Ferrous fumarate fortification of a chocolate drink powder

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An evaluation was made into the usefulness of ferrous fumarate as an iron fortificant for an experimental chocolate drink powder targetted to children and adolescents. Organoleptically ferrous fumarate was acceptable when the chocolate drink powder was reconstituted in milk or water that was heated to < 80°. Unacceptable colour changes occurred, however, when boiling milk or water were used. In human Fe absorption studies when the Fe compounds were added to the chocolate drink immediately before consumption, ferrous fumarate was 3·31 % absorbed compared with 2·82 % for ferrous sulphate and 2·11 % for ferric pyrophosphate. When the Fe compounds were processed during the manufacture of the chocolate drink powder, the absorption of ferrous fumarate was 5·27 %, ferrous sulphate 2·62 % and ferric pyrophosphate 0·55 %. Ascorbic acid had little or no effect on the absorption of ferrous fumarate. It is concluded that food processing can influence the relative absorption of fortification Fe and that, if not reconstituted with boiling milk or water, ferrous fumarate could be a useful compound for the fortification of chocolate drink powders.

Iron fortification: Iron bioavailability: Food processing

Iron deficiency continues to be a common nutritional problem at the present time. There is evidence, however, that its prevalence is declining in certain segments of the population in industrialized countries due mainly to more effective intervention programmes. For example, in the US consumption of fortified infant formulas instead of cow's milk has resulted in a significant reduction in the prevalence of Fe deficiency during infancy (Yip *et al.* 1987*a*,*b*; American Academy of Pediatrics, Committee on Nutrition, 1989), one of the most vulnerable periods of life with respect to Fe balance. There is also some evidence that the prevalence of Fe deficiency in menstruating women is declining in industrialized countries (Pilch & Senti, 1984). The one segment of the population that remains highly vulnerable to Fe deficiency, however, is school-aged children because their Fe requirements for growth often exceed the dietary supply of Fe (Bothwell *et al.* 1979). Fe requirements for adolescents may be increased further by strenuous athletic programmes and, in turn, their athletic performance may be impaired by the development of Fe deficiency (Rowland *et al.* 1988; Rowland & Kelleher, 1989).

Several strategies have been proposed to reduce the prevalence of Fe deficiency in schoolchildren. Fe supplements are effective, but the prevalence of Fe deficiency is not usually high enough to justify the use of medicinal Fe. The Fe intake of children can be increased by fortifying a dietary staple such as wheat products or by fortifying a widely

consumed foodstuff such as sugar, but this may be too costly for Third World countries. The most cost-effective approach to increasing the Fe intake of children is targetted fortification of a speciality food item that is used selectively in this age-group. The vehicle should be appealing to children and ideally should provide other nutritional benefits. One vehicle that has several potential advantages is a chocolate-flavoured milk beverage. The consumption of this product is largely limited to school-age children and would not be consumed extensively by adult men who seldom require additional Fe. Furthermore, this product would also increase the intake of vitamins and other minerals such as zinc and calcium which is also desirable in children.

Organoleptic problems, however, are common when foods are fortified with Fe (Hurrell, 1984), and chocolate milk drinks have proved particularly difficult to fortify because of unacceptable colour problems with ferrous sulphate and other highly soluble Fe compounds (Douglas *et al.* 1981). In the present study, we have assessed the usefulness of ferrous fumarate as an Fe fortificant in a chocolate drink powder. This Fe compound was recently found to be suitable for the fortification of infant cereals, another foodstuff which is difficult to fortify with Fe because of organoleptic problems (Hurrell *et al.* 1989*a*). After first evaluating the colour and flavour of the chocolate drink with added ferrous fumarate, radio-Fe absorption measurements were made in human subjects to determine the bioavailability of ferrous fumarate relative to ferrous sulphate and ferric pyrophosphate, to evaluate the influence of heat processing on the relative absorption (RA) of the different fortification Fe compounds, to quantify the influence of added ascorbic acid, and to estimate the amount of absorbed Fe that would result from fortification.

MATERIALS AND METHODS

Test materials

Chocolate drink powder. The test meal was based on an experimental malted chocolate drink powder designed to be added to hot or cold milk or water. The experimental batches were manufactured in a pilot plant (Linor, Orbe, Switzerland) by drying under vacuum for 3 h at 95° an aqueous slurry of malt, cocoa, skim milk powder, sugar, butter oil, mineral salts and vitamins. The mineral and vitamin mixtures were calculated to provide about 20% of the recommended dietary allowances (National Academy of Sciences, 1980) for 11- to 14-year-old boys in one serving.

Chocolate drink powders for Fe absorption studies. Seven different batches of chocolate drink powder were produced. All were of identical composition except for the levels of Fe and ascorbic acid. Batch 1 contained all the normal ingredients except the fortification Fe (the test Fe sources were added to the product before consumption). In batches 2 and 3, ^{55}Fe-labelled ferrous fumarate and ^{55}Fe-labelled ferric pyrophosphate respectively were added to the slurry before processing. Batches 4 and 5 contained ^{59}Fe-labelled ferrous sulphate added to the slurry before processing. Batch 6 contained ^{55}Fe-labelled ferrous fumarate but no ascorbic acid; and batch 7 contained no added Fe and no added ascorbic acid. The Fe and ascorbic acid contents of the different products are shown in Table 1; the amount of radioactivity was such as to provide about 37 MBq ^{59}Fe or about 80 MBq ^{55}Fe in two 25 g servings.

Radioactive Fe compounds. The ^{55}Fe-labelled ferrous fumarate and ferric pyrophosphate were the same compounds as used previously by Hurrell *et al.* (1989*a*). They were prepared by the Dr Paul Lohman Co. (Emmerthal, Germany) using a scaled-down version of the normal manufacturing procedures. The radioactive Fe sources were similar in appearance, Fe content, particle size, and solubility in dilute hydrochloric acid to their commercial counterparts. The measured Fe content of ferrous fumarate was 320 g/kg and of ferric

Table 1. *Iron and ascorbic acid contents (mg/25 g serving) of experimental chocolate drink powders*

Batch no.		Fe	Ascorbic acid
1	No added Fe	0·5	26·4
2	^{55}Fe-labelled ferrous fumarate	4·7	24·8
3	^{55}Fe-labelled ferric pyrophosphate	5·1	26·6
4	^{59}Fe-labelled ferrous sulphate	5·1	28·4
5	^{59}Fe-labelled ferrous sulphate	4·8	25·4
6	^{55}Fe-labelled ferrous fumarate no added ascorbic acid	4·7	4·2
7	No added Fe, no added ascorbic acid	0·5	8·6

pyrophosphate 251 g/kg. The labelled ferrous sulphate was prepared by dissolving 1·4 g hydrated ferrous sulphate ($FeSO_4.7H_2O$) in 50 ml deionized water, adding the requisite amount of ^{59}Fe-labelled ferrous sulphate (New England Nuclear), evaporating under vacuum to approximately 10 ml and then freeze-drying overnight. The Fe content of the dried radiolabelled ferrous sulphate was 330 g/kg, indicating that it was in the anhydrous form.

Organoleptic evaluation

The organoleptic tests were made with chocolate drink powders fortified with non-radioactive ferrous fumarate and ferric pyrophosphate. Ferrous sulphate has long been known to cause unacceptable colour changes in such products and was not investigated further. Ferric pyrophosphate, on the other hand, causes no organoleptic problems and is added to similar products sold commercially.

Non-radioactive ferrous fumarate and ferric pyrophosphate were obtained from the Dr Paul Lohman Co. The fortified chocolate drink powders were reconstituted at 150 g/l with cold, hot (80°) and boiling milk or water. Colour and taste were evaluated by an experienced panel.

Fe absorption studies

Four Fe absorption studies were made and, in each study, four separate Fe absorption measurements were performed on each of eight to eleven subjects. The feeding protocol for studies 1–3 is shown in Table 2. In the first study, the subjects were given the chocolate drink powder containing no added Fe (batch 1), but supplemented at the time of feeding with ^{55}Fe-labelled ferrous fumarate, ^{55}Fe-labelled ferric pyrophosphate or ^{59}Fe-labelled ferrous sulphate. Meal 4 in the first study was ^{59}Fe-labelled ferrous sulphate added before processing (batch 4). In the second study, the radioactive Fe compounds were added to the chocolate drink powders before heat processing (batches 2, 3 and 5). Meal 4 in the second study was a reference meal. In the third study the influence of added ascorbic acid was investigated with and without ^{55}Fe-labelled ferrous fumarate added before processing (batches 1, 2, 6 and 7). An extrinsic tag containing 37 MBq $^{59}FeCl_3$ with 0·1 mg $FeCl_3.6H_2O$ in 1 ml 0·01 M-HCl was added to the chocolate drinks made from batches 1 and 7 before feeding.

The fourth study was made with a semi-synthetic liquid formula meal and not with the chocolate milk drink. It was made to investigate whether non-processed ^{55}Fe-labelled ferrous fumarate consumed with a meal enters the common Fe pool and to determine to what extent the absorption of this Fe is influenced by added ascorbic acid.

Table 2. *Studies 1–3. Feeding protocol for chocolate drink powders*

Meal	Days	Study 1	Study 2	Study 3
1	1, 2	Batch 1* plus ^{55}Fe-labelled ferrous fumarate	Batch 2	Batch 1 plus extrinsic tag of ^{59}Fe-labelled ferric chloride
2	3, 4	Batch 1 plus ^{59}Fe-labelled ferrous sulphate	Batch 5	Batch 2
3	15, 16	Batch 1 plus ^{55}Fe-labelled ferric pyrophosphate	Batch 3	Batch 7 plus extrinsic tag of ^{59}Fe-labelled ferric chloride
4	17, 18	Batch 4	Reference meal	Batch 6

* For details of different batches, see Table 1.

Test meals. The test meals in studies 1–3 consisted of 25 g chocolate drink powder which was thoroughly mixed with 200 ml cold milk (20 g fat/l) immediately before serving. The Fe-fortified meals contained about 5 mg Fe and those fortified with ascorbic acid contained about 25 mg ascorbic acid (Table 1). To permit extrapolation of the absorption data obtained in the present report to other investigations, one group of subjects in study 2 was given a standard reference dose of inorganic Fe. This test dose contained 3 mg Fe as ^{59}Fe-labelled $FeSO_4.7H_2O$ and 18·9 mg ascorbic acid (molar ratio 2:1) in 50 ml water.

The semi-synthetic liquid formula meal fed in study 4 was the same meal as fed in previous studies (Hurrell *et al.* 1989*b*) and contained 35 g egg white (Monark Egg Corp., Kansas City, MO), 67 g hydrolysed maize starch (Fro-Dex 36, American Maize Products Co, Hammond, IN), 35 g maize oil (Nugget Brand, Stockton, CA), 12 ml vanillin extract (McCormick and Co., Inc., Baltimore, MD), and 200 ml deionized distilled water. This meal contained about 0·6 mg intrinsic Fe.

Subjects. Subjects ranged in age from 20 to 40 years with a mean age of 29 years. There were ten men and twenty-eight women. There was a wide range of Fe status as reflected by their serum ferritin levels. However, none was anaemic as defined by a packed cell volume below 0·36 in women and 0·39 in men. All of the subjects were in good health and denied a history of disorders that are known to influence gastrointestinal absorption of Fe.

Written, informed consent was obtained from each volunteer before beginning the investigation and all experimental procedures were approved by the human subjects committee at the University of Kansas Medical Center.

Absorption measurements. All radioactivity-labelled meals were given between 07.00 and 09.00 hours following a 10 h fast. Water, but not food, was allowed for 3 h following each meal. On the day preceding the first test meal, 15 ml blood were obtained for measurement of packed cell volume, serum ferritin, and background blood radioactivity. In studies 1–3, a test meal tagged with 41 MBq ^{55}Fe was given on the following two mornings and a second test meal tagged with 18·5 MBq ^{59}Fe was given on the third and fourth mornings. At 2 weeks following administration of the first test meal, 25 ml blood were drawn for measurements of blood radioactivity and serum ferritin. Two additional test meals labelled separately with the same amounts of ^{55}Fe and ^{59}Fe were again fed in the same way on four consecutive days and a final blood sample was drawn 2 weeks following the last test meal so as to measure the increase in blood radioactivity.

In study 4 only two meals were fed, each on one occasion only, and each was labelled simultaneously with both ^{55}Fe and ^{59}Fe. Thus in contrast to the previous three studies in which each meal contained one tag only, in this study both radio-Fe tags were present in

the same meal, one labelling the fortification Fe and the other labelling the native Fe. The semi-synthetic liquid formula meal labelled with 7·2 mg Fe as ^{55}Fe-labelled ferrous fumarate (74 MBq) and with 37 MBq $^{59}FeCl_3$ added as an extrinsic tag with 0·1 mg $FeCl_3.6H_2O$ was given on day 1. After 2 weeks 25 ml blood were withdrawn for analysis and the formula meal was again given, labelled simultaneously with 7·2 mg ^{55}Fe-labelled ferrous fumarate and 37 MBq $^{59}FeCl_3$. On this occasion 100 mg ascorbic acid were also added. The final blood sample was drawn 2 weeks later.

Measurements of erythrocyte ^{55}Fe and ^{59}Fe were performed on duplicate 10 ml samples of whole blood using a modification of the method of Eakins & Brown (Bothwell *et al.* 1979). Percentage absorption was calculated on the basis of blood volume estimated from height and weight (Wennesland *et al.* 1959; Brown *et al.* 1962). Erythrocyte incorporation of absorbed radioactivity was assumed to be 80% (Hosein *et al.* 1967).

Statistical analysis

Percentage absorption values were converted to logarithms before statistical analysis and the results reconverted as antilogarithms to recover the original units (Cook *et al.* 1969). Paired *t* tests were used to compare absorption from any two test meals within the same study by determining whether mean log absorption ratios differed significantly from zero.

RESULTS

Organoleptic studies

Chocolate drink powders fortified with ferric pyrophosphate and reconstituted with cold, hot (80°) and boiling water or milk were judged to be acceptable with respect to colour and taste. Similarly chocolate drink powders fortified with ferrous fumarate and reconstituted with cold or hot (80°) milk or water were acceptable. The ferrous fumarate-fortified products reconstituted with boiling water or milk, however, changed colour from red/brown to an unacceptable grey.

Fe absorption studies

In the first two studies, we investigated the effect of processing (vacuum drying) on the relative absorption of three different Fe sources from a chocolate drink powder. In the first study, the Fe sources (ferrous fumarate, ferrous sulphate and ferric pyrophosphate) were added to the chocolate drink powder just before consumption and thus received no processing. In the second study, the same Fe sources were added to the ingredients of the chocolate drink powder and were subjected to all the processing steps during the manufacture of the product.

The results of the first study are shown in Table 3. When added to the chocolate drink powder just before consumption, mean absorption was 3·31% for ferrous fumarate, 2·82% for ferrous sulphate, and 2·11% for ferric pyrophosphate. None of these differences was significant ($P > 0·10$). Moreover, there appeared to be no significant effect of processing on the absorption of ferrous sulphate which averaged 3·52% when added before processing and 2·82% when added to the final product ($P > 0·30$). The only pair of test meals from which absorption differed significantly was ferric pyrophosphate added after processing (2·11%) and ferrous sulphate added before processing (3·52%, $P = 0·008$). It should be noted that the sensitivity of the comparison between these meals was enhanced because they were given on consecutive days rather than separated by a 2-week interval. The variance of absorption ratios is usually appreciably lower when tests are performed simultaneously rather than sequentially. Compared with ferrous sulphate (RA = 1·00), the

Table 3. *Study 1. Absorption by adult subjects of iron-fortification compounds added to a chocolate drink powder immediately before consumption**

Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (ng/ml)	Fe absorption (% dose)				Relative absorption†		
					Ferrous fumarate (A)	Ferrous sulphate (B)	Ferric pyrophosphate (C)	Ferrous sulphate (heat processed) (D)	A:B	C:B	D:B
1	♂	22	0·50	200	1·31	1·36	2·05	3·53	0·96	1·51	2·60
2	♂	22	0·43	130	1·12	0·90	1·26	1·58	1·24	1·40	1·76
3	♂	24	0·49	76	0·78	1·66	1·05	1·75	0·47	0·63	1·05
4	♀	21	0·42	74	4·33	2·46	3·87	2·33	1·76	1·57	0·95
5	♀	35	0·44	45	5·43	1·15	1·33	1·66	4·72	1·15	1·44
6	♂	22	0·44	44	2·51	2·21	1·83	4·15	1·14	0·82	1·88
7	♂	22	0·47	31	2·81	3·02	1·75	3·00	0·93	0·58	0·99
8	♀	21	0·41	20	1·67	1·92	2·01	6·02	0·87	1·04	3·14
9	♀	28	0·39	17	12·60	11·63	2·72	7·71	1·08	0·22	0·66
10	♀	33	0·37	11	39·17	36·96	7·17	13·52	1·06	0·19	0·37
Mean‡		25	0·44	45	3·31	2·82	2·11	3·52	1·17	0·75	1·25
−1 SE					2·26	1·96	1·76	2·81	0·97	0·59	1·02
+1 SE					4·83	4·05	2·53	4·42	1·41	0·95	1·53

* For details, see pp. 272–275.
† Absorption relative to ferrous sulphate added after processing.
‡ Geometric mean except for age and packed cell volume.

Table 4. *Study 2. Absorption by adult subjects of iron compounds added to a chocolate drink powder during manufacture and subjected to processing**

Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (ng/ml)	Fe absorption (% dose)				Relative absorption†		
					Ferrous fumarate (A)	Ferrous sulphate (B)	Ferric pyrophosphate (C)	Reference dose (D)	A:B	C:B	D:B
1	♂	22	0·44	106	1·73	1·08	0·50	16·41	1·60	0·46	15·19
2	♀	31	0·39	83	2·03	0·93	0·07	19·13	2·18	0·07	20·56
3	♀	20	0·39	43	3·01	2·42	0·30	14·21	1·24	0·12	5·87
4	♀	29	0·41	31	1·56	1·42	0·35	31·28	1·09	0·24	22·02
5	♂	25	0·46	25	11·23	4·91	0·65	41·42	2·29	0·13	8·45
6	♀	29	0·43	16	5·20	4·41	0·31	101·63	1·17	0·07	23·04
7	♂	24	0·43	11	2·28	0·23	0·61	18·96	9·91	2·69	82·43
8	♀	24	0·39	9	67·23	24·93	6·35	73·73	2·69	0·25	2·95
9	♀	21	0·39	5	21·30	13·60	1·58	24·06	1·56	0·11	1·76
Mean‡		25	0·41	24	5·27	2·62	0·55	30·01	2·01	0·21	11·45
−1 SE					3·41	1·62	0·37	23·86	1·61	0·14	7·74
+1 SE					8·15	4·24	0·83	37·75	2·52	0·31	16·96

* For details, see pp. 272–275.
† Absorption relative to ferrous sulphate.
‡ Geometric mean except for age and packed cell volume.

Table 5. *Study 3. Effect of ascorbic acid (25 mg) on absorption by adult subjects of native iron or ferrous fumarate added to a chocolate drink powder**

Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (ng/ml)	Fe absorption (% dose)				Absorption ratio			
					No ascorbic acid		With ascorbic acid					
					Native Fe (A)	Ferrous fumarate (B)	Native Fe (C)	Ferrous fumarate (D)	B:A	C:A	D:B	D:C
1	♂	38	0·45	209	1·00	1·12	1·21	0·96	1·12	1·21	0·85	0·79
2	♂	39	0·43	185	0·12	1·30	0·92	0·47	10·83	7·66	0·36	0·51
3	♀	29	0·47	63	2·28	5·42	2·55	2·77	2·37	1·11	0·51	1·09
4	♀	22	0·41	41	1·17	1·20	2·20	1·86	1·02	1·88	1·55	0·86
5	♀	27	0·44	40	1·36	1·90	1·10	1·07	1·39	0·80	0·56	0·97
6	♀	24	0·38	34	2·52	2·31	2·60	1·93	0·91	1·03	0·83	0·74
7	♀	28	0·40	27	2·56	3·18	1·15	1·52	1·24	0·44	0·47	1·32
8	♀	22	0·43	26	1·00	1·28	5·16	4·02	1·28	5·16	3·14	0·78
9	♀	32	0·37	14	9·10	13·08	6·98	3·15	1·43	0·76	0·24	0·45
10	♀	20	0·42	6	3·00	11·78	9·06	7·45	3·92	3·02	0·63	0·82
11	♀	21	0·37	3	8·23	23·08	8·72	16·07	2·80	1·05	0·69	1·84
Mean†		27	0·42	31	1·80	3·35	2·71	2·33	1·86	1·51	0·70	0·86
−1 SE					1·26	2·41	2·09	1·73	1·49	1·16	0·56	0·76
+1 SE					2·56	4·66	3·52	3·14	2·33	1·96	0·86	0·97

* For details, see pp. 272–275.

† Geometric mean except age and packed cell volume.

Table 6. *Study 4. Effect of ascorbic acid (100 mg) on absorption by adult subjects of native iron or ferrous fumarate added to a synthetic liquid formula meal**

Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (ng/ml)	Fe absorption (% dose)				Absorption ratio			
					No ascorbic acid		With ascorbic acid					
					Native Fe (A)	Ferrous fumarate (B)	Native Fe (C)	Ferrous fumarate (D)	B:A	C:A	D:B	D:C
1	♀	28	0·40	103	4·77	4·58	2·15	2·13	0·96	0·45	0·47	0·99
2	♀	40	0·40	91	7·05	10·81	9·96	11·36	1·53	1·41	1·05	1·14
3	♀	28	0·42	78	0·60	1·16	4·18	5·30	1·93	6·97	4·56	1·27
4	♀	29	0·41	37	6·78	15·07	14·68	13·57	2·22	2·17	0·90	0·92
5	♀	32	0·41	22	3·25	5·26	6·16	7·03	1·62	1·90	1·34	1·14
6	♀	37	0·39	13	26·35	23·38	33·32	31·31	0·88	1·26	1·34	0·94
7	♀	38	0·36	12	2·36	4·17	9·43	13·17	1·77	4·00	3·16	1·40
8	♀	35	0·44	9	9·92	15·25	46·57	51·25	1·54	4·69	3·36	1·10
Mean†		33	0·40	31	4·78	7·14	10·21	11·26	1·49	2·14	1·58	1·10
−1 SE					3·23	5·07	7·11	7·91	1·33	1·57	1·20	1·05
+1 SE					7·09	10·07	14·66	16·02	1·67	2·90	2·07	1·16

* For details, see pp. 272–275.
† Geometric mean except age and packed cell volume.

RA of ferrous fumarate not subjected to processing was 1·17 and the RA for ferric pyrophosphate was 0·75.

In contrast to these small differences in absorption for the Fe compounds not subjected to processing, marked differences in their absorption occurred when they were added to the ingredients of the chocolate drink powder before processing (Table 4). Mean absorption was 5·27% for ferrous fumarate, 2·62% for ferrous sulphate and 0·55% for ferric pyrophosphate. The absorption of ferric pyrophosphate was < 1% in all but two subjects, and in none of the subjects was the absorption of ferrous sulphate or ferric pyrophosphate higher than that of ferrous fumarate. The mean absorption of ferrous fumarate was significantly greater than ferrous sulphate ($P = 0·016$) and ferric pyrophosphate ($P < 0·00001$). The RA of ferrous fumarate subjected to processing was 2·01 whereas that for ferric pyrophosphate was only 0·21. Reference dose absorption averaged 30% in these relatively-Fe-depleted subjects (mean serum ferritin 24 μg/l). This represents an eleven-fold inhibiting effect of chocolate milk powder on absorption of a comparable dose of ferrous sulphate.

The third study was performed to evaluate the influence of ascorbic acid on Fe absorption from the chocolate drink powder and to calculate the contribution of the absorbed ferrous fumarate to the Fe requirements of the subjects. In eleven subjects, absorption of the native Fe from the non-Fe-fortified milk drink ranged from 0·12 to 9·1%, with a geometric mean of 1·8% (Table 5). Surprisingly, mean absorption increased appreciably to 3·35% when the powdered formula was fortified with ferrous fumarate (absorption ratio 1·86; $P = 0·022$). The second pair of test meals was given specifically to examine the effect of fortifying the product with ascorbic acid. The ascorbic acid content of the chocolate powder without and with added ferrous fumarate was 26 and 25 mg/serving respectively. Nearly identical mean absorption values of 2·71 and 2·33% were observed and these did not differ significantly from values obtained without added ascorbic acid ($P > 0·10$), although the absorption of the native Fe was increased by 50%.

The absorption of Fe from the liquid formula meal to which the two isotopes had been added simultaneously was 4·78% for the native Fe and 7·14% for the ferrous fumarate (Table 6). These values were significantly different ($P < 0·05$). The addition of ascorbic acid increased the absorption of the native Fe to 10·21% ($P < 0·05$) and that of the ferrous fumarate to 11·26%. This latter increase was not significant ($P > 0·05$), neither was the difference in absorption between the two tags.

DISCUSSION

The Fe compounds of high bioavailability, such as ferrous sulphate and ferrous gluconate, cause unacceptable colour and flavour changes in many food vehicles. Less bioavailable, but more inert Fe sources, such as ferric pyrophosphate and certain forms of elemental Fe have thus been commonly used to fortify foods such as infant cereals and chocolate drink powders. Although these Fe compounds are less well absorbed than ferrous sulphate, they cause no organoleptic problems.

Recently, two alternative Fe compounds were proposed for the fortification of infant cereals (Hurrell *et al.* 1989*a*). These compounds, ferrous fumarate and ferrous succinate, were shown to be as well absorbed as ferrous sulphate but, unlike ferrous sulphate, did not cause organoleptic problems. These findings prompted the present re-assessment of Fe compounds used to fortify chocolate drink powders. From an organoleptic viewpoint, ferrous fumarate was judged to be an acceptable Fe fortificant for chocolate drink powders provided that the powder was mixed with cold milk for consumption, as in the present study, or was mixed with hot water or milk up to a temperature of 80°. Adding the ferrous

fumarate-fortified powder to boiling water or milk resulted in the typical red/brown colour of the product being transformed into an unacceptable grey colour. This latter finding would unfortunately exclude the use of ferrous fumarate in commercially sold chocolate drink powders.

The most unexpected finding from the present study, however, was the marked effect which processing (vacuum drying) had on the relative absorption of the different Fe compounds. When the Fe compounds were added to the chocolate drink immediately before consumption, thus avoiding processing, the RA values (relative to unprocessed ferrous sulphate = 1·00) were 1·17 for ferrous fumarate and 0·75 for ferric pyrophosphate. The RA value for ferrous fumarate was similar to that obtained in previous studies, although that for ferric pyrophosphate was somewhat higher than previously reported (Hurrell *et al.* 1989*a*). These small non-significant differences in RA of the unprocessed Fe compounds became highly significant differences after they had been processed with the chocolate drink powder. After processing, the Fe from ferrous fumarate was ten times better absorbed than that from ferric pyrophosphate and twice as well absorbed as that from ferrous sulphate, whose absorption changed little on processing (Table 3).

The influence of heat sterilization on the relative bioavailability (RBV) of Fe compounds in rats has already been studied to some extent in liquid products. In agreement with our own results, these studies have shown that the RBV of ferrous sulphate is unchanged by heat processing (Theuer *et al.* 1971, 1973; Wood *et al.* 1978). In contrast to our findings, however, the RBV of ferric pyrophosphate and other insoluble Fe compounds was shown to increase with processing. Theuer *et al.* (1971, 1973), using the haemoglobin repletion test in rats, determined the RBV of Fe salts in liquid soya-bean and milk-based infant formulas. In both types of formula heat sterilization increased the RBV of ferric pyrophosphate. The increase in RBV was from 39 to 93 in the soya-bean formula and from 78 to 125 in the milk formula. Wood *et al.* (1978) similarly found that heat treatment of a chicken diet as a liquid slurry increased the RBV of ferric pyrophosphate to chicks from 7 to 90. On the other hand, in human studies, the baking of bread seemed to have little positive effect on the RA of ferric orthophosphate (Cook *et al.* 1973), or of carbonyl Fe (Hallberg *et al.* 1986). Different methods of heat processing may, therefore, have opposite effects on the bioavailability of an Fe compound. This must be due to the influence of the processing method on the solubility of the food Fe in the gastrointestinal tract.

The Fe in the chocolate drink powder processed with ferrous fumarate was unusual as it did not appear to enter the common Fe pool. This is indicated by the finding that after processing the absorption from ferrous fumarate is almost double that of the native Fe from the chocolate drink powder (Table 5) and double that of ferrous sulphate (Table 4), and that it is not increased by the addition of ascorbic acid (Table 5). These findings are analogous to the behaviour of haem-Fe and NaFeEDTA (McPhail *et al.* 1985), two Fe compounds which do not enter the common pool. The absorption of Fe from these compounds is greater than the absorption of pool Fe in foods that strongly inhibit Fe absorption. It is believed that the Fe remains bound to the chelate rather than exchanging freely with all ingested Fe.

We first thought that a new complex had been formed from ferrous fumarate and the chocolate drink powder ingredients during processing. However, in the liquid formula meal, the unprocessed ferrous fumarate was also significantly better absorbed than the native meal Fe (Table 6) and, although ascorbic acid increased Fe absorption by about 60%, this increase was not statistically significant and was not as great as the increase in native Fe absorption on the addition of ascorbic acid. It seems, therefore, that ferrous fumarate, whether processed or not, does not completely enter the common Fe pool.

The addition of ascorbic acid to the non-Fe-fortified chocolate drink powder increased

mean Fe absorption from 1·8 to 2·7%, an increase of 50% (not significant). Each serving of chocolate drink contained 0·5 mg Fe and either 8·6 or 26·4 mg ascorbic acid (Table 1). The enhancing effect of ascorbic acid on Fe absorption depends on the amount of ascorbic acid added, the quantity of Fe present, and the amount and nature of the absorption inhibitors present in the meal. In a semi-synthetic liquid formula meal identical to that used in our studies, except for the addition of Ca, phosphorus and 4·1 mg Fe, Cook & Monsen (1977) reported an increase in absorption of 65% on addition of 25 mg ascorbic acid and a four-fold increase on the addition of 100 mg ascorbic acid. The same quantity of ascorbic acid (100 mg) added to a farina–milk meal fortified with 3 mg Fe as ferrous sulphate, ferric orthophosphate or electrolytic Fe also increased Fe absorption 2·5–4-fold (Forbes *et al.* 1989), whereas similar quantities of ascorbic acid added to infant formula or infant cereals have resulted in up to ten-fold increases in Fe absorption (Derman *et al.* 1980). Based on these results, the effect of 100 mg ascorbic acid on ferrous fumarate absorption from the synthetic liquid formula seems particularly low, although the modest increase in absorption of the native Fe from the chocolate drink powder on addition of 25 mg ascorbic acid would seem to be more or less as expected considering the inhibitors of Fe absorption present in the product (casein and calcium from milk, phytate from malt and polyphenols from cocoa).

Our studies indicate that ferrous fumarate is a useful Fe compound for the fortification of chocolate drink powders, and that the amount of Fe absorbed from a chocolate drink powder fortified with this compound would be likely to have a significant impact on Fe balance in children and adolescents. It could be included in school lunch programmes, for instance, and reconstituted with cold or warm milk or water. Mean absorption values of 5·27% were observed in subjects with an average serum ferritin of 24 μg/l (Table 4) and 3·35% in subjects with a mean ferritin of 31 μg/l (Table 5). These serum ferritin levels are higher than in school-aged children, indicating that even higher absorption levels will occur in this age-group. Based on a reference dose absorption of 40%, which is commonly taken to represent individuals with borderline Fe deficiency, absorption of 7% from the fortified drink would be predicted. Even this may be an underestimate of the absorption that would occur in Fe-deficient children. For example, absorption in six subjects with serum ferritin levels below 15 μg/l ranged from 2·3 to 67·2% in the present study with a composite mean of 9·5% (Table 4). This level is encouraging in view of the fact that milk is commonly regarded as inhibitory to Fe absorption and one might also expect the polyphenols in the cocoa and the phytate in the malt to inhibit absorption. Our study suggests that in the absence of food, one glass of chocolate milk drink will furnish approximately 0·5 mg absorbable Fe/serving. Two glasses/d, therefore, would cover a substantial part of the Fe requirement of school-aged children (1·0 mg absorbable Fe/d; National Academy of Sciences, 1980) and would be likely to have a significant impact on Fe nutrition.

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