A preliminary simulated iron fortification trial in South Indian preschool children

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1. The effect of fortification of food with iron to provide 10 mg elemental Fe/child per d was studied in preschool children maintained on a cereal diet, over a 5-month period.

2. The absorption of 5 mg Fe as ferrous sulphate mixed in one meal was 3·3 % of the test dose and when 3·3 mg was given with each of three meals over a 2 d period the corresponding value was 4·8 %.

3. The mean absorption of a test dose of ferrous ascorbate studied in twenty-four children midway through the trial was 42 %.

4. The only beneficial effect of Fe fortification in this time-period in the experimental group was the prevention of the decrease in packed cell volume which occurred in the control group.

Iron supplementation of 20–30 mg inorganic Fe/d, given between meals to preschool children was successful in significantly improving their Fe nutritional status within a period of 3 months (Pereira *et al.* 1978). However, supplements of this order cannot be provided by food fortification. If Fe deficiency is to be combated by fortification of foodstuffs it must be shown that the lower level of supplementation, which can be achieved by this means, is beneficial.

The following studies were undertaken to assess the effect of a feasible level of food fortification with Fe in preschool children maintained on a high-cereal diet.

MATERIAL AND METHODS

Preschool children 2–5 years of age living in a residential home were the subjects of the study. The children were admitted after a medical examination which included a skiagram of the chest. Children with tuberculosis and other chronic diseases were excluded. All were in apparent good health and none was infested with hookworm (*Ancylostoma duodenale*). Informed consent was obtained from the children's parents or legal guardians for their participation in the trial. Blood was taken without venous stasis, after an overnight fast, for the determination of haematological status. Haemoglobin was estimated by the cyanmethaemoglobin method using a photoelectric colorimeter, checked periodically against an international reference standard. Packed cell volume was measured by the use of a micro-haematocrit centrifuge. Serum Fe was determined by the method recommended by the International Committee for Standardization in Haematology (1971) and the unsaturated-Fe-binding capacity by the method of Herbert *et al.* (1966). Serum vitamin B_{12} was estimated using *Euglena gracillis* Z strain (Hunter *et al.* 1956) and serum folic acid by the technique of Waters & Molin (1961), using *Lactobacillus casei* as the test organism. The estimations were repeated 5 months later. Before the study was undertaken all the children had received supplements of cyanocobalamin and folic acid and the concentrations of these nutrients were at normal levels throughout the trial period.

The children were paired on the basis of their height, serum Fe and haemoglobin con-

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centration. One of each pair was assigned to an experimental group receiving an Fe-fortified diet and the other to a control group.

The diet, given to all the children, provided their requirements of energy and protein from cereals (millet (*Eleusine coracana*) and rice) pulses, vegetables and fruits (WHO, 1973). Millet was eaten at breakfast and rice at the other meals. Animal foods were not included. The average Fe content of the diet was 18 mg Fe/4·18 MJ eaten, as estimated by the method of Elvehjem (1930). The diet supplied an average (± SE) of 1·62 (±0·05) mg Fe/kg body-weight per d.

Ferrous sulphate, to provide a daily supplement of 10 mg inorganic Fe/child, was mixed into and cooked with the food given to the experimental group at breakfast and dinner when a weighed amount was served to each child and its consumption assured.

Heights were measured every month, the average of three readings being recorded. The average of the weights on three consecutive days was recorded every 2 weeks. A daily record of morbidity was maintained by trained resident staff.

The absorption of Fe was measured by radioisotopic studies using a moving-bed, shadow-shield, whole-body counter. In the twenty-four subjects in the experimental group the absorption of the supplement of 5 mg elemental Fe labelled with 1 μCi Fe^{59} was tested at the beginning of the experiment. The Fe was added to one curry dish before cooking and served as described previously. Each child was given an equal portion of this dish containing 5 mg inorganic Fe which he ate in a normal manner with the cereal (millet). The complete consumption of the meal was assured. In the same twenty-four subjects, 11 weeks after the start of the supplementation, the absorption of a reference dose of Fe was measured. This dose consisted of 3 mg elemental Fe in the form of freshly-prepared ferrous ascorbate, labelled with 1 μCi Fe^{59} given after an overnight fast. In the subjects in the control group, the absorption of ferrous sulphate supplying 10 mg inorganic Fe/d and mixed with 1 μCi Fe^{59}, was tested over a 2 d period. Inorganic Fe (3·3 mg) was added to a curry dish before cooking and fed to the children three times daily for 2 d.

RESULTS

The Fe absorption (mean ± 1 SE) from the 5 mg dose added to one meal was 3·3 ± 0·28% in twenty-four children. The Fe absorption (mean ± 1 SE) from the 3·3 mg added to each meal over the 2 d period was 4·8 ± 0·38% in the children in the control group. The absorption of the reference dose of 3 mg elemental Fe in the twenty-four children in the experimental group after 11 weeks of supplementation was 42·2% (SE 2·96).

Twenty-two pairs of children completed the 5 months on the trial. Their haematological status, height and weight at the beginning and end of the study are shown in Table 1.

In the control group the final value of the packed cell volume was significantly lower than the initial value ($P < 0·01$). The difference between the groups in packed cell volume and the other measurements were not significant.

The pattern of illness was similar in both groups of children (Table 2). Those in the control group had slightly more episodes of respiratory infection than the children in the group receiving extra Fe. In the control group, there were seven children who had had one to two episodes each, four children who had three episodes and two children who had four episodes each whereas in the fortified group, eight children had one to two episodes of upper respiratory infection each and only one child had three attacks. The differences in the episodes of respiratory infection between the two groups were not of statistical significance, probably because of the small numbers of subjects studied.

Table 1. *Heights, weights and haematological values of South Indian preschool children in the iron-supplemented (10 mg inorganic Fe/child per d) and control groups**

(Mean values and standard deviation for twenty-two children/group)

	Haemoglobin (g/l)		Packed cell volume		Serum Fe (mg/l)		Unsaturated-Fe-binding capacity (mg/l)		Percentage saturation of transferrin		Height (m)		Wt (kg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fe-supplemented														
Initial	103	14	0·361	0·035	0·540	0·247	0·359	0·075	14·4	8·2	0·901	0·062	11·62	1·85
Final	98	11	0·365	0·033	0·764	0·413	0·262	0·093	25·3	17·5	0·920	0·064	12·04	1·23
Control														
Initial	102	10	0·361	0·025	0·565	0·249	0·334	0·081	15·6	9·9	0·902	0·057	11·87	2·07
Final	95	11	0·345	0·022	0·778	0·385	0·286	0·097	23·5	14·4	0·922	0·059	12·48	2·35

* For further details, see p. 413.

Table 2. *The episodes of illness among the apparently-normal South Indian preschool children in the iron-supplemented (10 mg inorganic Fe/child per d) and control groups**

	Respiratory infection	Fever	Diarrhoea/ dysentery	Ear discharge	Impetigo/ scabies	Gingivitis	Others
Fe-supplemented (22 children)	16	23	13	7	13	7	2 (Hepatitis and urticaria)
Control (22 children)	31	20	10	4	10	3	1 (Hepatitis)

* For details, see p. 413.

DISCUSSION

There is a lack of information on the absorption of fortification Fe by preschool children consuming cereal- and vegetable-based diets. Comparisons have therefore to be made with results obtained in adults given cereal diets. In one study, salt (sodium chloride) fortified with ferrous sulphate to provide an Fe supplement of 3 mg, was cooked with rice, lentils and vegetables and eaten by Fe-deficient Indian women resident in South Africa. The average absorption of the supplement was 3·5% with a range of 0·6 – 11·7% (Sayers *et al.* 1974). In another study, 4·9% of a supplement of ferrous sulphate baked in wheat bread was absorbed by women who were not Fe deficient (Cook *et al.* 1973). The percentage absorption in the present study, where 3·3% of the supplement was absorbed at a single meal and 4·8% absorbed when the supplement was added to six meals fed over 2 d, was comparable to that found in these two studies in adults. In the present study better absorption was obtained when the supplement was fed over a 2 d period than when offered at a single meal. There are several factors which would account for the difference. The absorption of Fe is adversely influenced by the amount of phytin-phosphorus in the diet and favourably by the presence of ascorbic acid. The lower mean absorption of the supplement fed at one meal probably reflects the adverse effect of the negligible amount of ascorbic acid as compared with the better absorption over the 2 d period when the daily intake of ascorbic acid was 33 mg (Layrisse *et al.* 1974; Sayers *et al.* 1974). In fact the presence of phytin-P did not appear to influence absorption as the ratio phytin-P: Fe was relatively higher over the 2 d period (29 mg/mg Fe) than at the one meal (21 mg/mg Fe). Further, it is well established that the absorption of Fe varies from subject to subject and also from day-to-day in the same individual (Cook *et al.* 1969).

Assuming that the blood volume of preschool children is 80 ml/kg (Smith, 1972) the absorption of Fe, at 3·3% level, from the supplemental and food Fe would result in the accumulation of 142·5 mg over the trial period. Fe losses, estimated at 0·13 mg/d, amounted to 19·5 mg and the requirement for growth, estimated at 0·22 mg/d, was 33 mg (Bothwell & Finch, 1962), leaving 90 mg absorbed Fe, available for increases in haemoglobin. Allowing for the increase in blood volume resultant on growth, the haemoglobin value of the supplemental group should have increased by 24 g/l. In contrast to this theoretical projection, the children in the supplemented group showed a slight decrease in the average haemoglobin concentration though it was not as marked as that in the unsupplemented group. The only detectable benefit in the supplemented group was the prevention of the decrease in packed cell volume which occurred in the control children.

The absorption of the reference dose of ferrous ascorbate was carried out 11 weeks after the supplementation was started. It is obvious from the average absorption of 42% of the reference dose, that many of the children were still Fe deficient and that decreasing absorption as Fe nutritional status improved can probably not explain the failure of haemoglobin to increase. The children were affected by a variety of minor illnesses and it is possible that these interfered with Fe absorption or utilization or both. Other factors, as yet unknown, may also have played a part.

The difficulties of demonstrating positive benefits following iron supplementation and fortification in an industrialized country have been summarized by Elwood (1968). In the present study, despite theoretical considerations, the only demonstrable benefit was the maintenance of the packed cell volumes in the children receiving the fortified diet.

Longer-term trials are necessary to establish the benefits, if any, of food fortification in populations eating vegetarian diets.

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