the different studies were then normalized to 40% absorption from the reference dose. The mean values and SEs for the percentage absorption of iron from different meals corresponding to a 40% reference dose absorption were then calculated simply by multiplying the ratio values by 40. The amount of iron absorbed could then be calculated by multiplying the percentage absorption values by the nonheme iron content of the meal. Absorption values corresponding to a 40% dose absorption were chosen as these absorption values would then correspond to measurements made in subjects who are borderline iron deficient (8). It should be observed that the mean absorption percentage values A and R in Table 3 may be slightly divergent from the A_{40} values as these latter values are obtained from the mean A/R ratios (see "Materials and Methods"). The study was approved by the Ethical Committee of the University of Göteborg.

Results

Results from the different studies are given in Table 3 and Figure 1. In two studies (in study 1a and 3a) the same basal meal

was served. These results in 19 subjects were pooled together and are also given in Table 2. The pooled results are graphed in Figure 1.

The addition of 75 g lean meat containing 15 g protein increased the absorption of nonheme iron about 2½ times from 0.17 to 0.45 mg. Addition of the same amount of protein as defatted soy flour which was mixed into the dough of the maize tortillas increased the absorption from 0.17 to 0.51 mg (three times). A main cause for this increase is the additional amount of iron (6 mg) in this meal derived from the soy flour. Adding the same amount of iron, 6 mg, as ferrous sulphate increased the absorption to 0.64 mg (3.8 times). Cooked cauliflower (125 mg) providing 65 mg ascorbic acid when served increased the absorption more than three times from 0.18 to 0.58 mg.

Addition of 50 mg ascorbic acid in dry form to the black beans just before serving increased the absorption from 0.05 to 0.12 mg. In all these studies the increase in nonheme iron absorption was statistically highly significant ($p < 0.01$). Addition of citric acid reduced the absorption from 0.09 to 0.03 mg ($p < 0.05$). In eight of 10 subjects, lower absorption values were seen when adding citric acid and in two subjects no change was noted.

Discussion

Because there is a high prevalence of iron deficiency anemia in most developing countries that probably has important negative effects on health and well being, it is important to find effective and realistic methods to counteract the iron deficiency.

An increase of the iron content of a meal by iron fortification is one of the main techniques of increasing iron absorption. An alternative approach is to increase the bioavailability of the iron in the meal by modifying the meal composition. An example would be the addition of foods that promote the nonheme iron absorption such as fish, meat, or foods rich in ascorbic acid. The purpose of the present study was to compare the efficacy of different methods to increase dietary nonheme iron absorption using as a base a single simple meal from which iron

TABLE 3
Iron absorption from different meals

Study Meal	N	Iron content (mg)			Absorption (%)		A/R	A ₄₀ (%)		A ₄₀ (mg)	
		Heme Fe	Nonheme Fe		Meal (A)	Reference dose (R)		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
			Native	Added							
1 a Basal meal	9		4.7	0.6	5.3	3.5	38.9	0.08 ± 0.03	3.2 ± 1.2	0.17 ± 0.06	
b Basal meal with meat		0.7	5.3		5.3	8.0	38.9	0.21 ± 0.05	8.4 ± 2.0	0.45 ± 0.11	
2 a Basal meal with FeSO ₄	10		4.7	6.0	10.7	5.2	31.5	0.15 ± 0.03	6.0 ± 1.2	0.64 ± 0.13	
b Basal meal with fatfree soyflour			10.7		10.7	4.0	31.5	0.12 ± 0.03	4.8 ± 1.2	0.51 ± 0.13	
3 a Basal meal	10		4.4		4.4	2.7	30.4	0.10 ± 0.03	4.0 ± 1.2	0.18 ± 0.05	
b Basal meal with cauliflower			5.4		5.4	7.7	30.4	0.27 ± 0.09	10.8 ± 3.6	0.58 ± 0.19	
1a + 3a	19							0.09 ± 0.02	3.6 ± 0.8	0.17 ± 0.04	
4 a Basal meal	10		4.3		4.3	1.2	36.0	0.03 ± 0.002	1.2 ± 0.07	0.05 ± 0.003	
b Basal meal with ascorbic acid			4.3		4.3	3.4	36.0	0.07 ± 0.016	2.8 ± 0.64	0.12 ± 0.03	
5 a Basal meal	10		4.3		4.3	2.4	36.9	0.05 ± 0.02	2.0 ± 0.80	0.09 ± 0.03	
b Basal meal with citric acid			4.3		4.3	1.1	36.9	0.02 ± 0.007	0.8 ± 0.28	0.03 ± 0.01	

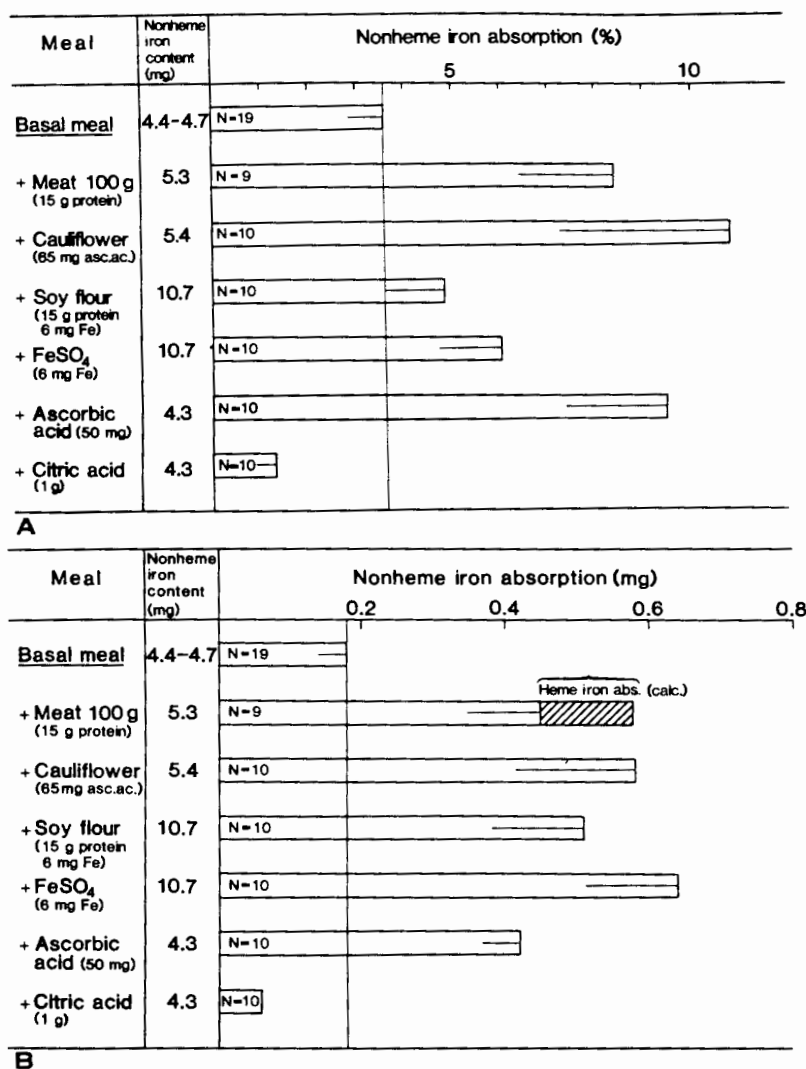


FIG 1. A, comparison of various methods to increase nonheme iron absorption from a simple meal of maize, rice and black beans (percentage absorption). B, comparison of various methods to increase nonheme iron absorption from a simple meal of maize, rice, and black beans. Amount of iron absorbed (mg). Absorption of heme iron in meat is calculated (see text).

absorption is known to be low. The type of meal chosen, composed of maize, rice, and black beans, is common in lower socioeconomic groups in Latin America, where iron deficiency is prevalent.

The addition of meat to a cereal vegetable-type of meal is an effective but unrealistic way of improving iron nutrition in developing countries. The present study included the effect of meat as a comparison with the other methods. The amount of meat added was about the same as in our previous stud-

ies on hamburger meals. Such meals have been used to compare the effect of various methods for improving iron absorption from Western-type meals (9). In the present studies the absorption of heme iron from the added meat was not measured. Based on our previous results the absorption of heme iron from the 0.5 mg heme iron contained in the meat can be calculated to be about 25% or 0.13 mg (10).

Soy is an important source of protein in human nutrition and is widely used both in

developing and industrialized countries. In previous studies on hamburger meals, where soy was partially substituted for meat, it was found that soy protein did not have the property of meat for stimulating the absorption of nonheme iron (1, 11). In fact, a decrease in percentage absorption was noted. The effect of soy protein on nonheme iron absorption could be different in meals not containing any animal protein, eg, the phytates present in soy protein products might be more inhibiting when meat is not present.

In these current studies the percentage absorption of nonheme iron was indeed slightly higher both when adding soy protein and ferrous sulphate. It has earlier been observed that the properties of the nonheme iron pool can be improved when adding a fairly large amount of an easily soluble iron salt. It is reasonable to assume that the iron in soy protein also slightly improved the poor absorption properties of the nonheme iron in the basal meal. The soy flour had a high iron content which led to a statistically significant increase in the total amount of iron absorbed. The contribution of soy to the iron content of a meal is important in evaluating the overall effect of adding soy protein products to diets in developing countries. While the iron content of diets in developing countries is often reported as high, it is important to recognize that a great proportion of such iron is usually derived from contamination of foods with soil iron. As a rule, this type of iron has a very low bioavailability. The iron in soy flour, however, does fully exchange with an extrinsic tracer and will thus form an integral part of the potentially available nonheme iron pool.

Ascorbic acid is one of the main dietary enhancers of nonheme iron absorption. The addition of ascorbic acid or foods rich in ascorbic acid is probably one of the most effective ways of improving iron nutrition (for reviews see References 12 and 13). This conclusion is supported in the current study by the findings that the addition of a piece of boiled cauliflower containing 65 mg ascorbic acid increased the bioavailability of iron in the present type of meal three to four times and that 50 mg pure ascorbic acid increased it three times.

Citric acid in an amount of about 1 g has been reported to enhance nonheme iron absorption. In the present study, however, no absorption promoting effect was observed. Actually, the absorption decreased significantly. The same effect has been observed in our laboratory when adding citric acid in the same amount to a hamburger meal. At present, no explanation for the divergent results can be given.

The studies with ascorbic acid and citric acid (studies 4 and 5) were made 1 yr after the other studies using other batches of maize and black beans which probably explains why the absorption from the basal meals in these series were different from the previous ones. To be able to compare the findings in all series the results in series 4 and 5 were adjusted to the same basal absorption (3.6%, mean value of series 1 and 3). The corresponding absorption figures for all series are given in Table 4 and Figure 1.

In some countries it is possible to fortify the diet with iron. Suitable vehicles and suitable iron compounds may be available to make iron fortification technically and economically feasible. In the present study ferrous sulphate was used as it is a well-absorbed iron compound often used as a reference when comparing the absorption of different iron compounds. The 6-mg iron dose used is high but was chosen to contrast the effect of adding 33 g soy protein which contained 6 mg iron.

Our results show that several methods are available to increase the absorption of iron from a simple Latin American meal. It is interesting to note that the methods compared gave fairly similar results: Meat increased the absorption 3.4 times when a calculated value for heme iron absorption was factored in; cauliflower as a source of ascorbic acid (65 mg) increased the absorption to the same extent, and the same was true for pure ascorbic acid (50 mg); soy flour increased the absorption three times when the amount of protein added was the same as in the meat added; and ferrous sulphate increased the absorption 3.8 times when the amount of iron added was the same as in the soy flour added. In combating iron deficiency one or more methods may be used. The choice depends on several factors, par-

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TABLE 4

Comparison of iron absorption from different meals; values for ascorbic acid and citric acid (series 4 and 5) are adjusted by relating absorption from basal meals in these series to the mean value for the basal meals in series 1 and 3; SE values are adjusted in the same way

Meal	n	Non-heme iron content	Non-heme iron absorption	
			Mean	SEM
		mg	mg	%
Basal meal	19	4.4-4.7	0.17 ± 0.06	3.6 ± 0.8
+ FeSO ₄ (6 mg)	10	10.7	0.64 ± 0.13	6.0 ± 1.2
Soy protein (6 mg Fe; 15 g protein)		10.7	0.51 ± 0.13	4.8 ± 1.2
Meat (75 g) (15 g protein)	9	5.3	0.45 ± 0.11	8.4 ± 2.0
Cauliflower (65 g ascorbic acid)	10	5.4	0.58 ± 0.19	10.8 ± 3.6
Ascorbic acid (50 mg)	10	4.3	0.41 ± 0.10	9.5 ± 2.1
Citric acid (1 g)	10	4.3	0.06 ± 0.02	1.3 ± 0.44

ticularly costs, and probability of reaching the target population.

In actual field programs smaller amounts of iron absorption enhancers will probably be used but yet a significant improvement of iron nutrition can be expected. 

References

- Hallberg L, Rossander L. Effect of soy protein on nonheme iron absorption in man. *Am J Clin Nutr* 1982;36:514-20.
- Björn-Rasmussen E, Hallberg L, Magnusson B, Rossander L, Svanberg B, Arvidsson B. Measurement of iron absorption from composite meals. *Am J Clin Nutr* 1976;29:772-8.
- Hallberg L. Food iron absorption. In: Cook JD, ed. *Methods in hematology*. London: Churchill, 1980:116-33.
- Björn-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B. Food iron absorption in man. Application of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. *J Clin Invest* 1974;53:274-355.
- Nordic Committee on Food Analysis (NCFA). Method no 57. Skelbaecksgade, Köpenhamn V: Danish Technical Press, 1965.
- Nordic Committee on Food Analysis (NCFA). Method no 17:2. Skelbaecksgade, Köpenhamn V: Danish Technical Press, 1966.
- Official methods of analysis. 12th ed. Washington, DC: Association of Official Analytical Chemists, 1975.
- Magnusson B, Björn-Rasmussen E, Hallberg L, Rossander L. Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurements. *Scand J Haematol* 1981;27:201-8.
- Hallberg L, Rossander L. Effect of different drinks on the absorption of nonheme iron from composite meals. *Human Nutrition: Applied Nutrition* 1982;36A:116-23.
- Hallberg L, Björn-Rasmussen E, Howard L, Rossander L. Dietary heme iron absorption. A discussion of possible mechanisms for the absorption promoting effect of meat and for the regulation of iron absorption. *Scand J Gastroent* 1979;14:769-79.
- Bothwell TH, Clydesdale FM, Cook JD, et al. The effect of cereals and legumes on iron availability. Report of the international nutritional anemia consultative group (INACG). Washington, DC: Nutritional Foundation, 1982.
- Hallberg L. Bioavailability of dietary iron in man. *Am Rev Nutr* 1981;1:123-47.
- Hallberg L. Effect of vitamin C on the bioavailability of iron from food. In: Counsell JN, Hornig DH, eds. *Vitamin C (ascorbic acid)*. London: Applied Science Publishers, 1981:49-61.
- Gillooly M, Bothwell TH, Torrance JD, et al. The effect of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *Br J Nutr* 1983;49:331-42.