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Iron availability from infant food supplements[1-3]

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ABSTRACT Radioiron absorption tests are performed in human volunteer subjects to measure iron absorption from five infant food supplements. These products included corn-soya-milk, corn-soy blend, wheat-soy blend, wheat protein concentrate blend, and whey-soy drink mix. In iron replete adult males, mean percentage absorption ranged from 0.6 to 1.4%. By relating these results to absorption from a reference dose of inorganic iron, it was estimated that iron-deficient infants would absorb between 1.7 and 4.1% of the iron contained in the infant foods. These results indicate that this fortification iron is poorly absorbed, and probably supplies only about half of the daily iron requirement for iron deficient children between 6 months and 3 yr of age. *Am. J. Clin. Nutr.* 34: 2630–2634, 1981.

KEY WORDS Iron, corn-soya-milk, infant foods

Introduction

Food supplements are distributed to undernourished infants and children in developing countries as part of the Food for Peace Program of the U.S. Department of Agriculture. Corn meal, wheat flour or protein concentrate, soy flour, nonfat dried milk, and sweet cheese whey are combined in varying proportions. The foods are distributed in dry form and are either diluted in water and consumed as a drink or porridge, or mixed with other foods to enhance protein and caloric intake.

Iron, as ferrous fumarate, is added at a level of 7.8 mg iron/50 g. Ferrous fumarate is well absorbed in the fasting state (1), but its assimilation when added to food has not been adequately studied. In the present study, extrinsic radioiron tagging was used to measure iron absorption from the following five infant food supplements: corn-soya-milk (CSM) (2), corn-soy blend (CSB) (3), wheat-soy blend (WSB) (4), wheat protein concentrate blend (WPC) (5), and whey-soy drink mix (WSD) (6). The protein content of these foods is listed in Table 1.

Methods

Studies were performed in 27 adult male volunteers ranging in age from 18 to 45 yr. All subjects gave written informed consent before their participation in the study and procedures followed were in accord with the ethical standards of the Human Subjects Committee of the University of Kansas Medical Center. None had a history of hematological abnormalities or disorders known to influence gastrointestinal absorption. Iron status was judged to be normal in 25 of the subjects on the basis of hematocrit, transferrin saturation, and serum ferritin. Two had reduced iron stores as determined by serum ferritin levels of 12 and 13 μg/l.

The subjects were divided into two groups of 13 and 14 individuals each. Four separate iron absorption tests were performed in each subject using dual radioiron labels. In both groups, one of the test meals consisted of CSM, the most widely distributed infant food supplement. The second and third test meals differed in the two studies, consisting of WSB and WPC in study 1 and CSB and WSD in study 2. In both, the fourth test meal was a reference dose (REF) of inorganic radioiron containing 3 mg iron as ferrous sulfate and 19 mg freshly dissolved ascorbic acid. The effect of differences in iron status between the two study groups was minimized by expressing iron absorption from the infant foods as a ratio with respect to REF absorption. CSM was administered in both studies to gauge the accuracy of this adjustment.

All test meals were administered between 7 and 9 AM after an overnight fast and water alone was allowed for 3 hr. The infant foods were prepared the day before serving, weighed into individual portions, and refrigerated overnight. Each meal contained 50 to 59 g dry product, 20 g sucrose, 1 g salt, and 170 to 225 ml water. Mineral and vitamin mixes were included in the product formulation and brought the total content to 9 mg iron and 20 mg ascorbic acid per serving. The meals were

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TABLE 1
Percentage of total protein contributed by major ingredients

	CSM	CSB	WSB	WPC	WSD
Soy	45	63	55	61	74
Cornmeal	27	37			
Milk	28				
Wheat			45	39	
Whey					26
Total %	100	100	100	100	100

tagged extrinsically by adding 0.1 mg iron as ferric chloride in 1 ml 0.01 N HCl and either 2 μCi ^{59}Fe or 5 μCi ^{55}Fe (7). WSD was served as a cold beverage and the remaining foods were heated in a microwave oven and served as a hot porridge.

Blood was drawn on the 1st day of the study for measurement of serum iron, iron-binding capacity, serum ferritin, and background radioactivity. Meals A and B, tagged separately with either ^{59}Fe or ^{55}Fe, were then administered on 2 successive days. Blood was obtained 14 days later to measure incorporated red cell radioactivity and meals C and D were then given on 2 successive days. A final blood sample was drawn 2 wk later to measure the rise in circulating red cell radioactivity. All radioiron assays were performed on duplicate 10-ml samples of whole blood using a modification of the method of Eakins and Brown (8). Percentage absorption in each subject was calculated on the basis of a blood volume estimated from the height and weight (9). Red cell incorporation of absorbed radioactivity was assumed to be 80% (10). Serum iron was measured by the ICSH method (11), iron-binding capacity by a magnesium carbonate technic (12), and serum ferritin by a 2-site immunoradiometric assay (13).

Because of the highly skewed distribution of iron absorption data when expressed as a percentage of the administered dose, statistical analysis was performed on logarithms and the results transformed to antilogarithms to recover the original units (14). One-way analysis of variance was used to compare absorption from the five infant foods after adjustment for REF absorption.

Results

Iron status measurements and iron absorption values for both studies are listed in Table 2. Iron absorption from all supplements was remarkably low; nearly two-thirds of the absorption values were less than 1%. Geometric mean values ranged from 0.6% for CSM in study 2 to 1.4% for the same food in study 1. Absorption from the REF dose averaged 26.8 and 13.4% in studies 1 and 2, respectively. This difference is presumably related to differences in iron status even though mean serum ferritin levels in the two groups do not reflect such a difference.

Absorption data were corrected for differences in iron status by expressing each value as a ratio with respect to REF absorption (Fig. 1). The accuracy of this correction can be assessed by comparing the absorption ratios obtained for CSM in study 1 and 2. Uncorrected percentage absorption from CSM differed by 60% in the two studies whereas the absorption ratios of 0.052 and 0.043 differed by less than 20% (Table 3). One-way analysis of variance was performed on logarithms of absorption ratios and showed marginal significance ($F = 2.70$, $P < 0.05$). However, Duncan's multiple range tests (15) failed to show a significant difference between any of the infant supplements.

Discussion

An important advantage of expressing absorption in relation to a reference dose is that percentage absorption can be predicted in subjects with differing iron status. The relatively low means for REF absorption of 27 and 13% in the present study are typical values for iron replete individuals. A much higher percentage REF absorption of about 60% would be observed in the iron-deficient children who would receive these food supplements. Iron absorption from each of the foods has been calculated for REF absorption of 60% in Table 3. Values range from 1.7% with both WSB and WPC to 4.1% for CSB; intermediate values of 3.1 and 2.6% were obtained with CSM and 2.9% for WSD. The corrected values are remarkably similar despite variations in the composition of each supplement. Thus, children who take 100-g supplement daily might derive only 0.30 to 0.74 mg iron daily from these infant foods.

The extremely low assimilation of iron contained in infant foods was unexpected. Nevertheless, these results are comparable to those obtained in a previous study in Jamaican infants reported by Ashworth and March (16). Absorption from CSM averaged 6% in that study compared to mean REF absorption of 64%. When adjusted to 60% REF absorption, CSM absorption was 5.7% in comparison with lower values of 2.6 and 3.1 observed in the present study. Much of the disparity between the two studies can be explained by the fact that arithmetic means rather than geometric means were calculated in the report by Ashworth and March (16). Geometric values are invariably lower than arithmetic means, especially when percentage

TABLE 2
Iron bioavailability from infant food supplements

Subject	Age	Packed cell volume	Serum iron	TS*	Serum ferritin	Iron absorption			
		%	μg/dl	%	ng/ml	% of dose			
						CSM	WSB	WPC	REF
Study 1									
1	18	48	164	41	56	0.58	0.72	0.71	10.86
2	18	47	104	23	58	0.62	0.70	1.58	32.33
3	24	51	113	31	113	0.65	0.28	0.42	18.96
4	20	48	172	46	105	0.70	0.40	0.45	27.18
5	18	46	84	21	37	0.71	0.47	1.51	42.88
6	31	45	142	39	107	1.00	0.61	0.33	11.46
7	19	50	106	29	39	1.03	0.35	0.67	23.40
8	19	46	119	34	44	1.28	1.22	0.47	19.66
9	20	46	100	25	54	2.16	0.91	1.20	51.46
10	20	49	123	34	40	2.17	0.58	0.40	23.00
11	21	49	89	24	67	2.22	0.45	0.31	33.25
12	18	50	134	40	41	4.10	1.93	0.97	46.83
13	20	45	150	39	13	12.51	9.68	7.08	49.35
Mean†	21	48	123	33	52	1.40	0.76	0.77	26.82
						CSM	CSB	WSD	REF
Study 2									
1	26	44	144	35	126	0.03	0.58	0.37	11.35
2	45	41	136	49	86	0.12	0.28	0.13	3.11
3	27	41	156	46	62	0.27	0.45	0.38	11.37
4	24	48	118	28	24	0.33	0.50	0.62	12.87
5	27	44	99	25	81	0.41	0.25	0.23	1.22
6	22	42	118	36	79	0.47	0.38	0.17	5.70
7	27	42	69	21	26	0.66	0.46	0.28	17.00
8	25	46	173	44	28	0.71	1.28	0.80	14.71
9	25	44	140	38	30	0.76	0.75	0.68	23.78
10	24	47	86	23	72	1.13	1.86	1.25	18.42
11	29	40	108	30	43	1.32	1.77	1.50	32.82
12	24	42	112	32	65	1.56	1.80	5.67	41.53
13	42	46	168	57	56	2.17	3.73	0.82	17.27
14	31	45	94	25	12	4.22	10.40	4.63	62.47
Mean†	28	44	123	35	48	0.57	0.92	0.65	13.36

* Transferrin saturation.
† Values for serum ferritin and iron absorption tests are geometric means.

absorption is low. Their data can be corrected to geometric means by assigning values of 0.1% to two subjects reported as absorbing no iron. When adjusted to REF absorption of 60%, absorption from CSM in iron-deficient children averages 3.1% as compared to values of 2.6 and 3.1% obtained in iron-replete males in the present study. The remarkable similarity in results obtained in subjects with widely different iron status underscores the importance of REF corrections. It also demonstrates the validity of measuring food iron availability in subjects who may differ greatly from the population that will consume the food. Further validation will be difficult because of the concern in administering radioisotopes to children.

Another aspect of methodology relating to the present study is that absorption of the fortification iron, ferrous fumarate, was measured by adding an extrinsic radioiron tag (ferric chloride) rather than using radiolabeled ferrous fumarate. Previous studies have shown that complete exchange occurs between dietary iron and fortification iron when the latter is added as ferric chloride or ferrous sulfate (17, 18). However, an extrinsic tag may not exchange completely with fortification iron when the latter is highly insoluble such as ferric orthophosphate or sodium iron pyrophosphate (17). There is no evidence that an extrinsic tag is *less* well absorbed than conventional forms of added fortification iron. If the extrinsic tag did not exchange

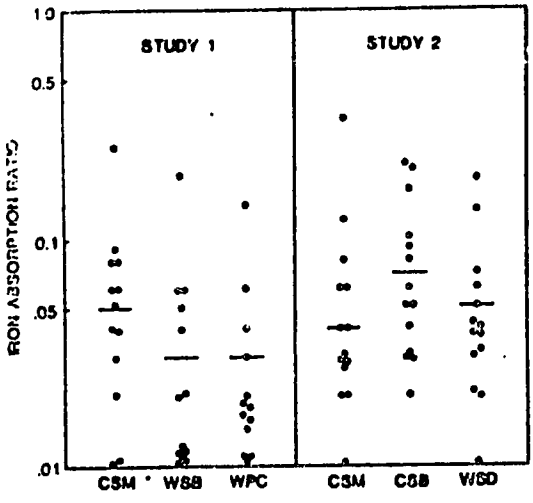

FIG. 1. Absorption of iron from infant food supplements expressed as a ratio with respect to absorption from a standard reference dose of inorganic iron. Ratios are plotted on a logarithmic scale. The *horizontal bars* depict geometric means.

TABLE 3
Iron absorption from infant food supplements adjusted for iron status

Study	Test meal	Iron absorption	Meal:REF	Adjusted absorption*	
				%	mg iron
		% of dose			
1	CSM	1.40	0.052	3.12	0.56
	WSB	0.76	0.028	1.68	0.30
	WPC	0.77	0.028	1.68	0.30
2	CSM	0.57	0.043	2.58	0.46
	CSB	0.92	0.069	4.14	0.74
	WSD	1.96	0.049	2.94	0.52

* Corrected to 60% REF absorption and 18 mg Fe/100 g dry product.

completely with ferrous fumarate in this study, falsely high rather than low absorption values would have been observed.

The recommended intake of iron is 15 mg/day for children 6 months to 3 yr of age (19, 20). These values are based on the assumption that 10% of dietary iron is absorbed by the average child although two to three times this amount may be absorbed by iron-deficient children (21, 22). The results of the present study indicate that the iron added to infant food supplements may not fulfill the daily requirement of 0.7 to 1.0 mg absorbed iron (23). Since we estimated that iron-deficient children would absorb only 0.3 to 0.7 mg iron from 100 g of product, the WHO/UNICEF recommended quantity of 80 to 100 g CSM/day might satisfy only about one-half of their iron requirements. It is unlikely that the typical diet in a developing country would furnish the balance of daily iron needs.

There are at least two factors that could account for the low availability of fortification iron added to infant foods. Calcium and phosphorous salts impair the absorption of food iron when combined in the same meal but not when added separately (24). Specifications for the mineral mixes used in each of the five products tested in this study require the inclusion of either tricalcium phosphate or a combination of calcium carbonate and dicalcium phosphate. We have recently observed a substantial increase in iron absorption from CSM when these minerals are omitted (T. A. Morck, S. R. Lynch, and J. D. Cook, unpublished observation). A second possibility for low absorption in this study is the presence of soy protein. We have noted a striking inhibition of food iron absorption with a variety of soy products ranging from whole soybean to isolated soy protein (25). The importance of this inhibitory effect of soy on iron absorption from infant foods cannot be assessed from the data contained in this report because soy protein contributed between 45 to 75% of the total protein in each of the five infant foods examined.

Poor availability of fortification iron is not in itself adequate justification for eliminating calcium and phosphate or soy protein from infant foods. Availability could be improved by increasing the level of ascorbic acid in the vitamin mix (26). Alternatively, it may be possible to add a form of iron, the absorption of which is less inhibited by food. Recent studies suggest that sodium iron EDTA may be less inhibited than other iron salts in certain diets and may therefore enhance iron absorption from infant foods (27, 28).

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