

Iron Absorption during Recovery from Malnutrition

Edgar Vasquez Garibay, MD, Irene Santos Torres, MD, Steven E. Nelson, BA, Ekhard E. Ziegler, MD, FACN, Ronald R. Rogers, BS, Morteza Janghorbani, PhD, and Samuel J. Fomon, MD

Instituto de Nutricion Humana, Hospital Civil "Dr. Juan I. Menchaca," Universidad de Guadalajara, Guadalajara, Jalisco, MEXICO (E.V.G., I.S.T.), Department of Pediatrics University of Iowa, Iowa City, Iowa (S.E.N., E.E.Z., R.R.R., S.J.F.), BioChemAnalysis Corporation, Chicago, Illinois (M.J.)

Key words: iron absorption, malnutrition, iron status

Objective: In infants and children recovering from severe malnutrition, iron deficiency is common, and the ability to absorb iron during such recovery is uncertain. The objective of this study was to determine iron absorption during recovery from malnutrition.

Methods: During the later stages of recovery from malnutrition, erythrocyte incorporation of orally administered ^{58}Fe was determined as a surrogate for iron absorption. Based on four indices, subjects were classified as iron-sufficient, iron-deficient or indeterminate.

Results: Of the 25 subjects, 9 were classified as iron sufficient, 5 as indeterminate and 11 as iron deficient; all but 5 had evidence of inflammation or infection. Geometric mean erythrocyte incorporation of ^{58}Fe was 32.0% of the dose in the iron-deficient subjects, which was not significantly different ($p = 0.073$) than the 13.1% in the iron-sufficient subjects. Incorporation of ^{58}Fe by the iron-sufficient subjects did not differ significantly from that by normal subjects in the same age range. Surprisingly, we found no correlation of erythrocyte incorporation of ^{58}Fe and reticulocyte count.

Conclusions: Even in the presence of infection or inflammation, iron absorption by children during a late stage of recovery from malnutrition is not impaired.

INTRODUCTION

Iron deficiency with or without anemia is common in infants and children during recovery from severe malnutrition. As a cause of the iron deficiency, Massa *et al.* [1] suggested that a transitory abnormality in iron absorption may be present in infantile malnutrition, a suggestion based on the increase in ^{59}Fe in serum after oral administration of the isotope. However, iron absorption has been found to be unaffected in protein depleted rats [2], and an earlier study of iron absorption by iron-deficient malnourished subjects had failed to provide evidence of decreased iron absorption [3].

Our study was undertaken to provide further data on absorption of iron by malnourished subjects. It was a collaborative effort between a group of investigators working with malnourished infants and small children in Guadalajara, Mexico, and colleagues in Iowa City, Iowa, involved in studies of

erythrocyte incorporation of ^{58}Fe by normal infants and children. In normal and iron-deficient adults, 80% to 90% of the absorbed isotope is promptly incorporated into erythrocytes [4,5]. The data of Lynch *et al.* [3] indicated that in malnourished, iron-deficient infants and small children a high percentage of absorbed iron is also promptly incorporated into erythrocytes. Therefore, we used erythrocyte incorporation of ingested ^{58}Fe as a surrogate for iron absorption in young subjects recovering from malnutrition.

MATERIALS AND METHODS

Study Design

Subjects less than three years of age with birth weights of 2.5 kg or more and z-scores for body weight for age less than -1.8 were considered candidates for study. With few exceptions, they were studied after initial treatment for gastroenteritis

Address reprint requests to: Samuel J. Fomon, MD, Department of Pediatrics, University of Iowa Hospitals and Clinics, 200 Hawkins Drive, Iowa City, Iowa 52242. E-mail: samfomon@aol.com.

or bronchopneumonia. Each iron absorption study consisted of oral administration of an accurately known amount of ^{58}Fe . Blood was obtained by heel stick just before and 14 days after ^{58}Fe administration, and, from the ^{58}Fe enrichment of the erythrocytes, the extent of erythrocyte incorporation of the administered isotope was determined and expressed as percent of the ^{58}Fe dose. Data on erythrocyte incorporation of iron were compared with data from normal infants and children and were examined in relation to anthropometric indices, iron nutritional status and evidence of inflammation. Indicators of iron nutritional status were determined on both blood specimens, and the average of the two values was used for classification of iron nutritional status.

Subjects

A cohort of 25 subjects was studied. With the exception of two subjects at or near three years of age (Subjects 1 and 19), subjects ranged in age from 6 to 27 months at the time of isotope administration (Table 1). All of the subjects were believed by the parents to have been born at term, and birth weights reported by the parents for 18 of the 25 subjects ranged from 2.5 kg to 4.0 kg. Subjects 5, 7 and 14 had previously been treated in the hospital, were being followed as outpatients and were admitted specifically for the ^{58}Fe study. Subject 15 was referred by another department, was not acutely ill and was admitted for the ^{58}Fe study. The other subjects were studied after 9 to 76 days of hospitalization (only one subject more than 55 days). At the time of admission and subsequently when studied for erythrocyte incorporation (see RESULTS), the subjects were underweight and stunted as indicated by z-scores for weight and length. Z-scores of weight-for-length were also low.

Most of the subjects were iron-deficient and many were anemic at the time of admission and at the time of ^{58}Fe administration. Five of the subjects received one or two transfusions of packed erythrocytes (Table 1), generally 40 mL per transfusion, administered soon after admission (16 to 129 days before the ^{58}Fe study; one subject was transfused during an earlier admission to the hospital) and 15 of the subjects were given oral iron therapy (29 mg/day of elemental iron in the form of ferrous sulfate) for intervals of a few days to several weeks. Iron therapy was discontinued at least three days before administration of ^{58}Fe , except one day for subject 16.

Classification with Respect to Iron Nutritional Status

Despite other evidence indicating the presence of iron deficiency in quite a number of the subjects, the great majority of plasma ferritin concentrations were in or above the range encountered in normal subjects of similar age. The highest values (11 of 48 plasma ferritin values were more than 100 $\mu\text{g/L}$) were presumably a reflection of inflammation, except for Subject 6, who was six months old, had received a transfusion of packed erythrocytes and oral iron treatment. All but 9 of the

49 ESR values were greater than 13 mm/hour, the value considered to be the upper limit of normal [6]. ESR values were not significantly correlated with indices of iron nutritional status.

Because evidence of inflammation was present in so many of the subjects and because inflammation is associated with increase in plasma ferritin concentration, we classified each subject with respect to iron nutritional status on the basis of four relevant indices of iron nutritional status. If none or only one of the indices was abnormal, the subject was classified as iron-sufficient; if two indices were abnormal, the subject was classified as indeterminate (i.e., of uncertain iron nutritional status), and if three or four of the indices were abnormal, the subject was classified as iron-deficient. All four indices were abnormal in seven of the eleven iron-deficient subjects.

The indices with their cutoff values were as follows:

- (1) Plasma ferritin concentration less than 12 $\mu\text{g/L}$ or less than 50 $\mu\text{g/L}$ if the ESR was more than 13 mm/hour. An upper cutoff value of 50 $\mu\text{g/L}$ in the presence of inflammation has been suggested by Galan *et al.* [7].
- (2) Erythrocyte protoporphyrin content greater than 80 $\mu\text{g/dL}$ of erythrocytes [8].
- (3) Iron saturation of transferrin less than 12% [8].
- (4) Hemoglobin concentration less than 110 g/L.

Refeeding Program

With the exceptions already noted (Subjects 5, 7, 14 and 15), the refeeding program was similar for all of the subjects. Feeding of a milk-based infant formula was begun immediately after admission to the hospital or, when considered necessary, after intravenously administered rehydration therapy. The energy density of a 67 kcal/dL formula was increased to 80 kcal/dL by the addition of corn syrup and fed by continuous infusion by an infusion pump and nasogastric tube. Feeding volumes were gradually increased so that by the 6th day of hospitalization an energy intake of 200 kcal \cdot kg $^{-1}$ \cdot d $^{-1}$ and a protein intake of 4 g \cdot kg $^{-1}$ \cdot d $^{-1}$ were achieved. Thereafter, the same formula (80 kcal/dL) was fed *ad libitum* by bottle. Infants older than 12 months of age were also fed hospital-prepared gruels made from fruits, vegetables and cereals.

Anthropometric Measurements

Body weight and recumbent length were measured by trained examiners using standard methods [9]. Age appropriate z-scores for weight, length and weight-for-length were calculated on the basis of NCHS reference data [10].

Administration of ^{58}Fe

Elemental iron enriched with ^{58}Fe (84.58 atom% ^{58}Fe) was obtained from Oak Ridge National Laboratory (Oak Ridge, TN) and was prepared in the form of ferrous sulfate as previously described [11]. A precisely weighed amount of ^{58}Fe solution was added to 5 mL of a 50 g/L glucose solution

Table 1. Subject Characteristics, Iron Status and Erythrocyte Incorporation¹

Subj #	Age ² (months)	Hosp ³ (days)	Gender	W ⁴ (kg)	L ⁵ (cm)	W	L	W-L	Trans ⁷	Rx ⁸	Hb ⁹ (g/L)	Ferr ¹⁰ (μg/L)	Fe ¹¹ (μmol/L)	Sat ¹² (%)	EP ¹³ (μg/dl RBC)	ESR ¹⁴ (mm/hr)	Retic ¹⁵ (%)	Incorp ¹⁶ (% of dose)	
									(z-scores) ⁶										
IRON-SUFFICIENT																			
4	23	58	M	7.4	67.0	-3.90	-6.05	-0.48	32	7	114	84	14.2	13.1	144	30	5.0	34.6	
5	9	0	M	7.4	64.0	-1.81	-3.19	0.84	129		138	43	16.3	21.4	63	14	2.1	29.1	
6	6	40	F	4.9	64.0	-2.59	-0.66	-2.56	38	6	121	245	11.8	17.8	52	9	3.1	0.8	
7	10	0	F	7.0	64.5	-2.08	-2.79	0.17		7	117	14	15.5	20.7	75	10	2.0	88.7	
11	7	20	M	6.9	64.0	-1.83	-2.53	0.16			111	370	7.0	12.0	97	17	2.5	1.9	
14	21	0	M	8.6	72.0	-2.77	-4.08	-0.69		154	113	36	13.6	15.5	83	8	1.1	41.6	
22	14	12	M	7.0	66.0	-3.39	-4.44	-0.50			104	215	12.5	19.6	58	25	1.4	11.6	
24	7	15	F	5.0	63.0	-3.30	-2.05	-2.13			118	220	8.3	12.1	128	42	2.6	7.6	
25	7	14	F	5.0	61.5	-3.08	-2.38	-1.49		3	117	169	11.6	19.0	83	33	2.1	22.6	
Mean				6.57	65.1	-2.75	-3.13	-0.74			116.8	155.1	12.3	16.8	86.9	20.6	2.4	26.5	
SD				1.31	3.0	0.74	1.56	1.12			9.3	119.4	3.1	3.7	31.4	12.1	1.1	27.5	
Geometric											103.0	11.9	16.4	82.3				13.1	
-1SD											34.3	9.0	13.0	58.2				2.8	
+1SD											309.3	15.8	20.7	116.3				60.4	
INDETERMINATE IRON STATUS																			
8	21	35	F	7.7	73.0	-3.11	-3.53	-1.73		5	111	18	8.8	8.8	127	12	1.6	58.6	
9	17	35	F	7.3	73.0	-2.96	-2.44	-2.20		3	118	42	7.9	9.7	68	48	1.8	47.5	
16	24	26	M	7.8	77.2	-3.63	-3.24	-3.01	16	1	103	110	15.0	20.7	94	27	2.5	90.5	
17	13	28	F	5.7	69.0	-3.92	-2.54	-3.07			133	46	17.5	17.4	93	31	1.4	22.8	
23	14	10	F	7.0	70.0	-2.78	-2.34	-1.73			96	68	17.1	24.9	247	29	4.9	31.9	
Mean				7.10	72.4	-3.28	-2.82	-2.35			112.0	56.8	13.2	16.3	125.7	29.1	2.4	50.3	
SD				0.85	3.2	0.48	0.53	0.66			14.2	34.9	4.6	7.0	71.0	12.8	1.4	26.4	
Geometric											48.0	12.6	15.0	113.1				44.9	
-1SD											24.3	8.6	9.4	69.3				26.3	
+1SD											94.7	18.3	23.9	184.5				76.7	
IRON-DEFICIENT																			
1	34	41	F	7.6	70.0	-4.41	-6.92	-0.98		6	74	6	3.2		287	16	3.1	20.5	
2	16	34	M	7.8	71.0	-2.55	-2.45	-1.27		4	114	36	5.1	5.7	97	20	4.2	42.8	
3	25	26	F	7.8	74.0	-3.45	-4.04	-1.86	23	8	94	39	5.7	5.1	113	29	4.4	50.6	
10	22	39	M	8.7	72.5	-2.72	-4.10	-0.62			106	18	4.8	4.9	157	35	1.0	71.6	
12	17	11	F	6.8	70.0	-3.31	-3.24	-1.98			85	17	5.5	6.4	108	16	1.1	36.9	
13	20	16	M	6.2	69.0	-4.55	-4.86	-2.72			108	44	6.5	9.4	92	23	1.2	45.8	
15	27	3	F	8.4	77.0	-3.27	-3.64	-1.92		3	81	14	5.2	6.4	204	15	1.5	43.8	
18	24	10	F	9.5	76.0	-2.06	-3.35	-0.41			102	24	6.0	5.7	87	35	1.8	49.2	
19	36	19	M	11.3	90.0	-2.03	-1.58	-0.69			110	22	5.6	5.9	92	24	1.5	25.6	
20	12	76	M	7.2	76.0	-2.98	-0.09	-3.57		43	109	26	7.2	8.0	174	19	0.8	21.0	
21	9	38	M	5.8	62.5	-3.55	-3.81	-0.85		7	109	39	9.3	16.0	109	22	0.9	5.8	
Mean				7.92	73.5	-3.21	-3.55	-1.62			99.0	26.0	5.8	7.4	138.1	22.8	1.9	37.6	
SD				1.55	6.9	0.81	1.72	0.94			13.5	12.3	1.5	3.3	62.6	7.2	1.3	18.2	
Geometric											22.8	5.6	6.9	128.0				32.0	
-1SD											12.7	4.3	4.8	86.6				16.1	
+1SD											41.1	7.4	9.8	189.0				63.6	

¹ Subject characteristics are given for Day 0 (day of ⁵⁸Fe administration); iron status indicators are average for Day 0 and Day 14 values.

² Age at the time of ⁵⁸Fe administration (Day 0).

³ Time from current date of admission to the hospital until date of ⁵⁸Fe administration.

⁴ W is body weight.

⁵ L is body length.

⁶ z-scores based on NCHS data (10).

⁷ Trans indicates that the subject received 1 or 2 transfusions of packed erythrocytes with the entries in the column indicating the number of days elapsing between transfusion and ⁵⁸Fe administration.

⁸ Rx indicates oral iron therapy with the entries in the column indicating the number of days between termination of therapy and ⁵⁸Fe administration.

⁹ Hb is hemoglobin concentration.

¹⁰ Ferr is plasma ferritin concentration.

¹¹ Fe is plasma iron concentration.

¹² Sat is iron saturation of transferrin.

¹³ EP is erythrocyte protoporphyrin content.

¹⁴ ESR is erythrocyte sedimentation rate.

¹⁵ Retic is reticulocyte count.

¹⁶ Incorp is erythrocyte incorporation of ⁵⁸Fe.

containing 10 mg of ascorbic acid, 1.1 mg of ^{58}Fe and 1.3 mg total iron. This solution was delivered directly into the back of the oral cavity by syringe in a small volume to decrease the likelihood of regurgitation. For the next hour, during which no feeding was given, the subjects were observed closely for the possibility of regurgitation.

Laboratory Methods

Using a disposable spring-loaded device (Tenderfoot, International Technidyne Corp., Edison, N.J.), blood samples were obtained by heel stick. Hemoglobin concentration was determined by the cyan-methemoglobin method (catalog number 368555 Boehringer Mannheim Diagnostics, Indianapolis, IN). Blood was analyzed for erythrocyte protoporphyrin by the method of Piomelli [12]. Plasma was analyzed for concentration of ferritin by radioimmunoassay using the Micromedic Ferritin RIA Kit (Micromedic Systems, Inc., Horsham, PA), for concentration of iron with the Ferrochem II Analyzer (ESA, Inc., Bedford, MA) and concentration of transferrin as described by Borum *et al.* [13]. Based on an atomic weight of iron of 56, molecular weight of transferrin of 74,000, and two iron-binding sites for transferrin, total iron binding capacity was calculated as transferrin concentration (μg) \times 0.00151, and iron saturation of transferrin (%) was calculated as plasma iron concentration (μg) divided by total iron-binding capacity \times 100. Erythrocyte sedimentation rate (ESR) was determined by a micromodification of the method of Wintrobe [14]. Blood smears stained with brilliant cresyl blue were counted for reticulocytes with the aid of a Miller Disc (American Optical Company, Buffalo, NY). Four slides were counted and each value therefore represented 2000 erythrocytes.

Calculations

The $^{58}\text{Fe}/^{57}\text{Fe}$ ratio in erythrocytes was determined by inductively coupled plasma mass spectrometry (ICP/MS) using the Elan 250 ICP/MS system as described by Janghorbani *et al.* [11]. As described previously [15–17], the quantity of administered ^{58}Fe incorporated into erythrocytes ($^{58}\text{Fe}^*_{\text{inc}}$) at a specified time t after administration of the dose was calculated as follows:

$$^{58}\text{Fe}^*_{\text{inc}} = \frac{\text{MIR}^t_{58/57} - \text{MIR}^0_{58/57}}{\text{MIR}^0_{58/57}} \times \text{Fe}_{\text{circ}} \times 0.00322$$

where $^{58}\text{Fe}^*_{\text{inc}}$ is expressed in mg, $\text{MIR}^t_{58/57}$ is the determined $^{58}\text{Fe}/^{57}\text{Fe}$ ratio at time t , $\text{MIR}^0_{58/57}$ is the determined baseline ratio, Fe_{circ} is the quantity of total circulating iron (mg) at time t and 0.00322 is the natural abundance (weight fraction) of ^{58}Fe . The quantity of total circulating iron was estimated as follows:

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

where BV is blood volume in liters (assumed to be 0.065 L/kg body weight), Hb is hemoglobin concentration in g/L and 3.47

is the concentration of iron in hemoglobin (mg/g). In the remainder of this communication the term “ ^{58}Fe ” will refer to the administered isotope.

Statistical Analysis

Unless specifically noted, values for plasma ferritin, plasma iron, percent saturation of transferrin, erythrocyte protoporphyrin content and erythrocyte incorporation of ^{58}Fe were transformed by natural logarithms before statistical analyses. Descriptive, associative, and comparative statistics were performed using SAS version 6.12 (SAS Institute, Cary NC). Linear relationships were determined by Pearson correlations and regression analyses. Comparisons of iron deficient *versus* iron sufficient and of malnourished *versus* normal subjects were analyzed using general linear models procedures with and without covariate adjustment for log ferritin. Least significant squares comparisons of least-square means were performed for variables with a significant F-test for grouping factor.

Ethical considerations

The study protocol was reviewed and approved by the institutional review board of the University of Guadalajara and by the University of Iowa Committee on Research Involving Human Subjects. The study procedures were explained to one or both parents and written consent was obtained.

RESULTS

Weight, length and weight-for-length (Table 1) indicated that the subjects were underweight and stunted (mean weight z-score -3.06 , SD 0.74; mean length z-score -3.25 , SD 1.48) and underweight for length (mean weight-for-length z-score -1.45 , SD 1.11). The severity of underweight did not appear to be age related, as indicated by the lack of significant correlation of the weight z-score and age, but the correlation for weight-for-length z-score and age was significant ($r = -0.49$, $p = 0.013$).

The entries in Table 1 for indices of iron status and for ESR are the averages of two determinations, one obtained at the time of ^{58}Fe administration and the other 14 days later. The two values were significantly correlated with correlation coefficients ranging from 0.55 to 0.92, with the exception of plasma iron, for which the correlation coefficient was not statistically significant ($r = 0.38$).

That iron deficiency and severity of anemia were inversely related to age is evident from the inverse correlation with age of hemoglobin ($r = -0.58$, $p = 0.002$), log plasma ferritin concentration ($r = -0.61$, $p = 0.001$) and log percent saturation of transferrin ($r = -0.58$, $p = 0.003$), and the positive relation with age of log erythrocyte protoporphyrin content ($r = 0.41$, $p = 0.04$) and log percent erythrocyte incorporation of ^{58}Fe ($r = 0.52$, $p = 0.008$).

Erythrocyte Incorporation of ⁵⁸Fe.

Erythrocyte incorporation of ⁵⁸Fe was significantly correlated ($r = -0.53$, $p = 0.007$) with plasma ferritin concentration (Fig. 1). Geometric mean erythrocyte incorporation of ⁵⁸Fe was 13.1% of the dose by the iron-sufficient subjects and 32.0% of the dose by the iron-deficient subjects. The difference was borderline significant ($p = 0.073$). We have no explanation for the extremely high value for erythrocyte incorporation of ⁵⁸Fe (88.7% of the dose) by iron-sufficient Subject 7. Three published reports concerning normal infants and young children were available for comparison with the data on erythrocyte incorporation of ⁵⁸Fe by the iron-sufficient malnourished subjects. Combined data from two studies (29 infants 165 to 215 days of age) reported by Fomon *et al.* [18,19] gave a geometric mean ⁵⁸Fe erythrocyte incorporation of 14.4% of the dose, a value that did not differ significantly from that of the iron-sufficient malnourished subjects ($p = 0.77$). Neither did the values for ten infants 12 to 15 months of age studied by Abrams *et al.* [20] (10.6% of intake) differ from those of the iron-sufficient subjects.

Reticulocyte Count

Surprisingly, the reticulocyte count was not correlated with any of the indices of iron nutritional status. The mean reticulocyte count of subjects classified as iron-sufficient (2.4%) did not differ significantly from that of subjects classified as iron-deficient (1.9%; $p = 0.40$). Log erythrocyte incorporation of ⁵⁸Fe for subjects with reticulocyte counts in the first quartile were greater than for those with reticulocyte counts in the

fourth quartile but, even for this comparison, the difference was not significant ($p = 0.55$).

DISCUSSION

We studied 25 underweight and stunted infants and young children, during recovery from malnutrition, most of them after initial treatment for gastroenteritis or bronchopneumonia, and none during the acute stage of malnutrition associated with circulatory disturbances [21]. Many were iron-deficient and, as judged by ESR values, all but four had evidence of infection or inflammation, conditions known to be associated with elevated values for plasma ferritin concentration and erythrocyte protoporphyrin content and for decrease in the iron saturation of transferrin [8].

The finding that a number of the plasma ferritin concentrations were quite high (11 of 48 determinations more than 100 $\mu\text{g/L}$) is perhaps not surprising because even in presumably normal subjects in the age range of the malnourished subjects, concentrations greater than 100 $\mu\text{g/L}$ are sometimes found [22–24], and six of the seven subjects with values greater than 100 $\mu\text{g/L}$ had evidence of infection or inflammation. The finding of lesser concordance in malnourished than in normal subjects with respect to the relation of plasma ferritin concentrations obtained in the same subject at an interval of 14 days suggests an instability of the values in the malnourished subjects. We do not know whether this instability is related to inflammation or to other factors.

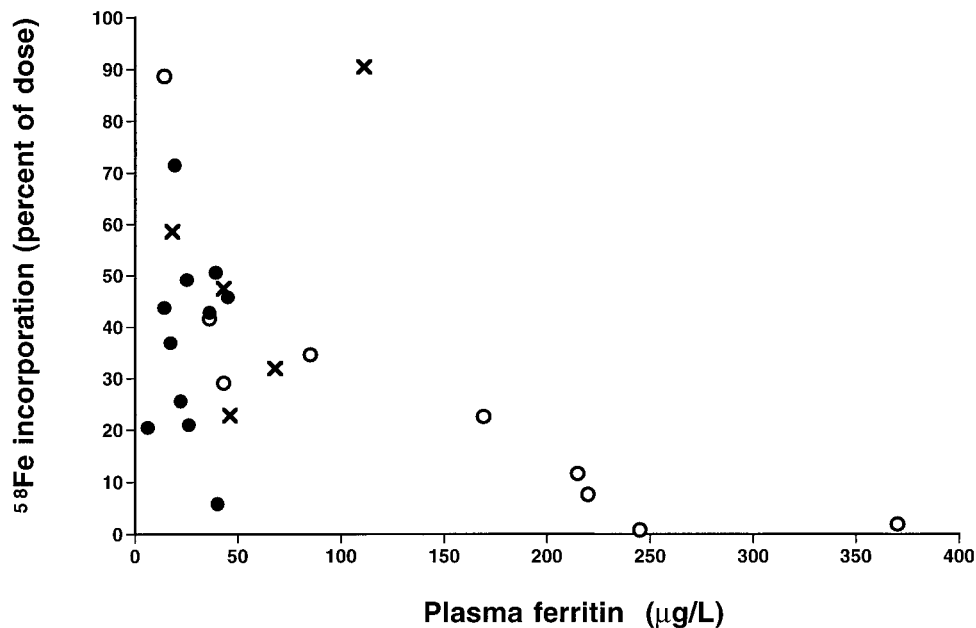


Fig. 1. Erythrocyte incorporation of ⁵⁸Fe in relation to plasma ferritin concentration of infants and children during recovery from malnutrition. Each symbol indicates the incorporation of ⁵⁸Fe as percent of the dose in one subject with the iron nutritional status of the subject as follows: ○ = iron-sufficient, × = indeterminate iron status, ● = iron-deficient.

That plasma ferritin concentrations were in the normal range for most of the iron-deficient malnourished subjects may not be entirely the effect of inflammation. Despite the high reliability of low concentrations of ferritin in plasma or serum as an indicator of iron deficiency, concentrations in the normal range in iron deficiency are not uncommon [7,8,25–27]. This perhaps explains the failure of Wickramasinghe *et al.* [28] to demonstrate a correlation between stainable iron in the marrow and plasma ferritin concentration.

Despite the general elevation of the plasma ferritin values, there was evidence that plasma ferritin concentration was related to iron nutritional status. Thus, erythrocyte incorporation of ^{58}Fe was inversely correlated with plasma ferritin concentration (Fig. 1) and log plasma ferritin was inversely correlated with log erythrocyte protoporphyrin and positively correlated with log percent saturation of transferrin.

It is recognized that in field studies, iron deficiency can best be identified by using several indices of iron-nutritional status [29,30], and we classified each subject with respect to iron nutritional status on the basis of four criteria (see RESULTS). Subjects were classified as iron-sufficient if none or only one criterion was abnormal, as of indeterminate iron nutritional status if two indices were abnormal and as iron-deficient if three or four indices were abnormal. Assuming that an elevated ESR value was an indication of current or recent infection or inflammation, we used a different cutoff value for plasma ferritin concentration in subjects with elevated ESR values than in subjects without elevated ESR values. We made no ESR-related adjustment in the cutoff values for hemoglobin concentration, transferrin saturation or erythrocyte protoporphyrin content because we are unaware of data on the quantitative nature of the effect of infection or inflammation on these indices. Nevertheless, by requiring that three or four of the four indices be abnormal for a subject to be classified as iron-deficient, we believed the identification of iron-deficient subjects to be strongly supported. Erythrocyte incorporation of ^{58}Fe was only slightly ($p=0.073$) greater by subjects we classified as iron-deficient than by those we classified as iron-sufficient. Lynch *et al.* [3] determined iron absorption by ^{59}Fe whole body counting in 10 subjects 6 to 20 months of age. The subjects were iron-deficient, as indicated by lack of stainable iron in the bone marrow, and were studied 34 to 76 days after initiation of treatment for kwashiorkor. Erythrocyte incorporation of ^{59}Fe was determined concurrently in six of the subjects. Geometric mean absorption was 35.2% of isotope intake and erythrocyte incorporation was 32.0% of intake. The value for erythrocyte incorporation of the iron isotope was identical to that in our study. The data of Lynch *et al.* [3] also indicate that, in malnourished iron-deficient subjects, in contradistinction to findings in normal infants [18], erythrocyte incorporation of iron accounts for a high percentage of absorbed iron, thus validating the use of erythrocyte incorporation as a surrogate for iron absorption in this group of subjects.

Our data and those of Lynch *et al.* [3] do not suggest that

iron absorption by iron-deficient subjects is impaired during recovery from malnutrition. In addition, erythrocyte incorporation of ^{58}Fe by iron-sufficient subjects recovering from malnutrition did not differ significantly from results with normal subjects in the same age range [18–20].

CONCLUSION

We conclude that at least for groups of subjects similar to those studied by Lynch *et al.* [3] and by us, iron absorption is unimpaired during recovery from severe malnutrition.

ACKNOWLEDGMENT

Supported in part by USPHS grant HD 07578 and by Programa de ayuda a la investigación de Nestlé Nutrition.

REFERENCES

1. Massa E, MacLean Jr WC, de Romana GL, de Martinez Y, Graham GG: Oral iron absorption in infantile protein-energy malnutrition. *J Pediatr* 93:1045–1049, 1978.
2. Beard JL, Huebers HA, Finch CA: Protein depletion and iron deficiency in rats. *J Nutr* 114:1396–1401, 1984.
3. Lynch SR, Becker D, Seftel H, Bothwell TH, Stevens K, Metz J: Iron absorption in kwashiorkor. *Am J Clin Nutr* 23:792–797, 1970.
4. Larsen L, Milman N: Normal iron absorption determined by means of whole body counting and red cell incorporation of ^{59}Fe . *Acta Med Scand* 198:271–274, 1975.
5. Heinrich HC, Fischer R: Correlation of postabsorptive serum iron increase and erythrocyte- ^{59}Fe -incorporation with the whole body retention of absorbed ^{59}Fe . *Klin Wochenschr* 60:1493–1496, 1982.
6. Behrman RE, Kliegman RM, Arvin AM (eds): “Nelson Textbook of Pediatrics,” 15th ed. Philadelphia, WB Saunders Company, p 1042, 1996.
7. Galan P, Etard JF, Borel E, Debenaze C, Hercberg S: Relationship between serum ferritin, erythrocyte protoporphyrin and transferrin saturation in Mauritanian free living children. *Internat J Vit Nutr Res* 59:214–218, 1989.
8. Dallman PR, Yip R, Oski FA: Iron deficiency and related nutritional anemias. In Nathan DG and Oski FA (eds): “Hematology of Infancy and Childhood,” 4th ed. Philadelphia: WB Saunders Company, pp 413–450, 1993.
9. Fomon SJ, Nelson SE: Size and growth. In Fomon SJ: “Nutrition of Normal Infants.” St. Louis: Mosby-Year Book, pp 36–84, 1993.
10. Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM: Physical growth: National Center of Health Statistics percentiles. *Am J Clin Nutr* 32:607–629, 1979.
11. Janghorbani M, Ting BTG, Fomon SJ: Erythrocyte incorporation of ingested stable isotope of iron (^{58}Fe). *Am J Hematol* 21:277–288, 1986.
12. Piomelli S: A micromethod for free erythrocyte porphyrins: the FEP test. *J Lab Clin Med* 81:932–940, 1973.

13. Borum PR, Bennett SG: A simple and inexpensive method for plasma transferrin determination. *Nutr Res* 5:937–940, 1985.
14. Wintrobe MM, Landsberg JW: A standardized technique for the blood sedimentation test. *Am J Med Sci* 189:102, 1935.
15. Fomon SJ, Janghorbani M, Ting BTG, Ziegler EE, Rogers RR, Nelson SE, Ostedgaard LS, Edwards BB: Erythrocyte incorporation of ingested ⁵⁸Fe by infants. *Pediatr Res* 24:20–24, 1988.
16. Fomon SJ, Ziegler EE, Rogers RR, Nelson SE, Edwards BB, Guy DG, Erve JC, Janghorbani M: Iron absorption from infant foods. *Pediatr Res* 26:250–254, 1989.
17. Fomon SJ, Ziegler EE, Nelson SE: Erythrocyte incorporation of ingested ⁵⁸Fe by 56-day-old breast-fed and formula-fed infants. *Pediatr Res* 33:573–576, 1993.
18. Fomon SJ, Ziegler EE, Serfass RE, Nelson SE, Rogers RR, Frantz JA: Less than 80% of absorbed iron is promptly incorporated into erythrocytes of infants. *J Nutr* 130:45–52, 2000.
19. Fomon SJ, Serfass RE, Nelson SE, Rogers RR, Frantz JA: Time course of and effect of dietary iron level on iron incorporation into erythrocytes by infants. *J Nutr* 130:541–545, 2000.
20. Abrams SA, O'Brien KO, Wen J, Liang LK, Stuff JE: Absorption by 1-year-old children of an iron supplement given with cow's milk or juice. *Pediatr Res* 39:171–175, 1996.
21. Viart P: Hemodynamic changes in severe protein-calorie malnutrition. *Am J Clin Nutr* 30:334–348, 1977.
22. Saarinen UM, Siimes MA: Serum ferritin in assessment of iron nutrition in healthy infants. *Acta Paediatr Scand* 67:745–751, 1978.
23. Sherriff A, Edmond A, Hawkins N, Golding J, the ALSPAC Children in Focus Study Team: Haemoglobin and ferritin concentrations in children aged 12 and 18 months. *Arch Dis Child* 80:153–157, 1999.
24. Edmond AM, Hawkins N, Pennock C, Golding J, the ALSPAC Children in Focus Team: Haemoglobin and ferritin concentrations in infants at 8 months of age. *Arch Dis Child* 74:36–39, 1996.
25. Dallman PR, Reeves JD, Driggers DA, Lo EYT: Diagnosis of iron deficiency: the limitations of laboratory tests in predicting response to iron treatment in 1-year-old infants. *J Pediatr* 98:376–381, 1981.
26. Herschko C, Bar-Or D, Gaziel Y, Naparstek E, Konijn AM, Grossowicz N, Kaufman N, Izak G: Diagnosis of iron deficiency anemia in a rural population of children. Relative usefulness of serum ferritin, red cell protoporphyrin, red cell indices, and transferrin saturation determinations. *Am J Clin Nutr* 39:1600–1610, 1981.
27. Madanat F, El-Khateeb M, Tarawaneh M, Hijazi S: Serum ferritin in evaluation of iron status in children. *Acta Haematol* 71:111–115, 1984.
28. Wickramasinghe SN, Gill DS, Broom GN, Akinyanju OO, Grange A: Limited value of serum ferritin in evaluating iron status in children with protein-energy malnutrition. *Scand J Haematol* 35:292–298, 1985.
29. Pilch SM, Senti FR (eds): "Assessment of the Iron Nutritional Status of the US Population Based on Data Collected in the Second National Health and Nutrition Examination Survey, 1976–1980." Bethesda, MD: Life Sciences Research Office, FASEB, 1984.
30. Baynes RD, Bothwell TH: Iron deficiency. *Annu Rev Nutr* 10:133–148, 1990.

Received January 23, 2001; revision accepted March 26, 2001.