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Iron absorption from rice meals cooked with fortified salt containing ferrous sulphate and ascorbic acid

BY M. H. SAYERS, S. R. LYNCH, R. W. CHARLTON AND T. H. BOTHWELL

South African MRC Iron and Red Cell Metabolism Unit, Departments of Medicine and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa

AND R. B. WALKER

Department of Botany, University of Washington, Seattle, USA

AND FATIMA MAYET

Department of Medicine, University of Natal, Durban, South Africa

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1. Iron absorption from rice-containing meals was measured by red cell utilization of radioactive Fe in sixty-six volunteer multiparous Indian women.

2. In all the studies salt added during the cooking process was used as the carrier for supplemental inorganic Fe and ascorbic acid.

3. Intrinsic Fe in the rice and supplementary inorganic Fe were absorbed to the same extent, with a wide range of absorption values.

4. There was a striking difference between the mean absorption of a 3 mg dose of ferrous Fe given to fasting subjects in a solution containing 30 mg ascorbic acid and that of Fe in a rice meal (48·7 and 3·5 % respectively).

5. When ascorbic acid was added during cooking there was a threefold increase in the absorption of both intrinsic Fe and supplementary Fe when a sufficient quantity (60 mg) was present.

6. It is concluded that the Fe nutrition of rice-eating communities could be improved significantly by the addition of ascorbic acid to the diet.

Although there is evidence to suggest that the iron content of predominantly vegetable diets is often adequate (Ramalingaswami & Patwardhan, 1949; Apte & Iyengar, 1970), its availability for absorption is poor (Hussain, Walker, Layrisse, Clark & Finch, 1965; Layrisse, Cook, Martinez-Torres, Roche, Kuhn, Walker & Finch, 1969; Ashworth, Milner & Waterlow, 1973). Lack of available Fe is therefore an important factor in the pathogenesis of Fe deficiency in populations subsisting on diets in which the staple foodstuffs are vegetables. Improvement of Fe nutrition by dietary supplementation with inorganic Fe in various forms has produced disappointing results (Elwood, 1968; Layrisse, Martinez, Cook, Walker & Finch, 1973), and it has become increasingly apparent that the poor absorption of both the intrinsic vegetable Fe and the supplementary Fe is due to the presence in these staple foodstuffs of substances, e.g. phytates, which inhibit Fe absorption. On the other hand, there is evidence that absorption may be increased if potentiating factors are present in the diet. For example, the addition of ascorbic acid to maize porridge before boiling increases the absorption of not only the intrinsic Fe but also supplementary inorganic Fe (Sayers, Lynch, Jacobs, Charlton, Bothwell, Walker & Mayet, 1973). It has been suggested that its failure to produce a similar effect when baked into whole-wheat bread is related to its destruction by the high temperatures required for baking.

Because rice is an important staple food that is prepared by boiling, we investigated the effect of ascorbic acid supplementation on Fe absorption from rice-based meals. In these studies cooking salt (NaCl) was used as the carrier for both supplemental Fe and ascorbic acid.

EXPERIMENTAL

Sixty-six Indian housewives living in Durban and belonging to a low socio-economic group were studied. They were aged between 35 and 50 years (mean 38 years) and had each had at least three children. The diet of these people is largely made up of cereal foodstuffs, and Fe-deficiency anaemia has previously been shown to be common among them (Mayet, Adams, Moodley, Kleber & Cooper, 1972). Written consent was obtained from all subjects after the nature of the investigation had been fully explained to them.

The absorption of Fe from a traditional meal of rice with dhal sauce (made from split pulses), or rice with potato and onion soup, was studied. In some experiments rice intrinsically labelled with ^{55}Fe by hydroponic culture (Hussain *et al.* 1965; Layrisse *et al.* 1969) was mixed with carrier rice to provide 7·0 μCi/subject, and the absorption of the ^{55}Fe was compared with that of 2·5 μCi ^{59}Fe added as $FeSO_4.7H_2O$ during cooking. In other studies two meals were eaten on consecutive days, the $FeSO_4.7H_2O$ added during cooking being labelled with 7·0 μCi ^{55}Fe on the 1st morning and with 2·5 μCi ^{59}Fe on the 2nd morning. Before each study the subjects were weighed and blood was taken for haematological measurements. The meals were eaten after an overnight fast. Water was allowed *ad lib.* during the meal and for 4 h afterward, but no other food or drink was consumed during this time. A blood sample was collected 14 d later, after an overnight fast, for determination of its ^{55}Fe and ^{59}Fe content. Immediately afterwards 150 ml of a freshly prepared solution containing 30 mg ascorbic acid and 3 mg Fe as $FeSO_4.7H_2O$ labelled with 3·5 μCi ^{59}Fe was drunk. After a further 14 d a second blood sample was collected and its ^{59}Fe activity measured. The absorption of this 'reference' Fe salt, which gave a measure of the absorption capacity of each individual, was then determined by difference.

We calculated that if the whole of each test dose had been retained, the total radiation dose averaged over a period of 13 weeks would have been approximately 25% of the permissible whole body burden for continuous exposure in the case of ^{59}Fe and 0·5% in the case of ^{55}Fe (International Commission for Radiation Protection, 1960).

Preparation of the meals

Sufficient rice was weighed out to provide 100 g dry rice/subject. It was washed in running tap-water and then boiled in two-and-a-half times its weight of tap-water containing 4 g NaCl (analytical grade; BDH Chemicals Ltd, Poole, England) per subject until it was soft, usually about 15 min. All cooking was done in aluminium pots. In some studies the added NaCl contained 4 mg Fe as $FeSO_4.7H_2O$ (analytical grade; BDH Chemicals Ltd) labelled with ^{59}Fe or ^{55}Fe (Radiochemical Centre, Amersham, England). Various amounts of L-ascorbic acid (analytical grade; BDH Chemicals Ltd) were also present in the NaCl in certain experiments. (The feasibility of using NaCl in this way was tested by adding $FeSO_4.7H_2O$ and ascorbic acid to NaCl in the concentrations used in the absorption experiments and leaving the mixture at room temperature in the laboratory. There was no discoloration or change in taste in a period of 6 months and the ascorbic acid content was unchanged.) When the grains were soft, the water was decanted and the rice was allowed to steam for a further 20 min. The cooked rice was divided into equal portions by weight, and either pea dhal or potato and onion soup was added. A portion was retained for the determination of chemical Fe and radioisotope contents. For technical reasons it was not possible to measure the ascorbic acid content of the rice after cooking. However, previous studies with boiled maize porridge indicate that about two-thirds of an ascorbic acid supplement is still present in the reduced form after cooking (Sayers *et al.* 1973).

Sufficient dhal was weighed to provide 50 g dry weight for each volunteer. It was boiled in four times its weight of water for 30 min with small quantities of tomato, onion and seasoning in the form of tumeric powder and caraway seeds. Curry leaves, garlic and jeera, a spice, were fried and then homogenized with the pea dhal. The final volume consumed by each individual was approximately 200 ml.

Sufficient onions and potatoes were weighed out to provide 50 g dry weight of each vegetable per subject. The onions were fried in oil and then added, with the potatoes, to twice their total weight of water. Pepper and curry leaves were added as seasoning and the mixture was boiled for 20 min. The approximate volume of soup consumed by each individual was 175 ml.

Isotope and chemical methods

Portions of food (1 g) and blood samples (10 ml) were prepared for differential radioactive counting using the method of Katz, Zoukis, Hart & Dern (1964). The quantities of ^{55}Fe and ^{59}Fe in the processed samples were determined in a liquid scintillation spectrometer (Packard Tri-Carb Model 3002) which had a counting efficiency of 3–4% for ^{55}Fe and approximately 50% for ^{59}Fe at optimal gain and window settings. The scintillant used was Insta-Gel (Packard Instrument Co., Downers Grove, Illinois, USA). Blood samples (4 ml) collected at the beginning and at the end of the 'reference salt' study were counted in a scintillation spectrometer (Packard Autogamma Tri-Carb Model 3001) against suitable standards. The counting efficiency was approximately 20%. The percentage absorption was calculated on the assumption that 100% of the absorbed radioactivity was in circulation and that the blood volume of each subject was 65 ml/kg body-weight.

Serum Fe concentrations were estimated by a modification (Bothwell & Finch, 1962) of the method of Bothwell & Mallett (1955) in which sulphonated bathophenanthrolene was used as the colour reagent. The unsaturated Fe-binding capacity was determined by the method of Herbert, Gottlieb and Lau (1967). The Fe content of digested samples of food was estimated by a modification (Bothwell & Finch, 1962)

Table 1. *Absorption values for intrinsic iron (^{55}Fe) and extrinsic Fe (given as $FeSO_4.7H_2O$ labelled with ^{59}Fe) from rice and vegetable soup meals consumed with and without 100 mg ascorbic acid by female subjects*

Supplement	Final Fe content of meal (mg)	Haemoglobin (g/l)	Plasma Fe (mg/l)	Percentage saturation of total Fe-binding capacity	Fe absorption (%) Reference salt	^{55}Fe	^{59}Fe	Ratio, extrinsic Fe: intrinsic Fe
4 mg added Fe no added ascorbic acid	5·5	134	1·23	33·2	29·2	0	0·6	—
		140	0·61	16·8	29·4	0·7	1·2	1·7
		140	0·93	31·7	50·6	1·3	1·4	1·1
		90	0·44	9·7	64·3	2·5	2·9	1·2
		92	0·53	10·5	45·3	3·5	5·1	1·5
		128	0·36	9·4	75·2	4·1	4·3	1·1
	Mean	121	0·68	18·6	49·0	2·0	2·6	1·3
4 mg added Fe 100 mg added ascorbic acid	6·6	107	1·42	50·2	10·8	0·9	1·2	1·3
		115	0·59	14·0	102·4	1·8	4·3	2·4
		148	1·50	—	40·9	5·5	7·5	1·4
		102	0·37	8·2	108·0	6·5	6·7	1·0
		132	0·98	30·2	34·8	8·9	10·2	1·2
		128	1·04	—	53·9	12·9	12·5	1·0
		134	0·73	17·5	43·7	15·5	18·2	1·2
		128	1·76	40·9	89·7	15·5	20·5	1·3
		134	0·96	20·3	65·9	38·8	34·0	0·9
	Mean	125	1·04	25·9	61·1	11·8	12·8	1·3

of the method of Lorber (1927). This was necessary since there was some variation in the Fe content of the foods that were used.

RESULTS

Comparison of the absorption of intrinsic rice Fe with that of supplemental Fe

It was first considered necessary to establish whether an Fe salt added to rice during cooking was absorbed to the same degree as was the intrinsic rice Fe. This has been shown to occur with several other staple vegetable foods (Björn-Rasmussen & Hallberg, 1972; Cook, Layrisse, Martinez-Torres, Walker, Monsen & Finch, 1972; Sayers *et al.* 1973). When six subjects consumed a single meal of rice intrinsically labelled with ^{55}Fe, which had been cooked with NaCl containing 4 mg Fe as $^{59}FeSO_4.7H_2O$ and the rice was given together with potato and onion soup, the absorption of each isotope was similar; the mean value for the intrinsic label was 2·0% (SD ± 1·6) and for the extrinsic label 2·6% (SD ± 1·8) (Table 1). The absorption of the dietary Fe was in marked contrast to the absorption of a reference Fe salt, the mean value for which was 49·0% (SD ± 18·5).

A second, similar experiment was done using nine subjects; the only difference was the presence in the NaCl of 100 mg ascorbic acid with 4 mg Fe as $^{59}FeSO_4.7H_2O$. The mean value for absorption of intrinsic Fe in the rice was 11·8% (SD ± 11·5) and of the extrinsic label 12·8% (SD ± 10·1); the value for the reference salt was 61·1% (SD ± 33·0) (Table 1). It was concluded from these two experiments that supplemental $FeSO_4$.

Table 2. *Absorption values for iron in rice meals given with 4 mg supplementary Fe as $FeSO_4.7H_2O$ with and without 35 mg ascorbic acid to female subjects*

(Final Fe content of meal: without ascorbic acid 7·8 mg; with ascorbic acid 7·0 mg)

Haemoglobin (g/l)	Plasma Fe (mg/l)	Percentage saturation of total Fe-binding capacity	Fe absorption (%) Reference salt	Without ascorbic acid	With ascorbic acid
128	1·30	28·5	7·4	0·7	0·1
128	1·55	29·9	29·4	1·1	2·6
116	1·03	29·6	74·5	2·0	7·9
128	0·93	20·9	10·6	2·1	0·4
120	0·93	20·8	43·0	2·8	6·6
136	1·05	21·4	70·7	3·4	4·8
128	0·70	23·5	10·9	5·3	1·6
112	0·31	6·7	102·0	6·3	13·4
122	0·62	17·4	61·2	8·1	7·4
88	0·54	10·4	101·3	11·7	15·3
Mean 121	0·90	20·9	51·1	4·4	6·0

Table 3. *Comparison of absorption values for iron added to rice+soup and Fe added to rice+dhal meals each containing 4 mg supplementary Fe as $FeSO_4.7H_2O$ and 35 mg ascorbic acid, eaten by female subjects*

(Final Fe content of meal: rice+dhal 6·2 mg; rice+soup 5·1 mg)

Hb (g/l)	Plasma Fe (mg/l)	Percentage saturation of total Fe-binding capacity	Fe absorption (%) Reference salt	Rice+dhal	Rice+soup
115	1·18	39·8	12·1	0·2	0·5
121	—	—	18·9	1·0	0·3
132	1·68	44·1	31·5	1·4	3·2
132	1·84	28·2	38·9	1·7	1·6
94	0·45	9·9	39·6	2·2	4·4
118	0·47	9·6	34·6	3·1	7·3
97	0·54	11·5	60·9	3·3	4·4
101	0·38	8·6	103·8	3·3	23·4
70	0·43	9·4	50·9	5·0	7·1
94	0·34	8·9	60·7	5·0	8·4
112	0·62	15·9	58·1	5·4	11·2
120	0·72	14·8	26·7	6·3	7·4
Mean 109	0·79	18·2	44·7	3·2	6·6

$7H_2O$ with or without added ascorbic acid was absorbed to the same extent as the intrinsic Fe in the rice. Furthermore, the addition of 100 mg ascorbic acid increased the absorption of both the added Fe and the intrinsic Fe. (These conclusions only hold if the intrinsically labelled rice is properly cooked. In a preliminary study using nine subjects, Fe absorption was measured when the rice was prepared with 35 mg added ascorbic acid. The mean value for absorption of the intrinsic label was significantly less than that for the extrinsic label; the values were 3·0% (SD ± 2·4) and 7·4% (SD ± 4·9) respectively ($t = 4{\cdot}24$, $P < 0{\cdot}005$). On inspection, some grains of rice were

Table 4. *Absorption values for iron in rice meals given with 4 mg supplementary Fe as $FeSO_4.7H_2O$ with and without 60 mg ascorbic acid to female subjects*

(Final Fe content of meal: without ascorbic acid 5·4 mg; with ascorbic acid 5·7 mg)

Haemoglobin (g/l)	Plasma Fe (mg/l)	Percentage saturation of total Fe-binding capacity	Fe absorption (%)		
			Reference salt	Without ascorbic acid	With ascorbic acid
148	0·84	25·2	4·9	0·2	1·0
108	0·51	11·9	51·1	0·8	3·6
128	0·50	10·7	8·6	1·9	6·1
134	0·82	19·6	35·6	2·0	7·7
121	0·76	23·2	51·1	3·6	16·9
87	0·35	5·9	52·9	4·7	15·3
108	0·53	21·7	30·4	5·4	12·8
121	0·46	10·5	98·5	7·2	31·4
Mean 119	0·60	16·1	41·6	3·2	11·9

obviously incompletely cooked and when these were separated they were found to contain the ^{55}Fe. The labelled rice therefore required a longer period of cooking than the carrier rice. Care was taken, therefore, to cook the labelled rice until it was soft.)

Effect of 35 mg ascorbic acid on the absorption of Fe in a rice meal

As the results of the first experiment suggested that the addition of 100 mg ascorbic acid to the diet increased the absorption of Fe in a rice meal, the effect of smaller doses was studied.

In the first experiment, ten volunteers ate a meal of rice and dhal sauce. On the 1st morning the meal was cooked with NaCl containing 4 mg iron as $FeSO_4.7H_2O$ and on the 2nd morning the same dose of Fe plus 35 mg ascorbic acid was added. The mean value for absorption of Fe from the rice meal which contained no supplementary ascorbic acid was 4·4% (SD ± 3·5), and the mean absorption of the Fe in the meal with added ascorbic acid was 6·0% (SD ± 5·2) (Table 2). The difference between the two means was not significant ($t = 1·52$, $P > 0·1$).

There may be one of two reasons for the failure to demonstrate significant increase in Fe absorption at this level of Fe fortification. Though it seemed probable that the dose of ascorbic acid was too low, the possibility that the dhal sauce contained some inhibitory factor not present in the potato and onion soup could not be excluded. A formal comparison was therefore made between the two meals. As before, 4 mg Fe as $FeSO_4.7H_2O$ and 35 mg ascorbic acid were present in the salt. Twelve subjects consumed the rice with dhal sauce on the 1st morning, the $FeSO_4.7H_2O$ being labelled with ^{55}Fe, and the rice with soup meal on the 2nd morning. The isotope label on the 2nd occasion was ^{59}Fe. The mean value for Fe absorption from the meal containing dhal sauce was somewhat less (3·2%, SD ± 1·9) than that for absorption from the soup meal (6·6%, SD ± 6·3), but the difference was not significant ($t = 2·15$, $P > 0·05$). The mean value for Fe absorption from the reference salt was 44·7% (SD ± 24·5) (Table 3).

Table 5. *Absorption values for iron in rice meals given with* 4 *mg supplementary Fe as* $FeSO_4.7H_2O$ *with and without* 100 *mg ascorbic acid to female subjects*

(Final Fe content of meal: without ascorbic acid 7·6 mg; with ascorbic acid 6·3 mg)

Haemoglobin (g/l)	Plasma Fe (mg/l)	Percentage saturation of total Fe-binding capacity	Fe absorption (%) Reference salt	Fe absorption (%) Without ascorbic acid	Fe absorption (%) With ascorbic acid
148	1·61	43·2	29·5	0·2	0·2
132	0·70	22·7	12·7	1·5	6·4
120	1·24	31·2	6·2	1·6	7·7
118	1·56	45·4	52·8	1·7	8·5
87	0·26	4·3	46·2	2·5	7·6
108	0·63	14·0	37·9	2·7	6·3
112	1·00	24·0	13·7	3·2	6·4
118	0·48	14·3	52·7	3·4	12·7
112	0·78	13·5	60·3	3·7	12·3
94	0·65	17·0	102·3	4·2	11·7
110	0·43	6·8	52·6	12·8	31·8
123	0·73	15·6	67·2	13·1	34·4
Mean 115	0·84	21·0	44·5	4·2	12·2

Effect of 60 *mg ascorbic acid on the absorption of Fe in a rice meal*

As the presence of dhal sauce was apparently insufficient to account for the relatively low absorptions obtained with 35 mg of added ascorbic acid, the effect of adding 60 mg ascorbic acid was studied. This was done with eight volunteers given a rice and soup meal. The addition of 60 mg ascorbic acid significantly increased the percentage absorption of Fe from 3·2 (SD ± 2·4) to 11·9 (SD ± 9·7) ($t = 3·25$, $P < 0·02$). The mean absorption of the reference salt in the group was 41·6% (SD ± 29·6) (Table 4).

Effect of 100 *mg ascorbic acid on the absorption of Fe in a rice meal*

Finally, a study was made of the effect on Fe absorption of increasing the ascorbic acid supplement to 100 mg. Eleven volunteers consumed rice meals with potato and onion soup on two successive mornings: on the 1st morning the supplementary $^{55}FeSO_4.7H_2O$ was added without ascorbic acid; on the 2nd morning the Fe source was $^{59}FeSO_4.7H_2O$ and 100 mg ascorbic acid were also added. The mean value for Fe absorption from meals without added ascorbic acid was 4·2% (SD ± 4·2), whereas with added ascorbic acid it was 12·2% (SD ± 10·2); the difference was significant ($t = 4·42$, $P < 0·005$). The mean absorption of Fe from the reference Fe salt was 44·5% (SD ± 27·0) (Table 5). It was concluded that 100 mg ascorbic acid were no more effective than 60 mg in increasing Fe absorption from a rice meal.

DISCUSSION

Our results indicate that the intrinsic Fe in rice is absorbed to the same extent as is a small quantity of an inorganic Fe salt consumed at the same time if the rice is well cooked. The results are similar to those obtained for a number of vegetable foodstuffs

(Björn-Rasmussen & Hallberg, 1972; Cook *et al.* 1972; Sayers *et al.* 1973). These results are of both theoretical and practical importance. First, they suggest that the two forms of Fe form a common pool with respect to absorption and, secondly, they indicate that there is little purpose in fortifying a foodstuff that contains strong inhibitors of Fe absorption. For example, mean values for absorption of intrinsic Fe in rice of less than 1 % have been reported (Layrisse & Martinez-Torres, 1971); any Fe added to this rice should be similarly absorbed. The situation can be put into better perspective by our results. The subjects studied were multiparous Indian women living in a community where Fe-deficiency anaemia is common. Fe deficiency in the group was confirmed by giving each volunteer a small dose of a ferrous salt; in most of the subjects the values for Fe absorption were high. However, even in this group of women, absorption of dietary Fe was low, with a mean value of less than 4 % for both intrinsic Fe in rice and supplementary Fe. Values calculated from these results gave a mean Fe absorption value of less than 0·3 mg from a supplemented meal containing a total of 7 mg Fe. The addition of an adequate amount of ascorbic acid before boiling the rice had a marked effect on Fe absorption, producing a threefold increase in total Fe absorption. By varying the amount of added ascorbic acid it was possible to show that 60 mg was a satisfactory dose, whereas 35 mg was insufficient. The results of our study together with those obtained with other vegetables (Sayers *et al.* 1973) suggest that Fe nutrition of populations subsisting on vegetable diets could be improved significantly if ascorbic acid could be added to the diet.

In our study, common salt (NaCl) was used as the carrier for both the ascorbic acid and ferrous sulphate. Although this study was not primarily concerned with the storage properties of supplemented salt, it was possible to show that both supplements could be added to pure NaCl without discoloration or change in taste. However, the environment was temperate and it is possible that salt would prove a less satisfactory carrier under hot, humid conditions.

A supplementary study which gave interesting results was the determination of Fe absorption in each subject by means of a small dose of a reference salt. This was done to measure the Fe status of individual subjects. The Fe absorption rates were high for most subjects, which showed that they were Fe-deficient. A correlation between these reference Fe absorptions and the absorption of dietary Fe has been reported (Cook *et al.* 1972; Layrisse *et al.* 1973). We determined correlation coefficients for subjects receiving no ascorbic acid and for those receiving 35, 60 or 100 mg. The values were: no ascorbic acid $r+0{\cdot}53$, $P < 0{\cdot}001$; 35 mg $r+0{\cdot}77$, $P < 0{\cdot}001$; 60 mg $r+0{\cdot}86$, $P < 0{\cdot}01$; 100 mg $r+0{\cdot}29$, $P > 0{\cdot}1$. In general, these results confirm the usefulness of the measurement of 'reference' salt absorption as a method of defining the Fe absorption capacity of individual subjects and also its use in comparing results obtained in different laboratories. Also important was the finding that the increase in Fe absorption produced by a given dose of ascorbic acid could be predicted on the basis of the Fe absorption from an unsupplemented meal. The correlations between unsupplemented meals and meals supplemented with different amounts of ascorbic acid were: 35 mg $r+0{\cdot}76$, $P < 0{\cdot}01$; 60 mg $r+0{\cdot}93$, $P < 0{\cdot}001$; 100 mg $r+0{\cdot}98$, $P < 0{\cdot}001$.

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