The Bioavailability of Iron in Different Weaning Foods and the Enhancing Effect of a Fruit Drink Containing Ascorbic Acid

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ABSTRACT

There is limited information on the bioavailability of Fe in infant weaning foods, mainly because of the difficulties of measuring Fe utilization directly in infants. The aim of this study was to develop a safe and relatively noninvasive method for studying Fe bioavailability (measured as percent Fe incorporation into red blood cells) in infants using ^{54}Fe , ^{57}Fe , and ^{58}Fe stable isotopes. Four commonly used weaning foods were selected for study, labeled extrinsically with ^{57}Fe - or ^{58}Fe -enriched ferrous sulfate, and fed to five female and five male 9-mo-old fasting infants, using a multiple-dosing technique. Each food was given three times, labeled with one isotope, with a fruit juice drink containing 50 mg of ascorbic acid, and three times, labeled with a different isotope, with an ascorbic acid-free drink. Fourteen days after the last test meal, a blood sample was obtained from a heel-prick, spiked with a known amount of ^{54}Fe , digested, and purified by ion exchange; isotopic enrichment and total Fe content were measured by quadrupole thermal ionization mass

spectrometry. The proportion of administered dose of isotope circulating in the blood was calculated from an estimate of blood volume. The geometric mean bioavailability (range) was 3.0% (1.2–9.5%) in a proprietary dehydrated vegetable product, 3.0% (1.1–21.2%) in Weetabix whole-wheat breakfast cereal, 3.1% (1.2–15.4%) in wholemeal bread, and 4.3% (1.7–10.3%) in baked beans. When taken with the drink containing ascorbic acid, there was a 2-fold increase in bioavailability in all foods except the vegetable meal, presumably because this was already fortified with ascorbic acid. Thus, drinks containing 50 mg of ascorbic acid, taken with a meal, can significantly improve Fe bioavailability to infants from weaning foods low in ascorbic acid. (*Pediatr Res* 37: 389–394, 1995)

Abbreviations

ZPP, zinc protoporphyrin

TIMS, thermal ionization mass spectrometry

A normal full-term infant is born with sufficient body Fe stores to satisfy physiologic requirements until at least 4 mo of age. By this time the stores will be depleted, and although Fe losses from the body are small, growth is very rapid; thus, the infant's needs for Fe must be met almost exclusively by the diet. In breast-fed infants, solid foods are the primary source of Fe, because breast milk contains very little Fe. Formula-fed infants, however, have a higher total intake of Fe because the amount of Fe added to infant formulas is much higher than that found in breast milk to compensate for the lower bioavailability (1). Reports in the literature indicate that the intake of Fe-fortified formulas is one of the key determinants of Fe status of infants (2). However, if the Fe intake is not adequate, then nutritional Fe deficiency will begin to appear, usually after the age of 6 mo in a child with normal growth rate (3).

Fe deficiency is still very common among infants and young children, both in industrialized and developing countries. Although inadequate intakes of Fe may result in Fe deficiency, the most likely cause is low bioavailability of ingested Fe. Dietary Fe is inefficiently absorbed by humans, and a number of dietary factors have been shown to inhibit or enhance the absorption of Fe (1). Present knowledge indicates that the most important dietary factors include ascorbic acid and meat (enhancers) and phenolics and phytate (inhibitors). From the limited number of studies already performed, the bioavailability of Fe in different weaning foods appears to be very variable (1). The demand for Fe in infants aged 6 mo or more is particularly high, yet little is known about the suitability of individual weaning foods and mixtures of foods with regard to Fe nutrition. One of the important questions this study was designed to answer was "what simple dietary advice can be given to mothers to improve their child's Fe nutrition?"

The most accurate technique for determining the bioavailability of Fe uses radioisotopes (^{55}Fe and ^{59}Fe) to label extrinsically the native food Fe (4), but, because of the hazards associated with unnecessary exposure to ionizing radiation,

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this method is not ethically acceptable in healthy infants. An alternative approach is to replace radioisotopes with stable isotopes of Fe (5). The present study was designed to measure Fe bioavailability using ^{54}Fe , ^{57}Fe , and ^{58}Fe stable isotopes, in commonly used weaning foods, and to test whether or not a fruit juice drink containing ascorbic acid enhances Fe bioavailability from the foods.

METHODS

Subjects. Forty full-term apparently healthy infants (20 girls and 20 boys) aged 9 mo were recruited for the study and divided equally into four groups of 10 (five girls and five boys). None had been given Fe supplements immediately before the study. Blood was removed by heel-prick, and a portion of the whole heparinized blood was analyzed for hematologic indices using a CBC5 Coulter Counter (Coulter Electronics, Hialeah, FL) and ZPP using a hematofluorimeter (AVIV Associates, Bedford, MA). The remaining blood was centrifuged, and the plasma was removed and stored at -18°C until analysis for ferritin by ELISA. Subjects with Fe-deficiency anemia, $\text{Hb} < 110 \text{ g/L}$ (6), were not accepted for the study. Mean values for body weight, ZPP, and geometric means for plasma ferritin are given for boys and girls separately in Table 1, together with the numbers of infants in each group who were regularly consuming human milk, formula, or cow's milk during the period immediately before the study. Each group was given one of four selected foods, labeled with a stable isotope of Fe, with and without a drink containing ascorbic acid, and Fe bioavailability was assessed from the measured isotopic enrichment of red blood cell Hb 14 d after the final dose.

Foods. Four of the most commonly used weaning foods that contribute significantly to Fe intake were selected for study, using information generated from a parallel study investigating the relationship between infant feeding practice and body Fe status, and fed to the different groups as follows: group 1, garden vegetables dinner (Milupa, Friedrichsdorf, Germany); group 2, Weetabix breakfast cereal (Weetabix, Burton Latimer, Northants, UK) with full-fat milk; group 3, wholemeal bread (Allisons, Maidenhead, UK) with margarine and marmite [CPC (UK) Ltd., Esher, Surrey, UK]; and group 4, low sugar

Table 1. Body weight (kg), ZPP ($\mu\text{mol/L}$ red blood cells), plasma ferritin ($\mu\text{g/L}$), and type of milk consumed (number of infants per group) in 9-mo-old boys and girls

Group	Weight	ZPP	Ferritin	Human	Formula	Cows
1						
Boys	8.9 (0.8)	1.6 (0.7)	26 (−11, +63)	1	3	4
Girls	8.0 (0.9)	1.7 (0.8)	24 (−15, +65)	2	2	2
2						
Boys	9.1 (0.9)	1.7 (0.7)	19 (−9, +41)	0	5	3
Girls	9.0 (1.7)	2.0 (0.5)	31 (−22, +44)	1	4	3
3						
Boys	8.9 (0.8)	2.2 (0.5)	12 (−6, +26)	3	2	3
Girls	8.4 (0.9)	1.7 (1.0)	21 (−11, +38)	0	5	5
4						
Boys	9.1 (0.6)	2.1 (0.9)	22 (−12, +39)	2	2	4
Girls	8.6 (1.2)	1.4 (0.5)	20 (−10, +35)	1	3	4

Values are arithmetic means (geometric mean for ferritin) for five individuals ($\pm\text{SD}$).

Table 2. Iron and ascorbic acid content of test meals fed to infants

Food	Group	Wt (g)	Fe (mg/meal)	Ascorbic acid (mg/meal)
Vegetable dinner	1	25	0.95	63
Water		75		
Weetabix	2	20	0.72	3.8
Milk (full-fat)		85		
Wholemeal bread	3	22	0.59	0.3
Margarine		6		
Marmite		0.5		
Baked beans	4	75	0.90	0.9
Apple juice drink		8		
Water		92	0.0	50

(Weight Watchers) canned baked beans in tomato sauce (H. J. Heinz Co. Ltd., Hayes, Middlesex, UK).

Two of the foods are normally fortified with Fe; Weetabix contains ferrous sulfate (5.4 mg Fe/100 g) and Milupa meals contain ferric pyrophosphate (5 mg Fe/100 g). In this study, products without fortification Fe were specially prepared for us by the manufacturers, so that we were able to add isotopically enriched Fe in place of the fortification Fe. The Fe concentration was measured by atomic absorption spectrometry (7) and the ascorbic acid by HPLC (8), using 3% metaphosphoric acid for the extraction. The portion sizes and Fe and ascorbic acid contents are given in Table 2.

Labeling of meals. Isotope solutions used for the study were prepared from ^{57}Fe -enriched (95.2 atom %, Technical and Optical Equipment, London, UK) and ^{58}Fe -enriched (93.13 atom %, AEA Technology, Harwell, UK) elemental Fe. The Fe was dissolved by adding 5 mL of concentrated HNO_3 (Aristar, BDH Poole, Dorset, UK) to each isotope (103 mg of ^{57}Fe and 39 mg of ^{58}Fe) in silica crucibles, leaving it to stand overnight, warming it, and adding 5 mL of concentrated H_2SO_4 (Aristar, BDH Poole, Dorset, UK). The solutions were heated to drive off the HNO_3 , then taken to dryness and placed in a muffle furnace at 500°C for 30 min. The resulting white powders were taken up in minimum volumes of 0.5 M H_2SO_4 , and ascorbic acid was added (0.83 mg of ascorbic acid/mg of Fe) to reduce all Fe to Fe^{2+} . Natural abundance Fe as ferrous sulfate was added to the ^{58}Fe solution (1 mg of Fe per 0.5 mg of ^{58}Fe). The ferrous sulfate solutions were dispensed in 5-mL amounts, sealed in glass vials, autoclaved, and subjected to routine sterility testing in the Pharmacy Department of the Norfolk and Norwich Hospital (St. Stephens, Norwich, UK). The final solutions contained 1.5 mg of $^{57}\text{Fe}/5 \text{ mL}$ and 0.5 mg of ^{58}Fe plus 1.0 mg of natural abundance Fe/5 mL. The oxidation state of the Fe in ampoules stored for the longest time (12 mo) was checked using Ferrozine (Sigma Chemical Co., Poole, Dorset, UK) and found to still be 100% Fe^{2+} .

Experimental protocol. A single portion of each weaning food, containing 0.55–0.95 mg of native Fe, was mixed with 1.5 mg of Fe as ferrous sulfate (enriched with ^{57}Fe or ^{58}Fe) as an extrinsic label at least 1 h before consumption. Infants were fasted for at least 2 h before and after the test meals, with only

water being allowed. Each meal was given with a commercial apple juice drink (Baby Ribena, SmithKline Beecham, Brentford, Middlesex, UK), containing 50 mg of ascorbic acid per 8-g (undiluted) portion, or a placebo apple drink, containing no ascorbic acid. To ensure adequate isotopic enrichment of red blood cells and at the same time smooth out day-to-day variations in the efficiency of Fe absorption (9), a multiple-dose approach was adopted. Each infant received six labeled meals (three containing added ^{57}Fe , given with one drink, and three with ^{58}Fe , given with the other drink, different isotopes being given on alternate days) over a period not exceeding 10 d. The total quantities of isotopes given to each infant were 4.5 mg of ^{57}Fe and 1.5 mg of ^{58}Fe . The order of isotope administration was reversed in every other subject to eliminate any possible bias. All uneaten/spilt food was collected and analyzed by quadrupole TIMS (Finnigan-Mat, Bremen, Germany) to quantify enrichment with ^{57}Fe or ^{58}Fe . This figure was deducted from the administered dose to calculate the quantity of isotope ingested by each infant. A blood sample was obtained from a heel-prick 14 d after the final test meal, and enrichment with ^{57}Fe and ^{58}Fe was measured.

Blood analysis. Blood (~250 μL) was collected into a heparinized polypropylene tube from a heel-prick 14 d after the last test meal. Subsamples of approximately 0.12 g were cleaned up for TIMS analysis. One milliliter of concentrated nitric acid (Aristar) was added to the subsample and left overnight in a loosely capped vial. To measure the total Fe content by isotope dilution mass spectrometry, an isotopic spike was added to the blood sample (approximately 0.16 g, accurately weighed, of ^{54}Fe solution, 0.182 mg Fe/mL, 96.59 atom %). The mixture was transferred to a Teflon-lined digestion vessel (CEM Microwave UK Ltd., Buckingham, UK), and the vial was washed with 1 mL of concentrated HNO_3 (Aristar) and 1 mL of quartz-distilled water. The digestion vessel was capped and heated in a microwave MDS 2000 oven (CEM Microwave Ltd.) slowly to 90 pounds per square inch over 30 min. The vessels were cooled, 1 mL of H_2O_2 (Aristar) was added, and the vessels were heated again to 90 pounds per square inch slowly over 60 min. The digest was removed to polytetrafluoroethylene beakers, the acid was evaporated in a laminar flow fume cupboard, and the digest was redissolved in 4 M HCl (Aristar).

Acid-washed glass columns, measuring 100 mm and 5 mm internal diameter, were packed with previously swelled Bio-Rad AG 1-X8 resin (Bio-Rad Laboratories, Richmond, CA) and washed with 60 mL of 2 M HNO_3 in an airtight system using a peristaltic pump. The resin was regenerated to the chloride form using 60 mL of 4 M HCl, and the samples were loaded. After loading, the columns were washed with an additional 30 mL of 4 M HCl, and the samples were eluted with 0.5 M HCl, collected in a 1.5-mL polyethylene microtube, and dried in a laminar flow cabinet under a 1-kW lamp. The samples were redissolved in 0.2 M HNO_3 and analyzed by TIMS (10). A standard and a solution of the ^{54}Fe spike were run for each batch of samples.

Calculation of Fe bioavailability. The isotopic ratios (R) ($^{54}\text{Fe}/^{56}\text{Fe}$, $^{57}\text{Fe}/^{56}\text{Fe}$, $^{58}\text{Fe}/^{56}\text{Fe}$) of samples, standards, and

^{54}Fe spike were measured, and from these the Fe concentration and ^{57}Fe and ^{58}Fe enrichment per g of blood was calculated using the following formula:

$$R(^{54}/^{56}) = \frac{\frac{A_{n54}}{W_n} n + \frac{A_{x54}}{W_x} x + \frac{A_{y54}}{W_y} y + \frac{A_{z54}}{W_z} z}{\frac{A_{n56}}{W_n} n + \frac{A_{x56}}{W_x} x + \frac{A_{y56}}{W_y} y + \frac{A_{z56}}{W_z} z}$$

where A = isotopic abundance, W = average molecular weight, n = weight of all isotopes of natural Fe, x = weight of all isotopes of ^{54}Fe , y = weight of all isotopes of ^{57}Fe , and z = weight of all isotopes of ^{58}Fe . Similar equations were derived for $R(^{57}/^{56})$ and $R(^{58}/^{56})$. The values of R and x were known, so by combining the three simultaneous equations, values for n , y , and z could be calculated.

The total blood volume was estimated as 81 mL/kg of body weight (11), and thus the total circulating Fe derived from the doses administered was calculated for each infant. This was expressed as a fraction of administered dose to give an estimate of bioavailability.

Statistical analysis. The percent incorporation of administered isotope into Hb (bioavailability) values were converted to logarithms for statistical analysis, and the results were reconverted to the antilogarithms to recover the original units (12). The incorporation of isotope into Hb (percent of administered dose) data were analyzed by two-way analysis of variance (13), using a computer program, with variables food type and ascorbic acid. Where a significant treatment effect was shown, differences in individual groups were examined using a one-tailed t test (14). The relationship between $\log_{10}$ plasma ferritin and Fe bioavailability ($\log_{10}$ % incorporation of Fe isotope into Hb) from the meals was examined by regression analysis (14) using a computer program.

Ethical approval. Approval for this study was granted by the Institute of Food Research Human Research Ethics Committee, and every mother signed an informed consent form.

RESULTS

There were no differences in the weight, ZPP, or plasma ferritin between the four groups. Although Hb values were normal range, ZPP values were elevated, *i.e.* >1.42 suggested cut-off values (6), in 65% of the infants. The ferritin values were low, (see "Discussion") but only 17.5% had values <12 $\mu\text{g/L}$. This suggests that the cut-off value for ZPP is perhaps too low.

The mean percent Hb incorporation of isotope from the four meals is illustrated in Figure 1, given with and without a drink containing 50 mg of ascorbic acid. In two subjects (one from the Weetabix group and one from the vegetable dinner group), the isotopic analysis gave poor duplicate results, and the size of blood sample obtained from these infants was insufficient to perform repeat analysis, thus these data were excluded from the results. The geometric mean percent incorporation of absorbed isotope into Hb from the foods given with the placebo drink ranged from 3.0 to 4.3%. This increased to 4.0–8.6% when the foods were given with the apple juice drink contain-

Figure 1. Fe bioavailability (percent incorporation of isotope dose into red blood cells) of selected weaning foods, given to 9-mo-old infants, with and without a drink containing 50 mg of ascorbic acid.

ing ascorbic acid. No difference was found between boys and girls.

The data were subjected to analysis of variance (Table 3), and it was shown that there were no significant differences in bioavailability between the foods, but that ascorbic acid significantly affected Fe uptake into red blood cells. When each food was examined separately, it was found that ascorbic acid enhanced Fe bioavailability from meals of Weetabix ($p < 0.05$), wholemeal bread ($p < 0.05$), and baked beans ($p < 0.01$), but not the garden vegetables dinner. The ascorbic acid content of each of the four meals is shown in Table 2, where it can be seen that the garden vegetables dinner contained 63 mg of ascorbic acid (added by the manufacturers together with other micronutrients), whereas none of the other meals contributed a significant amount of ascorbic acid. The native Fe content of the test meals ranged from 0.59 mg in the wholemeal bread to 0.95 mg in the garden vegetables dinner.

The multiple dosing technique, together with ^{54}Fe spiking of samples, was sensitive enough to measure the total Fe content and detect 5% changes in isotopic enrichment of whole blood, and it required only very small volumes of blood (approximately 250 μL). Capillary blood can be obtained from a heel- or finger-prick which requires a lower level of training than is needed for venipuncture, and in our experience, some mothers find heel-pricks more acceptable than venipuncture.

DISCUSSION

The determination of Fe absorption by chemical balance is not reliable and does not provide information about individual foods or meals. The preferred methods of measuring Fe absorption and bioavailability use radioisotopes, but because of the potential hazards associated with ionizing radiation, studies of infant nutrition using radioisotopes have often been carried out in adults, thus there is limited knowledge about how well infants use Fe from different foods (15). There are, in fact, only

Table 3. Effect of type of food and ascorbic acid on percent incorporation of Fe into Hb in 9-mo-old infants

Food	- Ascorbic acid	+ Ascorbic acid	Significance
Vegetable dinner	1.2	2.7	NS
	4.1	1.5	
	1.6	3.2	
	1.4	0.9	
	2.5	3.4	
	4.7	13.8	
	3.2	5.0	
	9.5	10.3	
	4.2	8.4	
	Mean 3.0 (1.5, 5.9)	4.0 (1.6, 9.7)	
Weetabix	1.1	3.3	$p < 0.05$
	1.6	13.4	
	3.7	4.6	
	2.1	2.9	
	1.1	2.4	
	1.6	3.1	
	7.5	10.3	
	21.2	24.7	
	5.7	19.1	
	Mean 3.0 (1.1, 8.3)	6.5 (2.7, 16.0)	
Wholemeal bread	15.4	8.0	$p < 0.05$
	1.6	3.1	
	2.0	11.2	
	5.5	9.5	
	1.2	4.4	
	3.9	9.3	
	3.8	11.6	
	1.3	8.9	
	3.8	6.8	
	3.4	7.7	
Baked beans	Mean 3.1 (1.4, 6.7)	7.5 (5.0, 11.4)	$p < 0.01$
	3.6	6.4	
	10.3	11.1	
	1.9	9.9	
	3.3	2.0	
	5.7	13.0	
	1.7	5.8	
	4.4	10.4	
	8.0	9.3	
	3.1	23.7	
	Mean 4.3 (2.3, 7.8)	8.6 (4.5, 16.2)	$p < 0.01$

Individual and geometric mean ($-SD$, $+SD$) values.

a few isotopic studies in which Fe absorption from foods has been determined in infants, either by whole body counting (16), fecal balance (17), or Hb incorporation techniques (18). In recent years, efforts have been made to develop the methodology required to use stable instead of radioisotopes, so as to enable studies to be performed directly on infants by fecal monitoring (19) or red blood cell enrichment (10, 20–22). The present report presents results of a unique study in which two stable isotopes have been administered orally to infants, and a third one was used to quantify blood enrichment with the isotopes (bioavailability) from a small volume of capillary blood using isotope dilution mass spectrometry.

There was a large interindividual variation in percent Fe incorporation into Hb in the infants. In adults this is generally attributed to differences in Fe stores (measured by serum/plasma ferritin concentrations). Fomon *et al.* (20) found a weak but significant relationship ($r = -0.337$, $p < 0.03$) between

erythrocyte incorporation of ^{58}Fe added to infant foods and serum ferritin in 22-wk-old infants. A better correlation was reported in a smaller study involving nine infants aged 18 wk (geometric mean $46.2 \mu\text{g/L}$, $+\text{SD } 97.7$, $-\text{SD } 21.4$) (Spearman $r = -0.867$, $p < 0.01$) (21). However, in the present study, when the relationship between Hb incorporation (all foods fed without ascorbic acid) and plasma ferritin levels was examined (23), there was no significant correlation (Spearman $r = -0.212$). Infants had lower body Fe levels and a smaller range in plasma ferritin concentrations (geometric mean $21.2 \mu\text{g/L}$, $+\text{SD } 39.9$, $-\text{SD } 11.3$). None of the infants in the smaller study was Fe deficient, whereas 17.5% of our 9-mo-old infants had depleted stores (plasma ferritin $<12 \mu\text{g/L}$). Using rats as an experimental model for man, we have shown that there is indeed a significant correlation between percent Fe absorption and Hb concentration (24) but that, when Fe-deficient and normal animals are examined as two separate groups, the correlation disappears. These results suggest that there is a threshold response whereby the efficiency of Fe absorption increases only when body stores are depleted. Most infants 6 mo of age or older are Fe-depleted, therefore perhaps the same phenomenon is occurring in the infants in the present study as was observed in rats. Our findings confirm those of Kastemayer *et al.* (10), in which no significant correlation between serum ferritin concentration and percent Fe absorption in 13- to 25-wk-old infants was found. Further work is required to determine at what age the well-established inverse relationship in adults between body Fe stores and efficiency of Fe absorption develops.

The chemical form of Fe that is added to weaning foods depends on a variety of factors, including organoleptic properties (taste, color), shelf-life and stability (oxidation of lipids), bioavailability, and cost. Often the most highly absorbable sources of Fe are unacceptably reactive in the food product, resulting in undesirable characteristics. The commercial whole-wheat breakfast cereal (Weetabix) is fortified with ferrous sulfate, but the dried garden vegetables dinner (Milupa) contains added ferric pyrophosphate. Because it was not possible to prepare stable isotopically labeled pyrophosphate, the well-absorbed salt ferrous sulfate was used for all of the test meals. In the case of Weetabix, the results obtained are a true measure of Fe bioavailability from the product as consumed by infants, but with the other foods the results are a measure of the effect of the food on Fe utilization from well-absorbed Fe. Absorption of ^{59}Fe -labeled ferric pyrophosphate added to infant cereal was reported to be 39% of the ferrous sulfate values in adults (25), thus it is likely that Fe utilization from commercial Milupa meals would be lower than found in this study.

Fomon *et al.* (20) measured ^{58}Fe uptake into erythrocytes from labeled infant foods (rice cereal, vegetables and beef, and grape juice) in 22-wk-old infants. Mean values (percent of label incorporated into erythrocytes) ranged from 2.5% (vegetables and beef) to 5.4% (rice cereal with apples and bananas). These results are very similar to the results obtained in the present study for the foods given with the placebo drink. They found no significant differences between the foods, nor did the presence or absence of infant formula affect Fe bioavailability. However, because of practical considerations, the test meals

contained differing quantities of Fe (1.1–4.6 mg). Because the efficiency of Fe absorption is known to be dose-related, direct comparison between meals of different Fe content can be misleading. In the present study we attempted to ensure that the total (native plus added isotope) Fe content of each test meal was similar (Weetabix, 2.2 mg; baked beans; 2.4 mg; bread, 2.1 mg; and garden vegetables dinner, 2.5 mg), but because Fe was added as a label, the Fe concentration of the foods was higher than that found commercially, even in the products to which no fortification Fe had been added. The Fe content (mg/100 g) in test meals and commercial products, respectively, was Weetabix, 11.1 and 5.4; baked beans, 3.2 and 1.2; bread, 9.5 and 2.7; and garden vegetables dinner, 9.8 and 5.0. The efficiency of Fe absorption from the commercial foods with a lower Fe concentration would probably be higher than was observed for the test meals in our study.

There are no published data on Fe bioavailability from the foods examined in this particular study, but some indication can be obtained from studies on similar foods. Hb incorporation of ^{59}Fe added to white flour was 3.68% with 10% added bran and 1.76 with 40% added bran in adult men (26). The difference in dietary fiber content between wholemeal and white flour would be provided by approximately 3% bran (27), thus the Hb incorporation results from the wholemeal bread sandwich given without ascorbic acid to infants (3.1% of dose) were in close agreement with adult values for white flour with 10% added bran. The Fe bioavailability from whole-wheat flour processed into Weetabix breakfast cereal, and given with milk, was similar (3.0%).

The proportion of baked beans to tomato sauce in the test meal was 2.5:1. Fe utilization from baked beans *per se* has not been investigated, but Fe absorption from soybean flour by children (5 mo to 2 y of age) has been shown to vary according to the way it is cooked (16). When boiled, 2.8% was absorbed, but when baked, absorption rose to 9.4%. In rapidly growing infants with low Fe stores, it is likely that almost all of the absorbed Fe is used for Hb synthesis, thus absorption data can be validly compared with utilization data. It would appear, therefore, that the bioavailability of Fe in baked beans with tomato sauce is somewhere between boiled and baked soybean flour.

Ascorbic acid has been recognized as a potent enhancer of Fe absorption for many years (28). There are two mechanisms by which it promotes Fe bioavailability: first, it can reduce Fe^{3+} to Fe^{2+} , which is more soluble and does not form insoluble hydroxides at such a low pH as Fe^{3+} , and second it forms a stable complex with Fe, thereby preventing ionic Fe from forming complexes with dietary constituents, such as tannins and phytate, from which the Fe cannot be removed for uptake into the gastrointestinal mucosal cell. The effect is dose-dependent, and with adults, a threshold of 25 mg is indicated, and doses of 50 mg per meal have been suggested as optimal (29). The ascorbic acid:Fe molar ratio is another important consideration. Molar ratios of about 1.5 can result in a 2- to 3-fold increase in Fe absorption from Fe-fortified cereals (30). The present study showed a 2-fold increase in Fe utilization from baked beans, wholemeal bread, and Weetabix. The ascorbic acid:Fe molar ratios were all well in excess of 1.5,

ranging from 6.7 for baked beans to 14.7 for the garden vegetables dinner, thus an enhancement of Fe bioavailability would be anticipated. However, no significant increase in Fe bioavailability was found with the garden vegetables dinner. This product is normally fortified with ascorbic acid, and the analyzed concentration was 63 mg/meal. Thus the ascorbic acid:Fe ratio was 8.1, without the additional ascorbic acid from the fruit juice drink, which was high enough to promote Fe absorption from the meal alone.

In conclusion, the results of this study showed no significant difference in Fe bioavailability to 9-mo-old infants from four different weaning foods, but demonstrated a significant (2-fold) increase when foods low in ascorbic acid were given with a fruit juice drink containing 50 mg of ascorbic acid.

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