Gender-Related Differences in Iron Absorption by Preadolescent Children

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ABSTRACT. In a study of absorption of iron from meals by preadolescent children (Tanner stage 1), we had noted that erythrocyte incorporation of the extrinsic iron label was somewhat greater by girls than by boys. Although the difference was not significant, the observation seemed to warrant further study. Study A: A precisely determined quantity of ferrous sulfate enriched with the stable isotope ^{58}Fe was given without food to 15 boys and 15 girls (Tanner stage 1) after an overnight fast and was immediately followed by a dose of 70 mg of ascorbic acid. ^{58}Fe enrichment of the erythrocytes was determined by inductively coupled plasma mass spectrometry at baseline and 14 and 42 d after administration of the ^{58}Fe dose. Geometric mean erythrocyte incorporation of the ^{58}Fe label was 35.2% of intake by boys and 45.0% of intake by girls. The difference was significant (analysis of covariance with serum ferritin as covariate, $p = 0.035$). Study B: Fifteen boys and 15 girls (Tanner stage 1) were fed a breakfast labeled with ^{58}Fe . Geometric mean erythrocyte incorporation of the ^{58}Fe label was 14.8% of intake by boys and 24.7% of intake by girls. The difference was significant (analysis of covariance with serum ferritin as covariate, $p = 0.004$). Because serum ferritin concentrations were similar in boys and girls, the gender-related difference in iron absorption (as reflected by erythrocyte incorporation of the label) does not appear to be explained by a difference in body stores of iron. We hypothesize that hormonal differences between boys and girls in Tanner stage 1 favor iron absorption by girls. (*Pediatr Res* 29: 435-439, 1991)

Abbreviations

MIR $_{58/57}$, mass isotope ratio $^{58}\text{Fe}/^{57}\text{Fe}$

MIR $_0$, MIR $_{58/57}$ at baseline

NHANES II, Second National Health and Nutrition Examination Survey

Data from NHANES II demonstrated that from 1976 to 1980 iron deficiency was more prevalent among males 11 to 15 y of age (12.1% iron deficient) than among females in the same age group (6.1% iron deficient) (1). This finding was surprising because in older adolescents and adults prevalence of iron deficiency is much greater among females than among males. For example, in NHANES II, in the age category 15 to 19 y, 14.2% of females and only 0.1% of males were iron deficient. Sex-

specific prevalence data for iron deficiency of subjects less than 11 y of age were not reported.

The more satisfactory iron nutritional status of girls than of boys in the age range 11 to 15 y was also surprising because the same survey demonstrated that dietary intake of iron in each age interval from 6 to 15 y of age was greater by boys than by girls (2). The mean iron intake in NHANES II for individuals from 12 to 15 y of age was 6.66 mg/d for boys and 6.21 mg/d for girls.

In a study of absorption of nonheme iron from a simulated school lunch (3), we noted that erythrocyte incorporation of the extrinsic nonheme iron tag was somewhat greater by girls than by boys. The subjects were 16 children, eight boys and eight girls, ranging in age from 7.3 to 10.2 y. Each child consumed lunches containing a beef patty for 3 consecutive d at one time and lunches with a beef-soy patty for 3 consecutive d at another time. Each lunch was labeled with an iron isotope. Based on average values for the two series of lunches for each child, geometric mean erythrocyte incorporation of the extrinsic tag was 1.85% of the dose for girls and 1.14% of the dose for boys. The difference was not statistically significant (analysis of covariance with serum ferritin concentration as the covariate, $p = 0.13$), and it was not possible to exclude an order of treatment effect.

Because we could find no report concerning a prepubertal sex-related difference in iron absorption in human subjects or in experimental animals, we decided to explore the possibility that iron absorption was different in boys and girls at the stage of sexual development before testicular enlargement was evident in boys or breast buds were detectable in girls.

MATERIALS AND METHODS

Two studies were carried out, study A from October 9, 1987 to January 28, 1988, and study B from October 27, 1989 to March 2, 1990. The protocols were reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects. Informed consent was obtained from at least one parent of each child and from the child.

The children were recruited through newspaper advertisements and personal contacts. All children were healthy. In each instance, height and weight were between the 5th and 95th centile for age of the NHANES II reference data (4). The children were admitted to the General Clinical Research Center of the University of Iowa in sex-specific cohorts of approximately five children. Subjects were admitted by 1600 h on Friday and discharged at approximately 1100 h on Saturday. After admission on Friday, a complete physical examination, including evaluation of sexual maturity (5), was performed, and a sample of venous blood (7 mL) was obtained. A second blood sample of similar size was obtained 13 d later.

Anthropometry and Tanner Staging. Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer. Weight was measured to the nearest 100 g on a calibrated scale.

Girls were identified as prepubertal (Tanner stage 1) if there

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was no evidence of breast bud. Boys were identified as prepubertal (Tanner stage 1) if examination of genitalia revealed testicular volume less than 4 cm³, as determined by measurement with a Prader orchimeter (6).

Study A—Meals and Stable Isotope Administration. Meals. Dinner consisted of turkey nuggets, french fried potatoes, corn, sliced peaches, and 2% fat milk. An evening snack consisted of potato chips and 7Up. Breakfast consisted of toaster waffles, applesauce, and hot chocolate with marshmallow. Foods other than the standard meals were not permitted. There was no restriction of water intake.

Isotope administration. Metallic iron powder (84.58 wt % ⁵⁸Fe) obtained from Oak Ridge National Laboratory (Oak Ridge, TN) was used to prepare an aqueous solution of ferrous sulfate (7), providing 1 mg of iron per mL. The ferrous sulfate solution was sealed in multiple-dose ampules under nitrogen and stored in the dark until used.

At 0700 on Saturday morning, each child received a precisely weighed quantity of the ⁵⁸Fe-enriched ferrous sulfate solution, providing approximately 2.0 mg of iron and 1.7 mg of ⁵⁸Fe. The ⁵⁸Fe-enriched ferrous sulfate solution (about 2 g) was drawn up into a tared syringe, and the weight of the syringe and solution was recorded. The solution was dispensed into 60 mL of orange-flavored Kool-Aid without added vitamin C; the syringe was rinsed twice, each time with 2 mL of distilled water; the rinsings were added to the Kool-Aid; and the Kool-Aid was consumed. An additional 60 mL of Kool-Aid with 70 mg of vitamin C was added to the glass, mixed by swirling, and consumed. At 0900 h, the previously described breakfast was eaten. At 1100 h, the children were discharged from the General Clinical Research Center.

Study B—Meals, Stable Isotope Administration. Meals. Dinner consisted of turkey nuggets, mashed potatoes, corn, a cupcake, and 2% fat milk. An evening snack consisted of potato chips, a peanut butter cookie, and 7Up. Breakfast consisted of white bread toast (25 g), grape jelly (14 g), reconstituted frozen orange juice (240 mL), and a lean ground beef patty (60 g). Foods other than the standard meals were not permitted. There was no restriction of water intake.

Isotope administration. ⁵⁸Fe-enriched ferrous sulfate was prepared as described for study A, except that the metallic iron powder obtained from Oak Ridge was 60.28 wt % ⁵⁸Fe.

At 0800 h on Saturday morning, each child received with breakfast a precisely weighed quantity (approximately 1.25 g) of ⁵⁸Fe-enriched ferrous sulfate solution providing approximately 1.9 mg of iron and 1.1 mg of ⁵⁸Fe. The solution was dispensed into 120 mL of orange juice, and the syringe was rinsed twice, each time with 2 mL of distilled water. Rinsings were added to the orange juice, which was then consumed. An additional 120 mL of orange juice were then added to the glass, mixed by swirling within the glass, and consumed.

Analyses of Blood. Hb concentration was determined by the cyanmethemoglobin method (catalog no. 368555; Boehringer Mannheim Diagnostics, Indianapolis, IN), and serum ferritin concentration was determined by RIA using the Micromedex Ferritin RIA kit (catalog no. D-4401; Micromedex Systems, Inc., Horsham, PA). MIR_{58/57} was determined by inductively coupled plasma mass spectrometry, using the Elan 250 ICP/MS System (Sciex Inc., Thornhill, Canada) operated in the isotope ratio mode. The method has been described in detail (7).

Calculation of Quantity of Administered ⁵⁸Fe Incorporated into Erythrocytes. MIR₀ and MIR_{58/57} at a specified time after isotope administration are used in the following equation to determine the quantity of administered ⁵⁸Fe incorporated into erythrocytes:

$${}^{58}\text{Fe}_{\text{inc}} = \frac{(\text{MIR}_t - \text{MIR}_0) \times \text{Fe}_{\text{circ}} \times 0.02183}{1 + (\text{MIR}_t - \text{MIR}_0) \times 0.02183}$$

where ⁵⁸Fe_{inc} is the quantity of the ⁵⁸Fe label incorporated into erythrocytes, expressed in mg, MIR_t is the determined MIR_{58/57} at time t after dosing, Fe_{circ} is the calculated quantity of total

circulating iron (mg) at time t, and 0.02183 is the natural abundance (wt fraction) of ⁵⁷Fe. The quantity of circulating iron (Fe_{circ}) was estimated as follows:

$$\text{Fe}_{\text{circ}} = \text{BV} \times \text{Hb} \times 3.47,$$

where Hb is Hb concentration in g/100 mL, 3.47 is the concentration of iron in Hb (mg/g), and BV is blood volume in mL calculated for each subject from weight in kg and height in cm with the regression equations of Linderkamp *et al.* (8):

$$\text{Boys: } \log \text{BV} = 0.6459 \log \text{wt} + 0.002743 \text{ ht} + 2.0324$$

$$\text{Girls: } \log \text{BV} = 0.6412 \log \text{wt} + 0.001270 \text{ ht} + 2.2169$$

Statistical Analysis. Because the data are normally distributed, arithmetic means are presented for serum ferritin concentrations and percentage incorporation of the ⁵⁸Fe label into erythrocytes. Geometric means are also included to facilitate comparisons with data in the literature.

Percentage incorporation of the ⁵⁸Fe label by males and females was compared by analysis of covariance with serum ferritin concentration as the covariate.

RESULTS

Study A. Fifteen boys and 16 girls were enrolled. One girl vomited soon after consuming the dose of iron and was therefore eliminated from the cohort. Thus, 15 boys and 15 girls completed the study.

Data on age, height, weight, Hb concentration, serum ferritin concentration, MIR₀, and MIR_{58/57} on the 13th d after receiving the ⁵⁸Fe-enriched ferrous sulfate are presented in Table 1. The difference in mean age of the boys (118 mo) and girls (108 mo) was not significant (*t* test, *p* = 0.102). The values for Hb and serum ferritin listed in Table 1 are the averages of two determinations—one at baseline and one 13 d after administration of the ⁵⁸Fe-enriched ferrous sulfate. Mean Hb concentration was 13.5 g/dL for boys, and 13.4 g/dL for girls. Mean serum ferritin concentration was 41.2 ng/mL for boys, and 39.5 ng/mL for girls. The differences in these values for boys and girls were not significant.

Five of the children (subjects A4, A9, A11, A23, and A27 in Table 1) had received meals labeled with ⁵⁸Fe during the summer of 1986 (June 24 through September 1) in a prior study (3). Four of these children (the exception was subject A27) had elevated MIR₀. Such elevated baseline values were to be anticipated. In adult subjects, at least 1 y is required for an iron label that has been incorporated into erythrocytes to become equilibrated between erythrocytes and storage sites (9).

The regression of percent of the administered dose of ⁵⁸Fe incorporated into erythrocytes on serum ferritin concentration was statistically significant for boys (*r* = −0.626, *p* = 0.013), but not for girls (*r* = −0.247, *p* = 0.37). The corresponding regressions on the logarithm of serum ferritin concentration gave similar results (*r* = −0.653, *p* = 0.008 for boys; *r* = −0.256, *p* = 0.36 for girls).

The geometric mean value for percent of administered ⁵⁸Fe label incorporated into erythrocytes was 35.2 for boys and 45.0 for girls. The difference was statistically significant (analysis of covariance with serum ferritin as covariate, *p* = 0.035).

Study B. Fifteen boys and 15 girls participated in the study. Data on age, height, weight, Hb concentration, serum ferritin concentration, and MIR₀, and MIR_{58/57} on the 13th d after receiving the ⁵⁸Fe-enriched ferrous sulfate are presented in Table 2. As in Table 1, values for Hb and serum ferritin are the averages of the two determinations. Mean Hb concentration was 13.8 g/dL for boys and 13.6 g/dL for girls. Mean serum ferritin concentration was 50.2 ng/mL for boys and 47.6 ng/mL for girls. The differences in these values for boys and girls were not significant.

Contrary to the findings in study A, the difference in mean age of the boys (114 mo) and girls (102 mo) was significant (*t* test, *p* = 0.002).

Table 1. Study A: erythrocyte incorporation of ^{58}Fe from reference dose of iron

Subject	Age (mo)	Weight (kg)	Height (cm)	Hb (g/dL)	Ferritin (ng/ mL)	MIR _{58/57}		⁵⁸ Fe incorporation	
						MIR ₀	MIR ₁₃ *	mg	% of dose
Males									
A1	127	30.3	143.0	13.1	52	0.1478	0.1806	0.795	44.6
A2	93	23.1	125.0	13.6	42	0.1475	0.1928	0.841	47.4
A3	118	29.3	134.0	13.6	46	0.1478	0.1759	0.663	38.7
A4	137	30.3	139.0	14.4	24	0.1503	0.1832	0.865	50.4
A5	139	38.2	145.0	13.2	30	0.1474	0.1653	0.514	29.4
A6	132	35.0	137.0	12.8	32	0.1477	0.1636	0.401	24.5
A7	121	33.6	146.0	13.4	25	0.1474	0.1810	0.927	54.2
A8	108	36.4	142.5	13.6	23	0.1476	0.1818	1.000	59.3
A9	106	30.6	135.0	13.1	43	0.1512	0.1740	0.545	31.5
A10	108	25.7	128.2	13.8	80	0.1474	0.1612	0.280	16.7
A11	124	36.5	139.6	13.9	46	0.1506	0.1704	0.550	31.8
A12	105	50.4	137.7	13.5	47	0.1473	0.1637	0.530	32.1
A13	103	40.1	134.7	13.4	50	0.1470	0.1651	0.491	28.4
A14	123	38.2	137.3	13.8	38	0.1478	0.1710	0.640	38.5
A15	123	31.4	146.7	13.6	38	0.1472	0.1664	0.484	28.2
Mean	118	33.9	138.0	13.5	41			0.635	37.0
SD	13	6.6	6.2	0.4	14			0.208	12.0
Geometric mean					39				35.2
Females									
A16	109	38.4	142.5	14.0	39	0.1475	0.1782	0.831	50.3
A17	114	48.8	144.0	13.9	60	0.1477	0.1708	0.754	42.5
A18	91	20.3	124.0	12.5	45	0.1472	0.2088	0.958	55.6
A19	104	36.1	134.5	13.4	43	0.1468	0.1895	1.070	61.8
A20	93	29.0	125.7	12.9	52	0.1478	0.1684	0.418	24.7
A21	91	27.2	128.3	12.0	30	0.1475	0.1972	0.885	53.2
A22	88	26.7	128.3	13.4	28	0.1478	0.2096	1.243	74.2
A23	122	43.5	143.7	14.6	60	0.1529	0.1796	0.815	48.1
A24	133	52.3	148.8	14.9	27	0.1474	0.1680	0.763	44.9
A25	130	39.6	145.4	12.9	36	0.1468	0.1670	0.530	30.8
A26	129	29.5	134.5	14.4	32	0.1476	0.1870	0.925	55.0
A27	130	49.9	154.0	13.7	28	0.1481	0.1672	0.647	37.4
A28	86	28.0	125.7	12.9	30	0.1473	0.1811	0.660	40.0
A29	95	27.8	134.3	13.8	38	0.1474	0.1846	0.817	48.8
A30	109	29.5	143.5	12.4	45	0.1475	0.1757	0.563	32.5
Mean	108	35.1	137.1	13.4	40			0.792	46.7
SD	17	9.8	9.5	0.8	11			0.214	12.8
Geometric mean					38				45.0

* MIR₁₃, MIR_{58/57} on the 13th d after receiving the ^{58}Fe -enriched ferrous sulfate.

Four of the children in study B (subjects B3, B4, B16, and B18) had participated 22 to 25 mo earlier in study A (subjects A2, A10, A22, and A18, respectively). Subjects B1, B4, B16, B26, and B27 had been given ^{58}Fe in a study (unpublished) carried out between study A and study B. All of these subjects except B1 had elevated values for MIR₀.

The regression of percent of the administered dose of ^{58}Fe incorporated into erythrocytes on serum ferritin concentration was significant both for boys ($r = -0.545$, $p = 0.035$) and girls ($r = -0.676$, $p = 0.006$). The corresponding regressions on the logarithm of serum ferritin concentration gave similar results ($r = -0.657$, $p = 0.008$ for boys; $r = -0.660$, $p = 0.007$ for girls).

The geometric mean value for percent of administered ^{58}Fe label incorporated into erythrocytes was 14.8 for boys and 24.7 for girls. The difference was statistically significant (analysis of covariance with serum ferritin as covariate, $p = 0.004$).

DISCUSSION

Iron exists within the body in two major categories: functional (in Hb, myoglobin, and iron-containing enzymes) and storage (in ferritin and hemosiderin) (10). The quantity of storage iron in adult subjects is indicated both by the serum ferritin concentration and the extent of absorption of iron from an orally administered reference dose of an inorganic iron salt (11). The

relationships established for adults are useful in interpreting our findings in preadolescent children.

The geometric mean serum ferritin concentration (ng/mL) in study A was 39 for boys and 38 for girls and, in study B, 48 for boys and 45 for girls. In adult subjects, when serum ferritin concentrations are >12 ng/mL, serum ferritin of 1 ng/mL reflects storage iron of 10 ng (12). Thus, the geometric mean serum ferritin concentrations of 34 ng/mL in premenopausal women and 94 ng/mL in men (11) reflect iron stores of 340 and 940 mg, respectively.

Assuming that serum ferritin concentration reflects iron stores per unit of fat-free body mass, it seems likely that, per unit of fat-free body mass, iron stores of the preadolescent boys and girls in our studies were somewhat greater than those of premenopausal women, but substantially less than those of adult males.

Several investigators have reported on the absorption of iron by nonanemic fasting adults given a reference dose of iron. Generally, 3 mg of iron in the form of ferrous sulfate or ferrous ascorbate and about 30 mg of ascorbic acid are given to fasting subjects and the extent of iron absorption is estimated from the radioactivity of blood obtained 2 wk after administration of the radioactively tagged reference dose. In calculating percentage absorption, it is assumed that 80 (11), 90 (13), or 100% (14, 15) of the absorbed iron has been incorporated into erythrocytes during the 2 wk after consumption of the reference dose.

Table 2. Study B: erythrocyte incorporation of ^{58}Fe fed with breakfast

Subject	Age (mo)	Weight (kg)	Height (cm)	Hb (g/dL)	Ferritin (ng/ mL)	MIR _{58/57}		⁵⁸ Fe incorporation	
						MIR ₀	MIR ₁₃ *	mg	% of dose
Males									
B1	111	25.7	131.4	12.3	57	0.1480	0.1534	0.101	8.8
B2	100	27.7	132.0	13.6	40	0.1463	0.1575	0.244	21.3
B3	118	28.3	134.5	14.9	64	0.1708	0.1795	0.215	18.8
B4	130	30.3	137.3	14.2	76	0.1591	0.1662	0.177	15.7
B5	105	24.3	128.2	13.0	43	0.1457	0.1507	0.094	8.4
B6	105	27.8	123.9	13.6	81	0.1464	0.1519	0.114	10.1
B7	121	33.2	144.8	14.5	50	0.1467	0.1562	0.269	23.8
B8	120	40.2	139.7	15.5	44	0.1467	0.1534	0.223	19.6
B9	119	36.0	135.9	13.5	46	0.1466	0.1529	0.166	14.7
B10	105	29.7	129.5	13.8	34	0.1463	0.1554	0.207	18.3
B11	131	36.8	143.5	14.6	61	0.1467	0.1507	0.121	10.7
B12	122	29.7	129.5	13.6	52	0.1460	0.1541	0.182	16.2
B13	112	31.9	139.7	14.2	22	0.1463	0.1643	0.472	42.2
B14	102	36.4	132.1	12.6	36	0.1467	0.1560	0.224	19.9
B15	110	32.4	132.8	13.1	48	0.1479	0.1499	0.047	4.1
Mean	114	31.4	134.3	13.8	50			0.190	16.8
SD	10	4.5	5.8	0.9	16			0.100	8.9
Geometric mean					48				14.8
Females									
B16	115	33.2	139.2	12.2	34	0.1786	0.1939	0.331	29.1
B17	98	27.7	125.9	14.2	64	0.1460	0.1561	0.217	18.9
B18	116	25.8	139.0	13.5	58	0.1811	0.1913	0.207	18.4
B19	114	28.9	138.0	13.9	66	0.1457	0.1554	0.219	19.2
B20	101	22.8	124.2	14.3	30	0.1451	0.1672	0.422	37.4
B21	86	25.4	123.7	14.8	63	0.1460	0.1581	0.254	22.6
B22	103	25.4	123.2	13.8	34	0.1466	0.1742	0.540	48.5
B23	100	28.8	129.0	14.2	48	0.1468	0.1547	0.177	15.6
B24	94	25.4	122.0	12.9	40	0.1459	0.1636	0.324	29.2
B25	91	21.5	115.6	12.5	80	0.1462	0.1543	0.127	11.3
B26	106	37.2	135.9	14.2	47	0.1486	0.1633	0.394	35.2
B27	106	35.8	135.9	13.8	42	0.1492	0.1636	0.367	32.8
B28	92	29.7	123.2	12.2	32	0.1468	0.1606	0.264	23.2
B29	92	24.8	127.0	13.4	44	0.1461	0.1638	0.336	29.8
B30	115	29.9	134.0	14.4	32	0.1465	0.1575	0.259	23.0
Mean	102	28.2	129.1	13.6	48			0.296	26.3
SD	10	4.5	7.4	0.8	15			0.107	9.6
Geometric mean					45				24.7

* MIR₁₃, MIR_{58/57} on the 13th d after receiving the ^{58}Fe -enriched ferrous sulfate.

Cook *et al.* (11) determined absorption of a reference dose of iron by 30 women 18 to 35 y of age. The subjects were in satisfactory iron nutritional status as judged by hematocrit greater than 36 and transferrin saturation greater than 20%. Geometric mean serum ferritin was 27 ng/mL. Geometric mean absorption of iron from the reference dose was reported as 28%—corresponding to erythrocyte incorporation of 22%. In most studies of erythrocyte incorporation of iron from a reference dose, some of the subjects were iron deficient or were frequent blood donors (13–16). In these studies, geometric mean absorption of the test dose of iron has often been greater than 40% of the dose.

It therefore appears that the preadolescent children who participated in study A absorbed a greater percentage of a reference dose of iron than would have been anticipated with adults of similar iron nutritional status. The greater absorption by the preadolescent children may be explained in part by the larger dose of ascorbic acid included with the reference dose of iron (the children received 70 mg; the adults about 30 mg).

In studies of adults, a substantially greater percentage of a radioiron tag is incorporated into erythrocytes when the tag is administered in the form of a reference dose (*i.e.*, without food) than when the tag is administered with a meal (13–16). The lesser erythrocyte incorporation of iron when iron is administered with food presumably reflects the inhibitory effect of food

on iron absorption. Thus, the greater percentage incorporation of ^{58}Fe in study A than in study B was to be anticipated.

Studies of adults fed meals containing enhancers of iron absorption (14, 17, 18) suggest that adult subjects generally absorb a lesser percentage of the iron from such meals than we observed in study B. The difference in iron absorption between study B and the studies of adults may be explained by the greater bioavailability of iron in the meal fed in study B, which included two major enhancers of iron absorption (meat and orange juice) and no major inhibitors of iron absorption.

Our findings both in study A and in study B suggest that at Tanner stage 1 girls absorb iron more readily than do boys. Because serum ferritin concentrations were similar in boys and girls, the difference in absorption does not appear to be a consequence of smaller iron stores in the girls. We hypothesize that hormonal differences between boys and girls in Tanner stage 1 favor iron absorption by girls.

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