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Use of the Stable Isotope, ^{58}Fe, for Determining Availabilty of Nonheme Iron in Meals

JEROLD C. WOODHEAD, JEAN M. DRULIS, RONALD R. ROGERS, EKHARD E. ZIEGLER, PHYLLIS J. STUMBO, MORTEZA JANGHORBANI, BILL T. G. TING, AND SAMUEL J. FOMON

Department of Pediatrics, College of Medicine, University of Iowa, Iowa City, IA 52242 and Department of Pathology, School of Medicine, Boston, University, Boston, MA 02118

ABSTRACT. Because of reluctance to use radioisotopes for studies of iron absorption in children, we have explored the feasibility of using the least abundant stable isotope of iron, ^{58}Fe (natural abundance, 0.322 weight %) in a study of nonheme iron absorption. With a balanced cross-over design, each of 16 school-age children was fed a standardized lunch on 3 consecutive days and, 28 days later, an alternate standardized lunch on 3 consecutive days. The lunch included either a beef patty or a beef-soy patty. The mass isotope ratio, $^{58}Fe/^{57}Fe$ ($MIR_{58/57}$), was measured in blood by inductively coupled plasma mass spectroscopy before and 14 days after (*i.e.* study day 15) consuming the three lunches. The $MIR_{58/57}$ on study day 15 was used as a baseline value for lunches fed on study days 29, 30, and 31. Incorporation of ^{58}Fe into erythrocytes was greater from the lunch with beef patty than from the lunch with beef-soy patty (geometric mean values 2.02 and 1.05% of the dose, $p < 0.03$). Based on the similarity of our results with those obtained in adults with radioisotopes, we conclude that ^{58}Fe is a satisfactory tag for studies of nonheme iron absorption from meals. (*Pediatr Res* 23: 495–499, 1988)

Abbreviations

GCRC, General Clinical Research Center
ICP/MS, inductively coupled plasma mass spectroscopy
$^{58}Fe_{inc}$, quantity of administered ^{58}Fe incorporated into erythrocytes
Fe_{circ}, quantity of total iron in the circulation
BV, blood volume

The understanding of factors that influence iron absorption by infants and children has been impeded by reluctance of investigators to use radioisotopes for such studies. We have demonstrated (1, 2) that the least abundant stable isotope of iron, ^{58}Fe, can be used in lieu of a radioisotope for certain studies of iron absorption. Thus far, our experience has concerned erythrocyte incorporation of ^{58}Fe by infants fed a test dose of ^{58}Fe-enriched ferrous sulfate administered between feedings in aqueous solution with ascorbic acid. The present study was undertaken to explore the feasibility of using ^{58}Fe as a tag for determining absorption of nonheme iron when an entire meal is fed to a child.

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Correspondence Samuel J. Fomon, M.D., Department of Pediatrics, University of Iowa Hospitals, Iowa City, IA 52242.
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Absorption of nonheme iron from meals with beef patty or beef-soy patty has been well studied in adults with the use of radioisotopes (3–5). We believed that a similar study in children with ^{58}Fe would permit an evaluation of the ^{58}Fe method. The substitution of soy for a portion of the beef in a meal is of particular interest because federal regulations (6) permit the substitution of certain vegetable proteins for 30% of the beef in Child Nutrition Programs.

METHODS

Study design. The protocol for the study was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects.

Each child was admitted to the GCRC of the University of Iowa College of Medicine on two occasions for 3½ days (from Monday morning until Thursday afternoon). A balanced cross-over design was used, each child receiving three lunches with a beef patty during one admission and three lunches with a beef-soy patty during the other admission. Twenty-eight days intervened between the first day of the first admission and the first day of the second admission. We have referred to the first day of the first admission as study day 1 and the first day of the second admission as study day 29. Approximately 10 ml of venous blood were obtained a few days before study day 1 and on study days 15 and 43. The noon meals on study days 1, 2, and 3 and on study days 29, 30, and 31 were tagged with ^{58}Fe.

A study of zinc absorption was carried out concurrently with the same subjects and necessitated admission of the children to the GCRC for quantitative collection of feces. Results of that study will be reported separately.

Subjects. Sixteen children in good health, eight boys and eight girls, were recruited through personal contacts and newspaper advertisements. A small stipend was provided. The subjects ranged in age from 7.3 to 10.2 yr and in weight from 25.3 to 40.8 kg (Table 1). Hemoglobin concentrations ranged from 12.1 to 14.1 g/dl and iron nutritional status appeared satisfactory as judged by concentrations of ferritin in serum (Table 1).

To accommodate family vacation plans, we assigned five girls to cohort I (first cohort admitted for study), six boys to cohort II, three girls to cohort III, and two boys to cohort IV. Cohorts I and III (girls) consumed the lunch with the beef patty during the first GCRC admission and the lunch with the beef-soy patty during the second admission. Cohorts II and IV (boys) consumed the lunch with the beef-soy patty during the first admission and the lunch with the beef patty during the second admission.

Nutrient intake from lunch. Weighed portions of foods were provided in the test lunches (Table 2) served on the first 3 days of each admission, and in each instance the entire lunch was consumed. The intakes of energy, protein, fat, carbohydrate, and iron from the lunches were calculated with the aid of a computer program based on USDA Handbook 8 nutrient values in foods

Table 1. *Subject characteristics, indices of iron status, and test dose of iron*

Subject	Sex	Age (yr)	Wt (kg)	Hb (g/dl)	Hematocrit (%)	Ferritin* (ng/ml)	Lunch† 1st administration	Iron in test dose‡ (mg) 1st administration	Iron in test dose‡ (mg) 2nd administration
Cohort I									
1	F	8.0	25.4	13.8	42.7	34	B	0.98	1.43
2	F	8.3	40.8	13.0	42.3	27	B	0.94	1.42
3	F	8.5	31.1	12.1	39.0	35	B	0.98	1.43
4	F	8.7	25.3	12.7	40.6	14	B	1.01	1.38
5	F	8.9	36.5	13.0	40.5	25	B	1.04	1.36
Cohort II									
6	M	7.5	27.0	12.5	39.7	32	B-S	0.99	1.44
7	M	8.0	25.8	14.1	43.4	47	B-S	0.99	1.41
8	M	8.3	31.3	12.9	41.5	23	B-S	0.97	1.45
9	M	8.9	33.5	13.1	39.9	38	B-S	0.97	1.42
10	M	9.5	28.3	12.7	39.7	33	B-S	0.99	1.43
11	M	10.2	27.3	13.4	41.7	36	B-S	0.98	1.40
Cohort III									
12	F	7.3	28.5	12.4	40.3	50	B	1.00	1.42
13	F	9.7	26.5	12.8	41.7	23	B	0.98	1.42
14	F	9.9	30.5	12.5	40.1	23	B	0.99	1.43
Cohort IV									
15	M	8.0	26.4	13.4	43.1	32	B-S	1.01	1.35
16	M	8.8	30.4	13.5	42.9	44	B-S	1.01	1.21

* Concentration of ferritin in serum.
† Lunch during first admission. B refers to lunch with beef patty; B-S refers to lunch with beef-soy patty.
‡ ^{58}Fe comprised 84.6% of doses in 1st admission and 76.7% of doses in the second admission.

Table 2. *Nutrient content of lunches*

	Wt (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Iron* (mg) Calculated	Iron* (mg) Determined
Beef patty meal							
Hamburger bun	28	83	2.3	1.5	14.8	0.75	
Beef patty	45	180	11.6	14.9	0.0	1.46	1.51
Catsup, tomato	14	15	0.3	0.1	3.6	0.09	
Carrot sticks, raw	28	12	0.1	0.3	2.9	0.14	
Cookie, chocolate chip	10	47	0.5	2.2	6.6	0.09	
Potato chips	15	79	1.0	5.5	7.9	0.18	
Milk, chocolate	240	174	7.7	5.1	25.0	0.58	
Total		592	23.4	29.5	60.8	3.29	3.44
Beef-soy patty meal†							
Beef-soy patty	50	197	13.3	15.5	1.0	2.00	1.87
Total		609	25.1	30.1	61.8	3.83	3.83

* Excluding approximately 1 mg of iron added in the form of ^{58}Fe-enriched ferrous sulfate (see Table 1).
† Same components as in beef patty meal except for substitution of beef-soy patty for beef patty.

as referenced by Hepburn (7). With the exception of a few mg of ascorbic acid that may have been provided in the catsup, the lunch was free of ascorbic acid. Also included in Table 2 is the iron content of the lunches and of the beef and beef/soy patties determined by laboratory analysis.

Because the extrinsic ^{58}Fe was a tag for nonheme iron, it was necessary, for purposes of subsequent calculations, to subtract the heme iron contribution of the beef from the total iron intake to arrive at the intake of nonheme iron. Various reports indicate that heme iron accounts for 50 to 78% of the iron in beef (8–10), and we have used a value of 60% in our calculations. Thus, we have assumed that the beef patty provided 0.91 mg of heme iron (*i.e.* 1.51 mg × 0.6) and the beef-soy patty (70% beef) provided 0.63 mg of heme iron (*i.e.* 0.91 mg × 0.7).

Stable isotope. The natural isotopic composition (weight %) of iron is as follows: ^{54}Fe, 5.60; ^{56}Fe, 91.9; ^{57}Fe, 2.18%; and ^{58}Fe, 0.322. ^{58}Fe-enriched metallic iron powder was obtained from Oak Ridge National Laboratory (Oak Ridge, TN). Of the two batches used, one provided 0.846 mg ^{58}Fe/mg Fe, and the other batch provided 0.767 mg ^{58}Fe/mg Fe. Each batch of metallic iron powder was converted to ferrous sulfate solution and the pH adjusted to 2, as previously described (1). The first solution contained 0.98 mg total iron and 0.83 mg ^{58}Fe/ml. The second solution contained 1.33 mg total iron and 1.02 mg ^{58}Fe/ml. The solutions were stored under nitrogen in the dark until used.

For administration of the ^{58}Fe label, 1 ml of solution was drawn up into a tared syringe and the weight of the syringe plus solution determined. The solution was dispensed from the syringe into 120 ml of chocolate milk and thoroughly mixed with the milk. The syringe was rinsed twice with cold tap water, and

the rinse water was added to the chocolate milk. When the 120 ml of labeled chocolate milk had been consumed, an additional 120 ml of chocolate milk were added to the glass, the contents of the glass swirled, and the milk consumed. The milk was consumed during the course of the meal. It is worth noting that the added iron accounted for 20 to 25% of the iron in the meal and therefore cannot be considered a true trace label.

Because each subject consumed the entire lunch on each of the 3 days during each admission, intakes of energy and iron from the foods provided in the lunches (exclusive of the test dose) were as indicated in Table 2. The lunch, including the test dose, provided about 40% of the daily intake of iron.

Laboratory analyses. Two entire lunches with the beef patty and two entire lunches with the beef-soy patty were analyzed for iron. These lunches were identical to those fed to the children except that no ^{58}Fe-label was added. Each lunch was homogenized in a Waring Blendor, using enough water to form a slurry. A weighed aliquot of the homogenate was removed, placed in a porcelain crucible, dried at 100° C, and ashed for 12 hr at 400° C. The ash was dissolved in nitric acid and the iron concentration determined by atomic absorption spectrophotometry using a Perkin-Elmer model 306 with HGA 2100 graphite furnace. A beef patty and a beef-soy patty were analyzed for iron in a similar manner.

Hemoglobin concentration in blood was determined by the cyanmethemoglobin method (catalog no. 368555, Boehringer Mannheim Diagnostics, Indianapolis, IN) and hematocrit with heparinized capillary tubes centrifuged at 13,000 × *g* for 10 min. Serum concentration of ferritin was determined by radioimmunoassay using the Micromedic Ferritin RIA kit (catalog no. D-4401, Micromedic Systems, Inc., Horsham, PA).

The ^{58}Fe/^{57}Fe isotope ratio ($MIR_{58/57}$) was measured by ICP/MS, using the Elan 250 ICP/MS System (SCIEX Inc., Thornhill, Canada) operated in the isotope ratio mode. The method has been described in detail (1) and has been summarized (2). The analytical precision (relative SD) is 1%.

Calculation of quantity of administered ^{58}Fe incorporated into erythrocytes. The $^{58}Fe_{inc}$ (expressed in mg) during the first admission was calculated as follows:

$$^{58}Fe_{inc} = \frac{MIR^{15}_{58/57} - MIR^{0}_{58/57}}{MIR^{0}_{58/57}} \times Fe^{15}_{circ} \times 0.00322$$

where $MIR^{15}_{58/57}$ is the determined $MIR_{58/57}$ on study day 15, MIR^0 is the baseline $MIR_{58/57}$, Fe^{15}_{circ} is the calculated quantity of total iron (mg) circulating on study day 15, and 0.00322 is the natural abundance (weight fraction) of ^{58}Fe. The quantity of administered ^{58}Fe incorporated into erythrocytes during the second admission was calculated as follows:

$$^{58}Fe_{inc} = \frac{MIR^{43}_{58/57} - MIR^{15}_{58/57}}{MIR^{15}_{58/57}} \times Fe^{43}_{circ} \times 0.00322$$

where $MIR^{43}_{58/57}$ and Fe^{43}_{circ} are values pertaining to study day 43.

The Fe_{circ} (expressed in mg) was estimated as follows:

$$Fe_{circ} = BV \times Hb \times 3.47$$

where BV is in ml, Hb is in g/ml, and 3.47 is the concentration of iron in Hb (mg/g). BV was derived from the equations of Linderkamp *et al.* (11) as follows:

$$\text{boys:} \quad \log BV = 0.6459 \log W + 0.002743\ H + 2.0324$$

$$\text{girls:} \quad \log BV = 0.6412 \log W + 0.001270\ H + 2.2169$$

where W is body weight in kg and H is height in cm.

Use of the $MIR^{15}_{58/57}$ as a baseline value for the second admission had the advantage of avoiding the need for a third venipuncture on or shortly before study day 29. The difference between the two values was considered to be inconsequential. Calculations based on the equation of Linderkamp *et al.* (11) and the mean weight of 8- and 9-yr-old boys (12) yielded BV of 1965 and 2139 ml for the respective ages, with a difference of 174 ml. This difference translates for a 14-day interval to 6.7 ml of blood, which represents about a 0.3% increase in circulating iron and thus in circulating ^{58}Fe and ^{57}Fe.

Statistical analysis. The two-tailed paired *t* test on log-transformed data was used to compare incorporation into erythrocytes of ^{58}Fe (% of dose) and of nonheme iron (mg/lunch) between the two lunches, and the two-tailed unpaired *t* test to compare incorporation of ^{58}Fe by boys and girls.

RESULTS

The calculated mass isotope ratio, $MIR_{58/57}$, at natural abundance is 0.1475 (*i.e.* 0.00322 ÷ 0.02183). The determined natural abundance ratio, MIR^0_{58-57}, varies slightly from day to day, and it is therefore desirable to use as a baseline for each subject the average of all $MIR^0_{58/57}$ determinations performed at one time (1, 2). Herein all determinations were initially carried out at one time. A few of the results seemed questionable and we therefore elected to repeat all values concerning five subjects (subjects 1, 6, 7, 13, and 14). The $MIR^0_{58/57}$ values presented in Table 3 thus represent determinations done on 2 days. In calculating $^{58}Fe_{inc}$ during the first admission, we have used one common $MIR^0_{58/57}$ value (0.1446) for 11 children. This value was based on nine rather than 11 determinations because the vials containing two blood samples were broken during shipment from Iowa City to Boston. A separate common $MIR^0_{58/57}$ value (0.1478) was used for the remaining five children. In the calculations to determine the extent of $^{58}Fe_{inc}$ during the second admission, individual $MIR^{15}_{58/57}$ values were used as baseline.

Table 4 presents data on $^{58}Fe_{inc}$ as a percentage ot the ^{58}Fe dose. $^{58}Fe_{inc}$ from the lunch with beef patty ranged from 0.38 to 6.39% of the dose with a geometric mean of 2.02%. From the lunch with beef-soy patty, the values ranged from 0.25 to 4.8%, with a geometric mean of 1.05%. The difference between the two meals was statistically significant ($p < 0.03$).

Also included in Table 4 are data on erythrocyte incorporation of nonheme iron (mg/lunch), calculated by multiplying the percentage of the ^{58}Fe dose incorporated into erythrocytes by the quantity of nonheme iron in the meal. From the lunch with beef patty values ranged from 0.01 to 0.23 mg/day (geometric mean 0.08 mg/day), and from the lunch with beef-soy patty values ranged from 0.01 to 0.20 mg/day (geometric mean 0.05 mg/day). The difference was not significant ($p = 0.07$).

Differences between subjects in the percentage of ^{58}Fe label incorporated into erythrocytes (Table 4) appeared unrelated to hematocrit or to concentrations of Hb (Table 1). For example, the only subject with serum ferritin less than 23 ng/ml (subject

Table 3. *$MIR_{58/57}$ at baseline and on study days 15 and 43*

Subject	$MIR^0_{58/57}$	$MIR^{15}_{58/57}$	$MIR^{43}_{58/57}$
1	0.1477	0.1501	0.1526
2	0.1441	0.1488	0.1500
3	0.1457	0.1521	0.1552
4	0.1446	0.1477	0.1508
5	0.1441	0.1470	0.1524
6	0.1478	0.1514	0.1527
7	0.1482	0.1481	0.1508
8	0.1449	0.1458	0.1476
9	0.1449	0.1494	0.1530
10		0.1450	0.1483
11	0.1441	0.1455	0.1474
12	0.1454	0.1508	0.1542
13	0.1479	0.1483	0.1491
14	0.1474	0.1521	0.1531
15	0.1437	0.1451	0.1481
16		0.1451	0.1473

Table 4. *Intake and erythrocyte incorporation of nonheme iron*

Subject	Lunch with beef patty			Lunch with beef-soy patty		
		Erythrocyte incorporation			Erythrocyte incorporation	
	Intake of nonheme Fe (mg/lunch)	^{58}Fe (% of dose)	Nonheme Fe (mg/lunch)	Intake of nonheme Fe (mg/lunch)	^{58}Fe (% of dose)	Nonheme Fe (mg/lunch)
1	3.55	1.79	0.06	4.64	1.45	0.07
2	3.51	4.61	0.16	4.63	0.94	0.04
3	3.54	6.39	0.23	4.64	1.89	0.09
4	3.58	2.28	0.08	4.60	1.81	0.08
5	3.60	2.26	0.08	4.57	4.22	0.19
6	4.00	0.74	0.03	4.20	2.78	0.12
7	3.97	1.73	0.07	4.21	0.25	0.01
8	4.01	1.29	0.05	4.19	1.17	0.05
9	3.99	2.61	0.10	4.18	4.80	0.20
10	3.99	2.12	0.08	4.20	0.34	0.01
11	3.97	1.27	0.05	4.20	0.78	0.03
12	3.57	4.78	0.17	4.64	1.96	0.09
13	3.54	0.38	0.01	4.64	0.46	0.02
14	3.55	3.57	0.13	4.64	0.62	0.03
15	3.92	1.98	0.08	4.23	0.40	0.02
16	3.78	1.84	0.07	4.23	0.46	0.02
Geometric mean	3.75	2.02	0.08	4.41	1.05	0.05
−1 Std	3.54	1.00	0.04	4.20	0.42	0.02
+1 Std	3.97	4.08	0.15	4.63	2.60	0.12

Table 5. *Intake and absorption of nonheme iron from meals with beef patty or beef-soy patty*

	Subjects	*n*	Beef			Beef-soy*		
			Intake	Absorption†		Intake	Absorption	
			(mg)	(%)	(mg)	(mg)	(%)	(mg)
Cook *et al.* (3)‡	Men	15	3.0	3.20	0.10	4.2	1.51	0.06
Hallberg and Rossander (4)	Men§ and women	78	3.0	11.2	0.34	3.8	7.2	0.27
Lynch *et al.* (5)	Men	12	3.0	5.05	0.15	4.2	1.90	0.08
Present study	Boys and girls	16	3.8	2.53	0.10	4.4	1.31	0.06

* 70% beef, 30% soy in three studies (3, 4, and present study), 50% beef, 50% soy in one study (4), textured soy flour in previous studies (3–5), isolated soy protein in present study.

† Erythrocyte incorporation of isotope assumed to be 80% of amount absorbed (3, 5 and present study) or absorption determined by whole body counting (4). Means are geometric (3, 5 and present study) or arithmetic (4).

‡ Intake of nonheme iron assumed to be as reported by Lynch *et al.* (5).

§ Included some regular blood donors.

4, Table 1) did not demonstrate unusually high erythrocyte incorporation of ^{58}Fe.

Mean erythrocyte incorporation of ^{58}Fe was somewhat greater for girls than for boys. Based on average values for the two lunches for each child, geometric mean incorporation of ^{58}Fe was 1.85% of the dose for girls and 1.14% of the dose for boys. This difference was not statistically significant ($p = 0.13$), and we cannot exclude an order of treatment effect. As indicated in Table 1, cohorts I and III (girls) received the beef lunch during the first admission and the beef-soy lunch during the second admission.

DISCUSSION

Herein, as in the previously reported studies of adults, each subject was fed at one time a meal (or meals) with beef patty and at another time a similar meal (or meals) in which textured soy flour or isolated soy protein was substituted for a portion of the beef. Results of the various studies are summarized in Table 5.

For purposes of comparing our results with those reported previously, we have assumed, as was done by Cook *et al.* (3) and Lynch *et al.* (5), that 80% of absorbed iron appeared promptly (*i.e.* within 14 days) in erythrocytes. Thus, geometric mean iron absorption in our study is presented in Table 5 as 2.53% of the dose (2.02% ÷ 0.8) for the lunch with beef patty and 1.31% of the dose for the lunch with beef-soy patty. Among the studies of adult subjects, that by Cook *et al.* (3), concerning iron-sufficient men (serum ferritin values 27–70 ng/ml), appears to be most similar to the present study of iron-sufficient preadolescent children. Geometric mean absorption of nonheme iron was reported to be 3.20% of the dose from the meal with beef patty and 1.51% of the dose from the meal with beef-soy patty. That our values were slightly less may be explained by the timing of the meals: after an overnight fast in the study by Cook *et al.* (3) (as was also the case for the other studies of adult subjects), and as midday lunch in our study. The greater mean values for absorption reported by Hallberg and Rossander (4) and by Lynch *et al.* (5) than by Cook *et al.* (3) probably reflect, at least in part, the less satisfactory iron nutritional status of some of the subjects. In addition, the mean values reported by Hallberg and Rossander (4) are apparently arithmetic rather than geometric means.

Based on the similarity between our results and those obtained with a radioisotope, we conclude that ^{58}Fe is a satisfactory tag for studies of nonheme iron absorption from meals.

Whether substitution of soy for a portion of the meat in the school lunch program is likely to exert a nutritionally significant adverse effect on iron nutritional status of children cannot be answered by the data thus far available. The requirement for

absorbed iron by children 5 to 11 yr of age has been estimated to be 1.0 mg/day (13). The mean difference in absorption of nonheme iron from the two meals in our study was only 0.03 mg/lunch, and in the studies of adults, the mean differences ranged from 0.04 to 0.07 mg/meal. Such differences are unlikely to be nutritionally significant. The data of Lynch *et al.* (5) indicate that the absorption of heme iron from such meals is quantitatively more important than absorption of nonheme iron. Further studies of children are needed to determine the extent of heme iron absorption from meals with beef patty or beef-soy patty. We believe that ^{58}Fe-enriched Hb can be used as a tag for studying absorption of heme iron from such meals.

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