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Bioavailability of Iron from Infant Foods: Studies with Stable Isotopes

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Iron deficiency is common among infants and young children both in industrialized and in developing countries (1). Besides inadequate intake of iron, poor availability of ingested iron is a major cause of poor iron nutriture. Development of strategies to combat iron deficiency has been impeded by lack of precise information about the availability to infants and young children of food iron and fortification iron. This lack of information is due to a reluctance to use radioisotopes in studies of infants and young children, although administration of radioiron poses a very negligible hazard. However, the use of stable (nonradioactive) isotopes has recently become feasible as a result of the development of suitable mass spectrometry instrumentation, such as inductively coupled plasma mass spectrometry (ICP/MS). The feasibility of using ^Fe, the least abundant stable isotope of iron (natural abundance 0.322 weight %), for studies of iron metabolism in infants has been demonstrated (2,3). Enrichment of circulating erythrocyte iron following administration of a tracer amount of ^Fe can be determined with sufficient precision to permit use of the erythrocyte incorporation approach for comparative studies. This approach is more convenient than the isotope balance method, which requires prolonged periods of stool collection.

GENERAL METHODOLOGY OF STABLE ISOTOPE STUDIES OF IRON

Whole body counting following the administration of radioiron (^Fe or ^Fe) is considered the most precise method to determine iron bioavailability (4). Obviously, an equivalent approach does not exist for stable isotopes. Since the cumbersome nature of isotope balance studies limits their use, erythrocyte incorporation studies are the approach of choice for availability studies with the use of stable isotopes. The main limitation of this approach is that the percentage of absorbed iron that is promptly incorporated into hemoglobin and that appears in circulating erythrocytes is unknown. In normal and in iron-deficient adults, that percentage is greater than 80% and usually around 90% (1), so that, to estimate actual absorption, an arbitrary correction must be made. However, this limitation is not an impediment in studies of the relative bioavailability of iron, where it is sufficient to state results in terms of the percentage of administered label found in circulating erythrocytes. Naturally, the conditions of administration and measurement must be rigorously standardized.

Two additional points concerning stable isotope studies must be mentioned. First, a background correction must always be made since the isotope used as a label is always naturally present, or it may even be present at higher than natural abundance in subjects who have served in earlier similar studies. Second, mass spectrometric measurements of iron yield isotope ratios. Therefore, to calculate the amount of the isotope of interest, the total amount of the element must be determined independently. In the case oferythrocyte incorporation studies of iron, the total amount of circulating iron is estimated from the determined blood hemoglobin concentration and an assumed blood volume.

Finally, in studies of iron bioavailability, wide interindividual variability of values is universally observed, even among subjects with proven good iron nutritional status. It is therefore customary in studies with radioisotopes of iron to administer a second isotope as a reference dose (e.g., as ferrous ascorbate) in close temporal proximity to the test dose, and to correct results with the first isotope for the value obtained with the reference isotope. In the case of stable isotopes, this approach is not yet possible.

FEASIBILITY OF STABLE ISOTOPE STUDIES IN INFANTS

In a feasibility study (3), nine normal healthy infants were studied at 126 days of age. Between 8 and 112 days of age, these infants had served as subjects in studies of food intake and growth and had received various formulas providing at least 1.8 mg of iron/100 kcal. From 112 to 128 days of age, a milk-based formula providing about 0.2 mg of iron/100 kcal was fed. At 126 days of age, the infants received the test dose of ^Fe. The dose consisted of 1.95 mg of iron containing 1.44 mg of ^Fe as ferrous sulfate and was administered as a solution containing 84 mg of ascorbic acid and 400 mg of sucrose in a volume of 5 ml. The dose was given 2 hr after a formula feeding and 2 hr before the next feeding. Venous blood was obtained at 84, 112, 140, 168, and 196 days of age for determination of hemoglobin concentration, serum ferritin concentration, and the ^Fe/^Fe mass isotope ratio (MIR58/5?), the latter by ICP/MS. The coefficient of variation for the isotope ratio determinations was 1 % or less. The amount of ^Fe label incor-

K^asTfoi:^" ••me'after •he adm•-8•ra‹

sspe, = MIR^g^T - MIR°.R,^
MIROsg^——— x Fe^e X 0.00322

^a5^!?^^^^^^^ is **the ^———d MIR^ at**

total circulating ^(^^i^TanT^^^^^^^ ^ is the amount of
(weight fraction) of5^ TUs^l^ the natura1 ^undance
of^Fe. Thus ^Fe rpm•Plc?l^a58 roectsforthenaturalabundance

centration of iron in hemoglobin (mg/g) g ' 47 ls the con-
Individual values for the ^Fe/^Fe ratin in ^ i »•

^n^^•,K^s>^H^
-.>.>. T.e ,L ^^S ?4S^^.^^^^^^ 2

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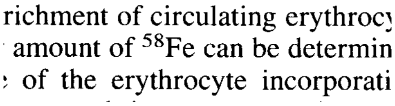
richment of circulating erythrocy
amount of ^{58}Fe can be determin
of the erythrocyte incorporati

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0:

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'^Fe

112^~^8^

AGE(days)

^..^V^'ss^^^^^^

of the ^Fe label 1 day after the first dose. It is also evident that enrichment is highest at 140 days of age and declines gradually thereafter. The decline is due to expansion of the circulating hemoglobin mass through addition of unlabeled erythrocytes, as evidenced by the fact that the calculated amount of circulating ^Fe label did not change significantly: the average circulating ^Fe label was 0.1852 mg at 140 days of age, 0.1882 mg at 168 days of age, and 0.1868 mg at 196 days of age. The data offered the opportunity to obtain an estimate of the overall methodological error, i.e., the error due to uncertainties in the measurement of isotope ratios and of hemoglobin concentrations, as well as uncertainties in the assumed blood volume. Table 1 presents for each subject the mean value (from determinations at 140, 168, and 196 days of age) for circulating ^Fe label, expressed both in absolute terms (mg) and as percent of the dose. The standard deviation represents an estimate of the uncertainty of the method. Expressed as percent of the dose, the standard deviations ranged from 0.11% to 1.28%. However, expressed as the coefficient of variation, uncertainties ranged from 1.0% to 30.5% of the dose, with a mean of 9.7%. For the six subjects with incorporation greater than 4% of the dose, the mean coefficient of variation was only 5.6%.

The wide interindividual variation of percent incorporation represents a formidable obstacle to comparative studies of iron bioavailability that will be overcome only when a second stable isotope becomes available as a reference dose. Interindividual variation is partly explained by variation in iron nutritional status, which, in turn, is reflected by serum ferritin concentration. Percent ^Fe label incorporated was inversely correlated with mean ferritin concentration ($r = -0.836$, Spearman rank correlation, $p < 0.01$).

TABLE 1. *Circulating ^Fe label"*

		Percent of dose		
Subject	Mean dose (mg)	Mean	SD	CV

isotope balance method, which requires prolonged periods of stool col-
tion.

ENERAL METHODOLOGY OF STABLE ISOTOPE STUDIES OF IRON

Whole body counting following the administration of radioiron (^{55}Fe or
e) is considered the most precise method to determine iron bioavailability

SD = standard deviation; CV = coefficient of variation.
••From ref. 3.
"Geometric mean.
'Arithmetic mean.

IRON ABSORPTION FROM INFANT FOODS

The bioavailability of iron from different infant foods and from different iron salts was assessed in a series of studies (5) using an approach generally similar to that previously described, except that test meals labeled with ^Fe were fed at 154 days of age. From 112 days of age until 4 days after the day on which the test meal was fed, a milk-based formula providing approximately 0.3 mg of iron/100 kcal was fed. From 140 days of age until 4 days after the test meal, a food similar or identical to that scheduled for use as the test meal was fed at least once daily. Subsequently, infants were permitted to receive other foods. Venous blood was obtained at 140, 168, and 196 days of age. Methods and procedures were as described above. Values for percent erythrocyte incorporation of ^Fe were calculated separately for each infant at 168 and 196 days of age, and the average value was used.

The results are summarized in Table 2. Four foods were fortified with ferrous sulfate, and one food was fortified with ferrous fumarate. Despite efforts to achieve similar intakes of iron with all test meals, intakes of iron from test meals differed with the different foods for technical reasons. Values for percent incorporation showed wide variability between individual infants. Differences between foods were not statistically significant. It may be concluded that rice cereal does not exert a substantial inhibitory effect on iron absorption. Grape juice, despite its ascorbic acid concentration of 31 mg/100 ml, was not associated with greater iron incorporation than that observed with other foods. Similarly, the relatively small amount of beef (about 1 % of wet weight) of the vegetables and beef preparation apparently did not enhance the availability of iron. Finally, the data suggest that iron was as available from ferrous fumarate as from ferrous sulfate. Ferrous fumarate is a promising iron salt for fortification of dry-packed cereals.

TABLE 2. *Iron absorption from infant foods*

Food	Fortification iron	Number of infants	Iron (mg) in test meal	^Fe incorporation (% of dose)	
				Geometric mean	±1 SD range
Rice cereal with apples and bananas	FeS04	12	2.8	5.4	2.9-10.0
Rice cereal with formula	FeS04	9	1.1	4.4	1.6-11.9
Vegetables and beef	FeS04	10	3.2	2.5	1.2- 5.3
Grape juice	FeS04	10	2.8	4.8	2.1-11.1
Rice cereal with formula	Fe fumarate		4.6	4.0	2.5-6.5

From ref. 5.

CONCLUSION

The use of stable isotopes now offers an approach to the assessment of iron bioavailability in normal infants and young children, the group at greatest *risk of iron deficiency. Results in these initial studies generally agree with* results from studies with radioisotopes. In the only previous study on iron absorption from infant foods, Rios et al. (6), using whole body counting after administration of radioiron (^Fe), found that the geometric mean absorption of ferrous sulfate fed with mixed grain infant cereal was 2.7%. Thus, stable isotope studies can now be used to obtain information on the bioavailability of iron from infant foods.

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Discussion

Dr. Filer: How was the iron dose administered?

Dr. Ziegler: It was given through a nipple. The nipple was placed in the infant's mouth and 5 ml of solution was given slowly through the nipple and then flushed. We were very careful to administer the dose quantitatively.

Dr. Filer: How did you decide to use 80 mg of ascorbic acid?

Dr. Ziegler: We used enough ascorbic acid to get a high molar ratio of ascorbic acid to iron of about 14. This is not unlike what is done in studies using radioisotopes. It does not make any difference exactly how much ascorbic acid is given as long as it is the same every time.

Dr. Oski: In your stable isotope studies comparing the different foods, did all of the subjects receive the same foods?

Dr. Ziegler: No, they were all different subjects. There were between 9 and 12 subjects per group.

Dr. Filer: Did you analyze the grape juice for its vitamin C content?

Dr. Ziegler: No, but the label stated its content as 31 mg/dl.

Dr. Filer: That means that it probably had double that amount.

Dr. Dallman: What was the cost of the isotope preparation per milligram?

Dr. Ziegler: A stable isotope-enriched preparation costs about $100.00 per milligram.

Dr. Oski: Were these children fasting before the test dose was administered?

Dr. Ziegler: No. They were brought from home to the metabolic unit. Approximately 2 hr after their morning formula feeding, they received the test dose and then nothing for 2 hr.

Dr. Dallman: The variability in your study is actually not more than what is observed in radioisotope studies.

Dr. Ziegler: That is right. However, in radioisotope studies the variability can be reduced by giving a reference isotope.

Dr. Filer: Apparently, between 140 and 168 days of age there is not a very large change in the circulating ^Fe: ^Fe ratio. Have you considered giving another dose of ^Fe enriched food to that same infant using ^Fe as a reference standard and just enriching it?

Dr. Ziegler: You could do that, but the second dose would need to be administered at least 2 weeks later, and during that time the physiological state of the infant could change. Technically, it could be done. I do not know whether that would solve the problem. We have not tried it.

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