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## EFFECT OF ASCORBIC ACID ON IRON ABSORPTION FROM DIFFERENT TYPES OF MEALS. Studies with ascorbic-acid-rich foods and synthetic ascorbic acid given in different amounts with different meals

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The effect of ascorbic acid on the absorption of non-heme iron was studied in 299 subjects. Different meals in which the non-heme iron was labelled with two different radio-iron isotopes were served with and without ascorbic acid to the same subject. Other meals containing foods with a known high content of ascorbic acid were also studied. Studies were also made giving different amounts of ascorbic acid with different meals. Marked differences in the enhancement of iron absorption were seen when ascorbic acid was given in different meals. It is suggested that ascorbic acid promotes iron absorption from the diet by reducing the negative effect on iron absorption of certain ligands such as phytates and tannins present in the diet. This interpretation is supported by observations that the most pronounced effects of ascorbic acid were found in meals with a high content of ligands known to inhibit iron absorption. Crystalline ascorbic acid and native ascorbic acid in foods appeared to have the same effect in promoting absorption of iron.

The results indicate that ascorbic acid has a key physiological role in facilitating the absorption of non-heme iron from the diet and that about 50 mg of the vitamin in each main meal is desirable for optimum effect.

### *Introduction*

Iron deficiency is one of the most common deficiency disorders of the world. It may be caused by increased iron losses due to pathological blood loss, eg resulting from infestation with hookworms, but the main cause of iron deficiency, both in developing and industrialized countries, is nutritional (Baker & DeMaeyer, 1979). The absorption of iron from the diet does not cover the physiological iron losses and the requirements for growth and pregnancy in a considerable proportion of otherwise healthy subjects.

Knowledge about the absorption of iron from the diet has increased markedly in recent years, and it has become evident that there are two kinds of iron in the diet with respect to mechanism of absorption, heme iron and non-heme iron (Hallberg, 1981a). These two classes of iron compounds can be uniformly labelled by extrinsic radio-iron tracers — heme iron with radio-iron-labelled haemoglobin and non-heme iron with an inorganic radio-iron tracer. An important implication of these studies has been the introduction of the pool concept in iron absorption. All non-heme iron compounds in the diet may be considered as part of a common pool and the amount of iron absorbed as a net effect of the properties of the iron compounds in the diet and the balance between different dietary factors which enhance or inhibit iron absorption. The latter factors operate by supplying ligands which make the dietary iron more or less available for the absorptive mechanisms of the intestinal mucosa (Cook *et al.*, 1972; Hallberg, 1974; Hallberg & Björn-Rasmussen, 1972).

Ascorbic acid seems to be the most potent factor in the diet enhancing the absorption of non-heme iron. The augmenting effect of ascorbic acid on food iron absorption was first reported by Moore and Dubach in 1951 using single, biosynthetically radio-iron-labelled foods (Moore & Dubach, 1951). The extrinsic tag method greatly facilitated studies on the effect of ascorbic acid on food iron absorption, especially when using two radio-iron isotopes giving the same meal on different days with and without ascorbic acid to the same subjects and labelling the non-heme iron with different radio-iron isotopes. Several reports using this method have been published which all show a marked and consistent effect of ascorbic acid in enhancing the absorption of dietary non-heme iron (Moore & Dubach, 1951; Apte & Venkatachalam, 1965; Björn-Rasmussen & Hallberg, 1974; Hallberg, 1981; Sayers *et al.*, 1973, 1974a). The amounts of ascorbic acid showing a clear-cut effect have been 50 mg or more per meal. In most of these studies the iron absorption was increased several times by ascorbic acid.

Early studies were usually made on meals with a low iron absorption, e.g. simple maize meals or rice meals. The effect of ascorbic acid was less marked when added to meals containing meat or fish (Cook & Monsen, 1977; Layrisse, Martinez-Torres & González, 1974; Hallberg & Rossander, 1982a) and the effect was less marked still when ascorbic acid was given with a simple continental-type breakfast containing no meat (Rossander, Hallberg & Björn-Rasmussen, 1979).

Reviewing all studies made on the effect of ascorbic acid on non-heme iron absorption, the variation in effect is quite remarkable. This is even true when comparisons are limited to studies where between 50 and 100 mg of ascorbic acid was given. In such studies, for no obvious reasons, the ratio of iron absorption with/without ascorbic acid varied between 1.5 and 6. These studies were made with different meals, in groups of subjects with different iron status, in different laboratories and applying different methods to calculate the enhancing effect of ascorbic acid. Since there is no doubt from these earlier studies that ascorbic acid has an important role in iron nutrition, it was considered important to try and acquire further knowledge about the effect of ascorbic acid and the reasons for the variation in its effect on non-heme iron absorption. We did this first in three ways: (i) by comparing the effect when ascorbic acid was given in crystalline form or as foods rich in ascorbic acid; (ii) by comparing the effect of ascorbic acid when the same amounts were given with different types of meals; and (iii) by studying the relationship between the effect and the amount of ascorbic acid given with different meals.

#### *Material and methods*

The investigation comprised 26 studies in a total of 299 subjects. The haematological and other data of the subjects are given in Table 1. The number of subjects, their sex and the number who were blood donors are also included in the table.

Studies 1-9 deal with the effect on non-heme iron absorption of adding different ascorbic acid rich foods from six different meals (Table 3). Studies

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Table 1. Details of subjects (mean and values  $\pm$  s.e.m.)

Series	No. and sex of subjects*	Age (years)	Height (cm)	Weight (kg)	Hb (g/l)	Hematocrit (%)
1	5 M (4)	32	184	80.6 $\pm$ 5.2	148 $\pm$ 5	45.2 $\pm$ 1.4
	7 F (1)	26	163	56.4 $\pm$ 2.1	127 $\pm$ 3	38.9 $\pm$ 1.4
2	5 M (2)	28	176	69.4 $\pm$ 3.4	144 $\pm$ 5	44.6 $\pm$ 1.3
	7 F	31	164	57.7 $\pm$ 2.8	130 $\pm$ 2	40.1 $\pm$ 0.6
3	7 M (2)	24	182.1	71.1 $\pm$ 1.8	152 $\pm$ 3	44.6 $\pm$ 0.6
	3 F	23	167.3	59.7 $\pm$ 3.0	129 $\pm$ 4	39.0 $\pm$ 1.2
4	8 M (6)	31	178	78.5 $\pm$ 9.7	142.8 $\pm$ 8.3	44.0 $\pm$ 2.9
	2 F (1)	29	166	62.5 $\pm$ 7.8	145.0 $\pm$ 7.1	44.5 $\pm$ 2.1
5	7 M (3)	25	178	72.9 $\pm$ 2.5	156 $\pm$ 5	46.6 $\pm$ 1.6
	5 F	27	168	63.6 $\pm$ 4.1	139 $\pm$ 3	42.6 $\pm$ 0.5
6	9 M (4)	29	185.6	80.3 $\pm$ 12.2	146.1 $\pm$ 8.0	46.0 $\pm$ 4.5
	1 F (1)	46	170	81	150	45
7	8 M (3)	27	177.1	68.4 $\pm$ 3.2	144 $\pm$ 3	45.0 $\pm$ 1.0
	4 F (1)	25	167.3	55.5 $\pm$ 1.2	130 $\pm$ 3	42.0 $\pm$ 0.8
8	5 M (3)	29	182.4	79.8 $\pm$ 5.5	143 $\pm$ 2	44.4 $\pm$ 0.8
	5 F (1)	29	167.2	59.4 $\pm$ 2.2	130 $\pm$ 3	40.4 $\pm$ 0.7
9	14 F (2)	20	157	49.6 $\pm$ 0.9	132 $\pm$ 0.3	40.6 $\pm$ 0.7
	1 M (1)	26	179	62	145	46
10	9 F (3)	23 $\pm$ 5	170	63.4 $\pm$ 9.8	130 $\pm$ 7	42.3 $\pm$ 3.2
	1 M (1)	23	174	73	141	42
11	1 M (1)	21 $\pm$ 3	166	58.9 $\pm$ 10.6	137 $\pm$ 8	42.2 $\pm$ 2.2
		26	188	100	144	46
12	1 M (1)	22 $\pm$ 6	169	59.0 $\pm$ 7.7	136 $\pm$ 6	41.9 $\pm$ 2.3
	9 F (2)	34	179	80.5 $\pm$ 8.2	152.0 $\pm$ 8.5	44.8 $\pm$ 1.7
13	4 M (3)	26	171	60.7 $\pm$ 8.1	140.3 $\pm$ 5.7	41.8 $\pm$ 1.6
	6 F (1)	26	184	81.6 $\pm$ 15.2	153.8 $\pm$ 6.9	45.4 $\pm$ 1.9
14	5 M (2)	24	173	67.8 $\pm$ 6.5	129.6 $\pm$ 10.0	39.0 $\pm$ 2.4
	5 F (1)	28	178	75	160	48
15	1 M (1)	27	167.6	59.3 $\pm$ 8.3	132.0 $\pm$ 6.3	39.8 $\pm$ 1.9
	8 F (2)	34	179.5	76.3 $\pm$ 6.7	154.3 $\pm$ 10.1	45.3 $\pm$ 3.0
16	4 M (3)	24	172.8	68.3 $\pm$ 6.3	137.8 $\pm$ 9.5	41.8 $\pm$ 2.3
	6 F (1)	27	175.4	71.6 $\pm$ 8.0	155.2 $\pm$ 6.3	44.4 $\pm$ 1.7
17	5 M (2)	27	164.8	67.2 $\pm$ 13.6	148.0 $\pm$ 6.4	43.0 $\pm$ 1.4
	5 F (2)	29	179.6	72.9 $\pm$ 8.4	148.0 $\pm$ 8.9	44.4 $\pm$ 3.5
18	7 M (3)	20	173.6	60.0 $\pm$ 3.6	132.0 $\pm$ 9.5	41.0 $\pm$ 2.6
	3 F (1)	26	179.5	72.8 $\pm$ 1.7	159.8 $\pm$ 4.3	46.8 $\pm$ 2.2
19	4 M (2)	24	166.8	60.3 $\pm$ 8.0	135.7 $\pm$ 4.7	39.8 $\pm$ 1.3
	6 F (1)	30	181.8	71.8 $\pm$ 4.8	154 $\pm$ 3	45.8 $\pm$ 1.3
20	3 M (2)	24	167.2	60.7 $\pm$ 3.1	140 $\pm$ 2	42.0 $\pm$ 0.6
	6 F (1)	30	178.8	70.3 $\pm$ 3.9	157.3 $\pm$ 5.3	48.0 $\pm$ 1.8
21	4 M (2)	24	166.3	56.7 $\pm$ 4.0	131.8 $\pm$ 8.7	40.0 $\pm$ 2.5
	6 F (1)	28	178.2	72.2 $\pm$ 2.7	154 $\pm$ 5	45.5 $\pm$ 1.7
22	6 M (3)	29	174	75.3 $\pm$ 8.1	140 $\pm$ 9	41.7 $\pm$ 1.8
	3 F (1)	26	177.5	70.3 $\pm$ 5.2	148.5 $\pm$ 11.6	45.5 $\pm$ 1.9
23	4 M (4)	23	169.0	58.3 $\pm$ 6.3	131.8 $\pm$ 5.3	40.7 $\pm$ 1.4
	6 F (1)	26	167.4	57.1 $\pm$ 2.6	159 $\pm$ 3	44.8 $\pm$ 0.8
24	11 M	25	155.0	49.9 $\pm$ 1.9	132 $\pm$ 1	40.4 $\pm$ 0.7
	9 F	28	166.1	61 $\pm$ 2.6	165 $\pm$ 6	47.9 $\pm$ 1.0
25	8 M	24	156.5	51.6 $\pm$ 1.6	135 $\pm$ 4	39.1 $\pm$ 0.6
	8 F	25	155.2	50.4 $\pm$ 1.0	129 $\pm$ 2	38.9 $\pm$ 0.6
26	25 F					

\*Number of regular blood donors in parentheses.

studies have previously been reported in four papers from our laboratory. The reason for including them here is that the method of calculating the results has been modified and all results are now presented in a directly comparable form. The previously reported studies are: studies 1 and 2 (Rossander *et al.*, 1979), study 5 (Hallberg & Rossander, 1982*b*), study 6 (Hallberg & Rossander, 1984) and studies 7 and 8 (Hallberg & Rossander, 1982*a*).

Studies 10-26 deal with the effect of adding different amounts of crystalline ascorbic acid to eight different meals (Table 4).

Studies 9 and 24-26 were made at Mahidol University in Bangkok, Thailand. The other studies were carried out at the University of Göteborg, Göteborg, Sweden, where radio-iron analyses for all studies were made.

#### *Experimental design*

In each series the subjects were served two kinds of meal, A and B, on four consecutive mornings (day 1 to 4) in the order ABBA or BAAB. The meals were labelled with two different radio-iron isotopes,  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$ . In all studies, the A and B meals were identical except that the B meals contained the iron absorbing factor under investigation. On day 18, a blood sample was drawn to determine the content of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  in blood and a whole body count of  $^{59}\text{Fe}$  was made. Immediately after this count on day 18 a reference dose of 3 mg iron as ferrous ascorbate labelled with  $^{59}\text{Fe}$  was given to the fasted subjects. A new whole body count of  $^{59}\text{Fe}$  was made 2 weeks later. Details of the procedures were as described in recent studies (Björn-Rasmussen *et al.*, 1976; Hallberg, 1980).

In studies 24 and 25, ascorbic acid was given with the meal, labelled with one isotope and the other isotope was given with the reference dose, served in the sequence ARRA. The effect of ascorbic acid was calculated by comparing the present results with those obtained in earlier studies on the same meals when no ascorbic acid was given (Hallberg *et al.*, 1978).

In studies 9 and 24-26, no whole-body count was performed. In series 9 and 26 a repeated blood sample was drawn on day 32 to measure the iron absorption from the reference doses given after drawing the first blood sample on day 18.

#### *Meal composition and preparation*

The breakfast meal in study 15 consisted of coffee (150 ml), one wheat roll with margarine (12 g), orange marmelade (10 g) and cheese (15 g). The roll was made from 40 g of unfortified white flour of 60 per cent extraction. In studies 1-2 and 10-14, the same amount of flour was fortified with 2.4 mg iron as ferrous sulphate.

In study 2, the breakfast was served with tea instead of coffee. Details as to how the coffee and tea were made have been presented previously (Rossander *et al.*, 1979).

The orange juice added in studies 1, 2 and 5 was freshly prepared frozen concentrate, reconstituted with water. In studies 1 and 2 150 ml was given and in study 5 250 ml.

four papers from our laboratory. The method of calculating the results are now presented in a directly reported studies are: studies 1 and 2 (Hallberg & Rossander, 1982b), study 6 (Hallberg & Rossander, 1982c), studies 7 and 8 (Hallberg & Rossander, 1982d).

of adding different amounts of iron to the meals (Table 4).

Mahidol University in Bangkok, Thailand and at the University of Göteborg, Sweden. The same analyses for all studies were made.

two kinds of meal, A and B, on four different orders ABBA or BAAB. The meals contained different amounts of iron isotopes,  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$ . In all studies except that the B meals contained  $^{59}\text{Fe}$  only. On day 18, a blood sample was taken to determine the content of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  in blood. Immediately after this count on a blood sample was taken. A known amount of ascorbic acid labelled with  $^{59}\text{Fe}$  was added to the blood. The body count of  $^{59}\text{Fe}$  was made 2 weeks after the blood count as described in recent studies (Hallberg *et al.*, 1980).

Ascorbic acid was given with the meal, labelled with  $^{59}\text{Fe}$ . The amount of ascorbic acid with the reference dose, served with the meal, was calculated by comparing the results obtained in earlier studies on the amount of ascorbic acid (Hallberg *et al.*, 1978).

A blood sample was obtained on day 32 to measure the amount of ascorbic acid after drawing the first blood sample.

150 ml), one wheat roll with margarine (15 g) and cheese (15 g). The roll was made from flour of 60 per cent extraction. In study 5 the flour was fortified with 2.4 mg iron.

instead of coffee. Details as to the amount of ascorbic acid have been presented previously (Hallberg *et al.*, 1978).

Study 5 was freshly prepared frozen meals. In studies 1 and 2 150 ml was given

The wheat-bran buns were made from unfortified wheat flour (27 g) and bran (12.5 g). Two buns were served with margarine (20 g) and water to drink (150 ml).

The hamburger meal consisted of hamburgers (110 g), string beans (60 g) and mashed potatoes (150 g). The hamburger served was a commercial product containing 66 g of minced meat. The fresh vegetable salad served with the hamburger meal in study 3 consisted of lettuce (50 g), tomatoes (35 g), cucumber (35 g) and sweet green pepper (25 g). A dressing (0.5 ml of vinegar, 2 ml of oil, salt, pepper) was poured over the vegetables.

The Latin-American 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.

The pizza meal was made from 84 g of unfortified wheat flour, yeast, water, oil and salt. The dough was rolled out in a round shape, covered with 40 g of tomato puree, black olives (25 g), fillets of anchovies (30 g) and finally covered with grated cheese (100 g). The pizza was baked in an oven at 250 °C for 20 min.

The rice meal consisted of 50 g of washed polished rice which was cooked under pressure with 125 ml of distilled water. The vegetables (20 g of each of string beans, Chinese cabbage and collard) were carefully washed and then finely chopped. Weighed amounts were put into aluminium forms with a measured amount of a hot mixture of chilli paste, fish sauce and coconut cream. In one of the series (No. 9) 150 g of fresh papaya was served with the rice meal. In study 26, the rice meal consisted of boiled rice, dried chilli, fish (50 g), fish sauce (Nam pla) and soup (300 ml).

The vegetarian 'low-ascorbate' meal (study 8). Dried navy beans (45 g) were soaked overnight in water and boiled for 1 h. Brown rice (45 g) was cooked with 125 ml of water (all the water was absorbed into the rice). A bread was made from corn-flour (25 g) and unfortified wheat flour (25 g) and spread with margarine (14 g). The meal also contained sliced apples (55 g), walnuts (8 g), almonds (8 g) and 225 g yoghurt (3% fat).

The 'high-ascorbate' vegetarian meal (study 7). Dried red kidney beans (42 g) were soaked overnight in water and then boiled for 1 h. The boiled beans (90 g) were mixed with tomato sauce (30 g) made from crushed canned tomatoes. A bread roll was made from unfortified wheat flour. Each roll had a total iron content of 0.9 mg, 0.6 mg of which was added as ferrous sulphate fortification iron. The bread was served with margarine (15 g). Cottage cheese (55 g), canned pineapple (125 g), and a banana (37 g) were also included in the meal.

Cauliflower (125 g) added to the meals in studies 4, 6-8 was served immediately after boiling for 10 minutes.

### Radio-iron labelling of meals

Each meal was labelled with 1.5  $\mu\text{Ci}$   $^{59}\text{Fe}$  or 2  $\mu\text{Ci}$   $^{55}\text{Fe}$ . In series 1, 2, 10-11, 23, the radio-iron was added to the rolls or the pizza when kneading the dough. In studies 3-5 and 17-19, the radio-iron was added dropwise to the three foods, hamburgers, mashed potatoes and string beans.

In study 7 two-thirds of the radio-iron was added to the tomato sauce and one-third was mixed into the dough of the wheat bun. In study 8 two-thirds of the radio-iron was added to the water when boiling the rice and one-third was mixed into the dough of the bun.

In studies 6 and 20-22, the radio-iron solution was added to each portion of water and rice, before boiling the rice. In studies 9 and 24-26, the radio-iron solution was added dropwise to each portion of boiled rice.

### 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 analysed for total iron (Björn-Rasmussen *et al.*, 1974), non-heme iron (Hallberg, 1980) phosphorus (Nordic Committee on Food Analysis, 1965), phytic acid (Nordic

Table 2. Composition of the meals used in 26 studies of iron absorption in man

Study No.	Meal	Iron content (mg)			Total Total	Energy (kcal)	Protein (g)	Phytic (mg)
		Heme Fe	Non-heme Fe Native	Added				
10-14	Breakfast	—	0.4	2.4	2.8	320	7	3
15	Breakfast without fortification	—	0.4	—	0.4	320	7	3
1-2	Breakfast with orange juice	—	0.7	2.4	3.1	390	8	3
23	Pizza	—	4.2	—	4.2	960	43	0
17-19	Hamburger meal	0.5	—	3.0	—	450	19	20
4	Hamburger meal with cauliflower	0.5	4.0	—	4.0	480	22	20
3	Hamburger meal with fresh salad	0.5	3.6	—	3.6	490	20	20
5	Hamburger meal with orange juice	0.5	3.5	—	3.5	565	21	20
20-22	Rice, black beans, maize	—	4.3	—	4.3	580	16	250
6	Rice, black beans, maize with cauliflower	—	5.4	—	5.4	615	19	250
24-25	Rice, vegetables, curry	—	1.8	—	1.8	250	7	5
26	Rice, vegetables, curry, fish	—	1.3	—	1.3	290	16	5
9	Rice, vegetables, curry, papaya	—	2.3	—	2.3	310	8	5
16	Wheat bran rolls with margarine	—	2.9	—	2.9	405	9	150
7	Vegetarian meal I with cauliflower	—	4.6	0.5	5.1	590	19	82
		—	5.3	0.5	5.8	620	22	82
8	Vegetarian meal II with cauliflower	—	5.8	—	5.8	730	22	271
		—	6.5	—	6.5	760	25	271

2  $\mu\text{Ci}$   $^{55}\text{Fe}$ . In series 1, 2, 10-16, for the pizza when kneading the iron was added dropwise to the and string beans. s added to the tomato sauce and wheat bun. In study 8 two-thirds en boiling the rice and one-third tion was added to each portion studies 9 and 24-26, the radio- portion of boiled rice.

then finely ground to a powder this powder were analysed for non-heme iron (Hallberg, 1980), analysis, 1965), phytic acid (Nordic

*Iron absorption in man*

Total	Energy	Protein	Phytic P
Total	(kcal)	(g)	(mg)
2.8	320	7	3
0.4	320	7	3
3.1	390	8	3
4.2	960	43	0
—	450	19	20
4.0	480	22	20
3.6	490	20	20
3.5	565	21	20
4.3	580	16	250
5.4	615	19	250
1.8	250	7	5
1.3	290	16	5
2.3	310	8	5
2.9	405	9	150
5.1	590	19	82
5.8	620	22	82
5.8	730	22	271
6.5	760	25	271

Committee on Food Analysis, 1966). Ascorbic acid was determined directly from foods as served (Official Methods of Analysis, 1975). The amount of energy and protein was calculated from a food composition table (Livsmedelstabelle, 1978). The chemical composition of the meals is shown in Table 2.

*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. Vials containing the iron solution (10 ml) 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 for 3 h after the reference dose. Each subject received a total of 1.5  $\mu\text{Ci}$   $^{59}\text{Fe}$ .

*Iron absorption measurements*

The relative absorption of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  was calculated from analyses of blood samples. The absolute absorption of the two tracers was calculated from a whole body count of  $^{59}\text{Fe}$  and the relative absorption of the two tracers. The analyses of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  in blood was made by means of a modification of the method described by Eakins & Brown (1966). All procedures and methods of calculation have been described previously (Björn-Rasmussen *et al.*, 1976; Hallberg, 1980).

*Expressing results of absorption measurements and statistical analyses*

In each subject, the ratio of the absorption of non-heme iron from a certain meal and from the reference doses is an expression of the bioavailability of non-heme iron in the meal. These ratio values show a normal distribution and mean and standard deviations are therefore calculated in the usual way. The ratio values in the different studies were then normalized to a 40 per cent absorption from the reference dose. The mean values of these ratios and their standard error of means were multiplied by 40 to obtain the percentage absorption of iron from the different meals corresponding to a 40 per cent reference dose absorption. The corresponding amount of iron absorbed could then be calculated by multiplying the percentage absorption values by the non-heme iron content of the meal. Values corresponding to a 40 per cent dose absorption were chosen as these absorption values would then correspond to measurements made in subjects who are borderline deficient (Magnusson *et al.*, 1981).

*Results*

*Effect of ascorbic acid rich foods (Table 3)*

Table 3 shows the results when ascorbic acid rich foods or drinks were given in different meals. Seven different meals were studied, adding four ascorbic acid rich foods. Three different foods containing varying amounts of ascorbic acid were given with the hamburger meal. In Fig. 1, the effect of such foods is compared with crystalline ascorbic acid given with the same hamburger meal. The results indicate that synthetic ascorbic acid or foods

Table 3. Effect of giving ascorbic-acid-rich foods on the absorption of heme and non-heme iron in human volunteers

No. Meal	Number of subjects	Ascorbic acid (mg)	Iron content (mg)		Absorption (%) Meal reference	A/R (R)	A <sub>40</sub> (%) Mean ± SEM	A <sub>40</sub> (mg) Mean ± SEM	Absorption ratio without/with ascorbic acid
			Heme Fe	Non-heme iron					
1 Breakfast meal with coffee + orange juice	12	70	0	0.4	2.4	2.8	2.4	5.7	1.96 ± 0.16
2 Breakfast meal with tea + orange juice	12	70	0	0.4	2.4	2.8	2.4	2.5	2.72
3 Hamburger meal + vegetable salad	10	45	0.5	3.0	0	3.0	0	6.0	1.56 ± 0.12
4 Hamburger meal + cauliflower	10	70	0.5	3.6	0	3.6	0	9.1	1.92 ± 0.18
5 Hamburger meal + orange juice	12	105	0.5	2.0	0	3.0	0	14.2	2.10 ± 0.27
6 Black beans, rice, maize + cauliflower	10	65	0	4.0	0	4.0	0	25.8	2.96 ± 0.26
7 Vegetarian meal I + cauliflower	10	70	0	3.0	0.5	3.5	0.5	8.7	2.10 ± 0.27
8 Vegetarian meal II + cauliflower	10	70	0	3.5	0	3.5	0	16.1	2.10 ± 0.27
9 Rice, vegetables, curry + papaya	14	75	0	4.4	0	4.4	0	2.7	2.96 ± 0.26

maize + cauliflower	65	0	4.4	0	4.4	0	2.7	30.4	0.10 ± 0.03	4.0 ± 1.2	0.18 ± 0.05	2.96 ± 0.26
7 Vegetarian meal	10	0	5.1	0.5	5.3	33.6	5.3	0.14 ± 0.03	5.6 ± 1.2	0.29 ± 0.06	2.96	
8 I + cauliflower	70	0	5.8	0.5	13.5	31.5	13.5	0.41 ± 0.05	16.6 ± 2.0	0.96 ± 0.12		
9 Vegetarian meal	10	0	5.8	0	1.9	39.9	1.9	0.05 ± 0.01	2.0 ± 0.4	0.12 ± 0.02	3.57 ± 0.56	
II + cauliflower	70	0	6.5	0	5.1	39.9	5.1	0.16 ± 0.05	6.4 ± 2.0	0.42 ± 0.03		
Rice, vegetables, curry + papaya	14	0	1.8	0	6.0	30.6	6.0	0.21 ± 0.04	8.4 ± 1.4	0.15 ± 0.03	3.61 ± 0.40	
	75	0	2.3	0	20.7	30.6	20.7	0.76 ± 0.21	30.4 ± 8.4	0.70 ± 0.19		

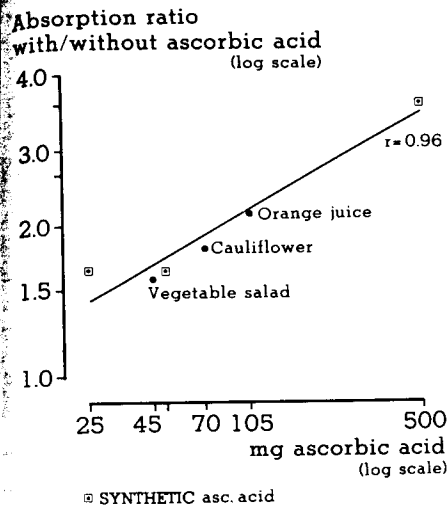


Fig 1. Effect of synthetic and various sources of natural ascorbic acid on non-heme iron absorption from a hamburger meal. The regression line drawn is based on all observations.

rich in ascorbic acid (fresh vegetable salad, cauliflower or orange juice) produce similar effect.

The same conclusion can be drawn from the studies of the Latin-American type of meal by comparing the effect of 50 mg ascorbic acid in pure form and the 65 mg present in cauliflower. In the breakfast meal, orange juice containing 70 mg of ascorbic acid and 75 mg of ascorbic acid in pure form also gave about the same absorption increase. Coffee was the drink given in breakfast meals except in one study when the breakfast was served with tea with and without orange juice (70 mg ascorbic acid). In the tea breakfast orange juice increased the iron absorption 2.7 times. When the same amount of orange juice was given with the same breakfast but served with coffee, the absorption increased 2.0 times. The difference in effect of orange juice may be due to different amounts of tannins in coffee and tea. The present ratio figures are slightly different from those reported earlier (Rossander *et al.*, 1979). This is due to the different way of calculating the effect in the present paper.

The effect of adding cauliflower (60 mg ascorbic acid) to two types of vegetarian meals with about the same iron content increased iron absorption 3.0-3.5 times. In one meal, iron absorption increased from 2.0 to 6.4 per cent (mean absorption ratio 3.6) in the other meal from 5.6 to 16.6 per cent (mean absorption ratio 3.0).

In summary, there is no doubt that foods rich in ascorbic acid markedly increase iron absorption and that this increase is of the same magnitude as obtained by adding the same amount of synthetic ascorbic acid.

#### Effect of synthetic ascorbic acid (Table 4)

Figure 2 shows a comparison of the effect of ascorbic acid on the absorption of iron, expressed as percent increase, from eight different meals giving the same amount (50 mg) of ascorbic acid (studies 12, 15, 16, 18, 21, 23 and 25-26). It is evident that there was a wide variation in the enhancing effect

Table 4. Effect of adding varying amounts of ascorbic acid on iron absorption from different meals

No Meal	Number of subjects	Heme Fe	Iron content (mg) Non-heme iron Native	Total	Absorption (%) Meal Reference (A)	A/R (R)	A <sub>50</sub> (%) Mean ± SEM	A <sub>50</sub> (mg) Mean ± SEM	Absorption ratio with/without ascorbic acid		
10 Breakfast meal with 12.5 mg asc. acid	10	0	0.4	2.4	2.8	5.7	41.7	0.13 ± 0.02	5.2 ± 0.8	0.15 ± 0.02	1.12 ± 0.11
11 Breakfast meal with 25 mg asc. acid	10	0	0.4	2.4	2.8	7.7	41.7	0.16 ± 0.04	6.4 ± 1.4	0.18 ± 0.04	1.52 ± 0.18
12 Breakfast meal with 50 mg asc. acid	10	0	0.4	2.4	2.8	7.7	41.3	0.17 ± 0.04	6.8 ± 1.6	0.19 ± 0.04	1.64 ± 0.20
13 Breakfast meal with 75 mg asc. acid	10	0	0.4	2.4	2.8	9.1	34.7	0.17 ± 0.02	6.8 ± 0.8	0.19 ± 0.02	1.61 ± 0.13
14 Breakfast meal with 500 mg asc. acid	10	0	0.4	2.4	2.8	8.6	34.7	0.19 ± 0.06	7.6 ± 2.4	0.21 ± 0.07	2.34 ± 0.23
15 Breakfast meal (unfortified) with 50 mg asc. acid	9	0	0.4	0	0.4	6.0	44.4	0.14 ± 0.04	11.6 ± 1.5	0.32 ± 0.00	1.68 ± 0.24
16 Wheat bran rolls with 50 mg asc. acid	10	0	2.9	0	2.9	9.2	30.8	0.13 ± 0.05	8.4 ± 2.4	0.03 ± 0.01	2.43 ± 0.06
17 Hamburger meal with 25 mg asc. acid	10	0.5	3.0	0	3.0	12.8	27.0	0.26 ± 0.06	10.4 ± 2.6	0.31 ± 0.06	1.63 ± 0.15
18 Hamburger meal with 50 mg asc. acid	10	0.5	3.0	0	3.0	18.2	30.0	0.38 ± 0.08	15.2 ± 3.2	0.46 ± 0.10	1.61 ± 0.12
19 Hamburger meal with 500 mg asc. acid	10	0.5	3.0	0	3.0	11.0	39.3	0.25 ± 0.04	10.0 ± 1.6	0.30 ± 0.05	3.47 ± 0.40
20 Rice, black beans, maize with 25 mg asc. acid	10	0	4.3	0	4.3	2.5	34.7	0.07 ± 0.17	2.7 ± 0.7	0.12 ± 0.03	2.05 ± 0.31
21 Rice, black beans, maize with 50 mg asc. acid	10	0	4.3	0	4.3	4.3	36.0	0.12 ± 0.025	4.8 ± 1.0	0.21 ± 0.04	2.44 ± 0.31
22 Rice, black beans, maize with 500 mg asc. acid	9	0	4.3	0	4.3	3.4	36.0	0.07 ± 0.002	2.8 ± 0.64	0.12 ± 0.03	6.52 ± 0.85
23 Pizza meal with 50 mg asc. acid	10	0	4.2	0	4.2	6.4	37.3	0.23 ± 0.08	9.2 ± 0.3	0.40 ± 0.01	2.64 ± 0.35
24 Rice, vegetables, curry with 25 mg asc. acid	20	0	1.8	0	1.8	13.3	33.9	0.17 ± 0.03	6.8 ± 1.2	0.29 ± 0.05	1.24
25 Rice, vegetables, curry with 50 mg asc. acid	16	0	1.8	0	1.8	10.1	31.7	0.27 ± 0.04	10.8 ± 1.5	0.19 ± 0.3	1.93
26 Rice, vegetables, curry, fish, with 50 mg asc. acid	25	0	1.3	0	1.3	14.7	34.8	0.48 ± 0.06	19.2 ± 2.4	0.25 ± 0.03	2.19 ± 0.13

Meal	Iron mg	Absorption increase %
19 Hamburger meal with 50 mg asc. acid	3.0	64 ± 0.14
20 Rice, black beans, maize with 25 mg asc. acid	2.8	68 ± 0.15
21 Rice, black beans, maize with 50 mg asc. acid	0.4	64 ± 0.02
22 Rice, black beans, maize with 500 mg asc. acid	1.8	68 ± 0.01
23 Pizza meal with 50 mg asc. acid	1.3	147 ± 0.03
24 Rice, vegetables, curry with 25 mg asc. acid	2.9	150 ± 0.02
25 Rice, vegetables, curry with 50 mg asc. acid	4.3	150 ± 0.04
26 Rice, vegetables, curry, fish with 50 mg asc. acid	4.2	170 ± 0.13

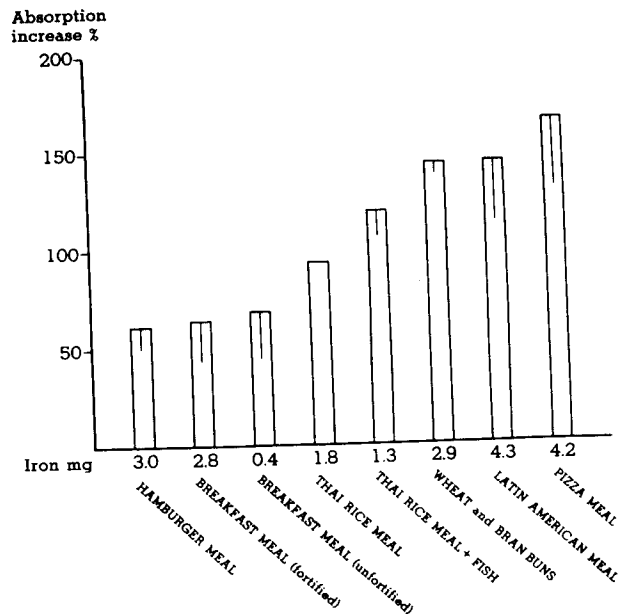


Fig 2. Comparison of the effect of 50 mg of ascorbic acid on the absorption of iron from eight different meals. Iron content of the meals are also given in the figure.

of ascorbic acid on different meals from about 60 to about 160 percent. The variation in the percent increase in iron absorption by ascorbic acid was not related to the iron content of the meals. This is evident from comparing the results from two breakfast meals in which the bread was baked from flour which was either unfortified (study 15) or fortified with ferrous sulphate (study 12). The total iron content in the two meals was 0.4 and 2.8 mg, respectively, but the percentage increase in iron absorption by 50 mg ascorbic acid was the same (64 and 68 percent, respectively). The effect of ascorbic acid was not related to the magnitude of iron absorption from the meals when ascorbic acid was not given, neither when expressed as the extra amount of iron absorbed nor as a change in percent absorption. The average extra amount of iron absorbed when 50 mg ascorbic acid was given varied from 0.01 to 0.33 mg in these eight meals.

Figure 3 shows the relationship between the amount of ascorbic acid added and the change in percentage absorption ratio with/without ascorbic acid for the different meals studied — the breakfast meal, the hamburger meal, the Latin-American type of meal and the Thai basal rice meal. Figure 3 also plots the results from two previously published studies where different amounts of ascorbic acid were added to a simple maize porridge meal (Björn-Rasmussen & Hallberg, 1974) and to a so-called semi-synthetic meal (Cook & Mosen, 1977). It is evident that the slopes of the regression lines are different for different types of meals. As the effect was about the same when adding both crystalline ascorbic acid and ascorbic acid rich foods, both kind of data were used in calculating the regression lines for the different meals.

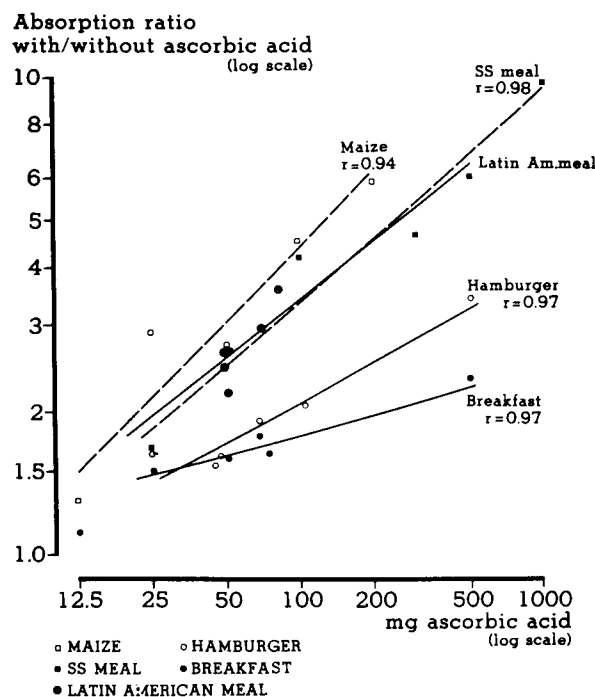


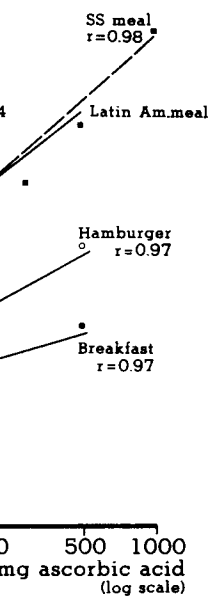
Fig 3. The relationship between the amount of ascorbic acid added and the ratio of the absorption of iron when different meals are given with or without ascorbic acid. The results of the maize meal and the SS meal (semi-synthetic meal) are published previously. (Björn-Rasmussen & Hallberg, 1974; Cook & Monsen, 1977).

It is evident that the regression lines are similar for the Latin-American meals, the previously reported maize meals and the semi-synthetic meals. The slope of the relationship for the Thai rice meals seems to be slightly different but it is based on only three observations. The slope is clearly different for the hamburger meals and the breakfast meals. The difference in slope between the latter two was also significantly different statistically ( $P < 0.05$ ).

#### Discussion

The present data clearly indicate that the same enhancement of iron absorption is obtained when synthetic ascorbic acid is given as when it is administered in its native form, as an integral component of a food (Table 3, Fig. 1). This suggests that ascorbic acid is rapidly and completely released from fruits and vegetables. An important implication of these findings is the feasibility of markedly improving the bioavailability of non-heme iron in the diet by including foods with a high content of ascorbic acid. The effect on iron balance can be expected to be especially marked when the vitamin is included in the main meals containing most of the iron.

The marked variation in the effect of ascorbic acid in promoting iron absorption is another important finding of the present paper. This was



added and the ratio of the absorption of iron. The results of the maize meal and the (Björn-Rasmussen & Hallberg, 1974;

similar for the Latin-American and the semi-synthetic meals. The rice meals seems to be slightly different. The slope is clearly different from the breakfast meals. The difference is significant statistically ( $P$

the same enhancement of iron absorption as when it is a component of a food (Table 3, rapidly and completely released. Application of these findings is the availability of non-heme iron in the presence of ascorbic acid. The effect on iron absorption is clearly marked when the vitamin is added to the iron.

Ascorbic acid in promoting iron absorption is the present paper. This was

evident both in the studies when the same amount of ascorbic acid was given with different meals and in the studies when different amounts of ascorbic acid were given with the same meal, where there were also marked differences between meals.

In the first group of studies, when iron absorption was measured from different meals served with and without 50 mg of ascorbic acid, absorption increase varied from 60 to 160 per cent (Fig. 2). An almost three-fold variation in the effect on iron absorption was also seen when the same amount of cauliflower, as a source of ascorbic acid, was added to different meals.

A relationship between the amount of ascorbic acid added to a meal and the increase in iron absorption (absorption ratio with/without ascorbic acid) was first demonstrated in a study on maize meals (Björn-Rasmussen & Hallberg, 1974). A very similar relationship was reported by Cook & Monsen (1977) with a semi-synthetic meal consisting mainly of dextrimaltose, corn oil and ovalbumin. In the present paper, a close relationship was also found between the amount of ascorbic acid in the meal and the absorption increase. As seen in Fig. 3, which contains both the present and the two previous studies mentioned, the slopes of the regression lines (log/log) are very different. The difference in the slopes of the regression lines is another expression for the difference in effect of ascorbic acid when given with different meals.

What then is the cause of the difference in effect of ascorbic acid? The mechanism of action of ascorbic acid on food iron absorption is usually related either to its ability to reduce ferric to ferrous ions or its ability to form soluble iron complexes. Both mechanisms will reduce the probability that iron ions are strongly bound to other ligands such as hydroxyl ions, and phosphate ions, and that iron is prevented from being absorbed. Ascorbic acid will thus counteract the influence of various ligands in the food that bind iron ions and inhibit non-heme iron absorption. Some of the most potent of these are phytates and tannins. The varying content in meals of such naturally occurring inhibitors of iron absorption may explain the variation in effect of ascorbic acid when added to different meals. Ascorbic acid could thus be considered as an anti-inhibitor in food iron absorption.

Ascorbic acid also counteracts the inhibitory effect of tannins on iron absorption. This has been demonstrated in previous studies by Rossander *et al.* (1979) and Gillooly *et al.* (1983). There are reasons to believe from qualitative tests that the curry included in the Thai rice meals had a high content of tannins, and this is also true for some of the ingredients in the Pizza meal. The anti-inhibitory effect of ascorbic acid on tannins is clearly demonstrated by the marked effect of orange juice on iron absorption from the breakfast served with tea (absorption ratio with/without orange juice was 2.72). Coffee also inhibits iron absorption (Hallberg & Rossander, 1982b; Morck *et al.*, 1983) probably mainly due to its content of tannins, but the inhibition is less marked than of tea (Hallberg & Rossander, 1982b). The enhancing effect on iron absorption of orange juice served with a breakfast with coffee was quite evident (absorption ratio 1.96), but was less marked than that of tea.

Soy protein products have been shown to diminish the fraction of non-heme iron absorbed from a meal (Cook, Morck & Lynch, 1981; Hallberg & Rossander, 1982c). This inhibition can also be counteracted by the addition of ascorbic acid (Morck, Lynch & Cook, 1982). The hamburgers used in the study contained 4 per cent of soy protein. Soy also contains phytates. These facts may possibly explain the more marked effect of ascorbic acid on non-heme iron absorption from the hamburger meals than from the breakfast meals. The present studies were not designed to study the relative importance of meat/fish and ascorbic acid in enhancing non-heme iron absorption, nor whether the effects of these two kinds of absorption promoters are competitive or additive. One study in the present paper however, is of interest in this connection. When ascorbic acid was given in a Thai rice meal, the absorption promoting effect was not decreased, but rather increased, when fish was also given with the meal. This observation supports the conclusions in previous studies that the mechanism of action is different for ascorbic acid and for meat and fish (Hallberg & Rossander 1982a).

In a recent study in 17 US college students (Cook *et al.*, 1984), only a negligible effect on iron stores (serum ferritin) was observed when as much as 1-2 g of ascorbic acid were given with one or two meals every day for several months. It was concluded that vitamin C has little effect on iron status when the diet contains substantial amounts of meat. These observations are quite compatible with the present results which suggest that a more marked long-term effect of ascorbic acid on iron balance can only be expected when the diet has a fairly high content of inhibitors. The composition of the main meals must therefore be considered when attempts are made to predict the effect expected of an increased intake of ascorbic acid.

Derman *et al.* (1980) have suggested that the molar ratio iron/ascorbic acid in a meal has a determining influence on the absorption effect of ascorbic acid. The present data partly fit this hypothesis for higher but not for lower dose levels of ascorbic acid. We found, for example, that 50 mg of ascorbic acid induced the same enhancement of iron absorption when given with two breakfast meals which were identical in composition, except for their markedly different iron content (0.4 and 2.8 mg Fe) due to iron fortification of one of the meals with ferrous sulphate. One explanation for the divergent results might be that each type of meal requires different minimal amounts of ascorbic acid to induce an effect. With an infant milk formula studied by Derman *et al.* (1980), 8 mg of ascorbic acid increased iron absorption almost three times (200 per cent), whereas we found that 12.5 mg ascorbic acid given with the breakfast meal induced a negligible effect (12 per cent).

It is reasonable to assume that the minimum amount of ascorbic acid needed to achieve an effect on the absorption of non-heme iron varies for different meals, possibly due to partial and varying destruction of the ascorbic acid by other food components. Studies on the effect of ascorbic acid on iron absorption from rice meals (Sayers *et al.*, 1974a) indicated that 35 mg of ascorbic acid was too low. The present series of studies, however, indicate that an amount of 25-50 mg of ascorbic acid always induces a measurable

diminish the fraction of non-heme iron (Cook & Lynch, 1981; Hallberg & Rossander, 1984). The counteraction is counteracted by the addition of ascorbic acid. The hamburgers used in this study also contains phytates. These phytates have an effect of ascorbic acid on non-heme iron absorption more than from the breakfast meal. The study is designed to study the relative effect of ascorbic acid in enhancing non-heme iron absorption. These two kinds of absorption are studied in the present paper. When ascorbic acid was given in a meal, the effect was not decreased, but rather increased with the meal. This observation suggests that the mechanism of action is different from fish (Hallberg & Rossander,

Cook *et al.*, 1984), only a small effect was observed when as much as one or two meals every day for a week. Ascorbic acid has little effect on iron status of meat. These observations are consistent with each other and suggest that a more marked effect can only be expected when ascorbic acid is given with the meal. The composition of the main meals in the present study attempts are made to predict the effect of ascorbic acid.

The molar ratio iron/ascorbic acid is 1:1. The absorption effect of ascorbic acid is more pronounced for higher but not for lower molar ratios. For example, that 50 mg of ascorbic acid increases non-heme iron absorption when given with two meals (12 mg Fe) due to iron fortification. The explanation for the divergent results is that different minimal amounts of ascorbic acid are present in the infant milk formula studied by Cook *et al.* and iron absorption almost doubled with 2.5 mg ascorbic acid (12 per cent).

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The amount of ascorbic acid in a meal and non-heme iron varies for different meals. The effect of ascorbic acid on non-heme iron absorption (Hallberg *et al.*, 1974a) indicated that 35 mg of ascorbic acid in a meal of studies, however, indicate that ascorbic acid always induces a measurable

effect. To ensure a more marked effect of ascorbic acid on the non-heme iron absorption from most meals the amount should rather be about 50 mg or more in each meal.

The observations that these relatively small amounts of ascorbic acid markedly increase the absorption of non-heme iron from a meal strongly suggest that one important physiological function of ascorbic acid is to facilitate the absorption of iron from the diet. This opinion is also supported by the observation that the destruction of naturally occurring ascorbic acid in a meal (eg by prolonged warming) significantly reduces the absorption of iron (Hallberg *et al.*, 1982). The role of ascorbic acid in iron absorption would thus be to counteract the effect of naturally occurring ligands in the diet which compete with the intestinal receptors for iron.

In most diets in developing countries the content of both phytates and tannins is often high. The addition of ascorbic acid and/or foods rich in ascorbic acid can then be expected to increase iron absorption markedly and may be considered a realistic alternative to iron fortification. This conclusion is strongly supported by the results of a recent study (Hallberg & Rossander, 1984) in which the effect of adding iron (as ferrous sulphate) to the same Latin-American type of meal (rice, maize, black beans) as studied in the present paper was compared with the addition of ascorbic acid and cauliflower (series 6 in the present paper). It could be estimated that an addition of 50 mg ascorbic acid in any form will increase iron absorption to about the same extent as an addition of 90-100 g meat or as a doubling of the iron content of the meal by iron fortification with ferrous sulphate.

In the industrialized society, a sedentary life style may change the iron balance situation for the worse. Energy requirements and thus food intake have become markedly reduced but iron requirements remain the same. The lower food intake has also led to a lower intake of dietary fibre and there is a clear trend today towards increasing the fibre content of the diet by various measures, eg use of flour with a higher extraction rate or the direct increase in the intake of bran and similar food items with a high fibre content. However, most of these measures, which seem to be physiologically desirable, increase the phytate content of the diet and thus reduce the bioavailability of non-heme iron. In some populations, fortification of certain foods with iron is probably necessary but it is also necessary to ensure that the bioavailability of the dietary iron is sufficient to cover the high iron requirements in children, teenagers, and women of child-bearing age. An adequate intake of ascorbic acid with the main meals is important to improve the bioavailability of the dietary iron and is a necessary complement to the intake of other enhancers of non-heme iron absorption such as fish and meat. For vegetarians, a high intake of ascorbic acid is a necessity for iron nutrition.

The present recommendation that each main meal should preferably contain about 50 mg of ascorbic acid implies an increase of the total daily intake of ascorbic acid. It is of interest, however, that the recommendation is compatible with the much higher intake of ascorbic acid in early man. This was emphasized in a recent critical review of paleolithic nutrition in man

(Eaton & Konner, 1985). In fact, studies in recent hunter-gatherer populations indicate average intake would have been as high as 400 mg each day.

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