

## Iron absorption from the whole diet in men: how effective is the regulation of iron absorption?<sup>1-3</sup>

Leif Hallberg, Lena Hultén, and Elizabet Gramatkovski

See corresponding editorial on page 419.

**ABSTRACT** Iron absorption from the whole diet, which contained a highly bioavailable form of iron, was measured for 5 d in 31 healthy men, including 12 blood donors. Nonheme iron in all meals was labeled with an extrinsic, inorganic radioiron tracer added in amounts to ensure uniform specific activity in all meals. Heme iron was labeled similarly by using hemoglobin biosynthetically labeled with another radioiron tracer. There was a good inverse relation between total absorption and concentration of serum ferritin up to  $\approx 60 \mu\text{g/L}$ . In subjects with serum ferritin  $> 60 \mu\text{g/L}$  there was no relation to iron absorption. At this serum ferritin concentration, absorption decreased to a level just sufficient to cover basal iron losses, implying that at a serum ferritin concentration  $\geq 60 \mu\text{g/L}$  no further accumulation of iron stores will occur by dietary iron absorption. The findings thus suggest that in normal subjects there is no risk of developing iron overload by iron absorption from the diet even if the diet is fortified. Similar findings were made previously in two studies in women, both of which indicated an effective control of absorption. At the same serum ferritin concentration the absorption per kilogram body weight was the same in men and women served identical diets with a high iron bioavailability. These new observations strongly suggest that translation of serum ferritin concentration into amounts of stored iron should be made with caution and that in subjects with high serum ferritin concentrations, other causes than increased iron stores should be considered. There was effective control of both heme- and nonheme-iron absorption but their relations to iron status were different. *Am J Clin Nutr* 1997;66:347-56.

**KEY WORDS** Iron absorption, heme iron, nonheme iron, men, iron overload, serum ferritin, diet, iron stores, bioavailability

### INTRODUCTION

It is well established that iron stores in some unknown way influence iron absorption. Several studies have shown that there is an inverse relation between serum ferritin concentrations and iron absorption (1-9). More iron is absorbed in a state of iron deficiency and less when iron stores are replete. How effective is the regulation of iron absorption to prevent iron deficiency and iron overload in otherwise normal subjects?

It is well known that iron absorption can increase severalfold in states of iron deficiency and that iron deficiency is common, for example, in women and teenagers in most populations. The obvious conclusion is that the regulation of dietary iron ab-

sorption to meet iron requirements is not sufficiently effective to prevent iron deficiency in those with high physiologic iron requirements, even when the diet may be considered to be generally adequate. The efficacy of this regulation in relation to iron requirements and diet was examined recently (8).

Regulation of iron absorption to prevent development of iron overload, when more iron than needed is in the diet, has been studied less extensively. There have been no direct studies of the regulation of iron absorption from the whole diet because no suitable method has been available. The chemical balance method, with which iron absorption is measured as the difference between dietary intake and fecal loss, is technically difficult, rather inaccurate, and requires long balance periods. Thus, this method is not suitable for studies on the regulation of iron absorption from the whole diet.

Recently, we developed a method to determine the total amount of dietary iron absorbed from the whole diet (10). The method is based on a separate uniform labeling of all nonheme iron and all heme iron in all meals. In the first two studies only nonheme-iron absorption was measured by adding an extrinsic inorganic iron tracer in amounts to achieve the same specific activity of all nonheme iron in all daily meals served over several days (10, 11). In these two previous studies, heme-iron absorption from the same meals was calculated from the absorption of an inorganic radioiron tracer and by using relations between heme- and nonheme-iron absorption observed in other studies. The method was validated in studies in women, in whom there was good agreement between total amounts of dietary iron absorbed and individual iron requirements calculated from body weights and measured menstrual iron losses (11).

In these two studies in women four diets with different iron bioavailabilities were served for 5 or 10 d. Linear relations were found between total amounts of iron absorbed and log

<sup>1</sup> From the Institute of Internal Medicine, Department of Clinical Nutrition, Göteborg University, Annedalskliniken, Sahlgrenska University Hospital, Göteborg, Sweden.

<sup>2</sup> Supported by the Swedish Medical Research Council (project B94-19X-04721-19A) and the Swedish Council for Forestry and Agriculture Research (50.0120/95 and 997/88L 113:3).

<sup>3</sup> Address reprint requests to L. Hallberg, Department of Clinical Nutrition, Göteborg University, Annedalskliniken, Sahlgrenska University Hospital, Göteborg S-413 45, Sweden.

Received January 21, 1997.

Accepted for publication March 26, 1997.

serum ferritin. The slopes of the four regression lines were significantly different. Of special interest was the observation that the lines converged to a point on the log serum ferritin scale corresponding to estimated iron stores of  $\approx 400$ –500 mg Fe. The low absorption obtained in this range did not exceed the estimated basal losses of iron from the body, suggesting that this serum ferritin range represented the maximal iron stores derivable from the diet in women. In men the distribution of serum ferritin concentrations were shifted toward higher values, suggesting that iron stores are higher in men than in women (12). This is also consistent with direct measurements of mobilizable iron stores by phlebotomy, showing larger stores in men than in women.

The purpose of the present study was thus to measure iron absorption from the whole diet over several days and to examine the relation between iron absorption and the serum ferritin concentration in men, including both healthy men, iron-replete men, and men with small or negligible iron stores as a result of being blood donors. We also compared the relation between iron absorption and serum ferritin in men and women because both groups were served an identical diet with a highly bioavailable form of iron. Attempts were also made to examine the validity of different methods to calculate heme-iron absorption from various indicators of iron status and to examine the relation between heme-iron absorption from the whole diet and iron status under realistic conditions.

## SUBJECTS AND METHODS

### Subjects

Forty men from the staff and student body of the Sahlgrenska University Hospital, the School of Dentistry, the Chalmers

School of Technology in Göteborg, and the Blood Donor Register at the hospital volunteered for the studies. They were informed that we would select only a limited number of them for the study because of the costs and extensive work involved and that we would first conduct a laboratory screening to select the suitable participants.

We took a careful medical history, including a history of the use of medicines. Nine subjects were excluded: one because he was taking an antipsychotic drug, one because he admitted to a high regular consumption of alcohol, three because of a moderate consumption of alcohol and high concentrations of serum  $\gamma$ -glutamyltransferase, one because of abnormal results from liver-function tests, one because of an infection they developed just before the study, and two because of laboratory signs of an ongoing infection or inflammation, an elevated erythrocyte sedimentation rate, and high carbohydrate-deficient transferrin (CDT) concentrations. These nine subjects were informed about the reasons for exclusion and the results of the examinations. The final sample was 31 men aged 20–59 y, 12 of whom were blood donors. The characteristics of the subjects and the results of the screening examinations of the participants are given in Table 1.

The project was approved by the Radioisotope Committee and by the Ethical Committee of the Medical Faculty of the University of Göteborg.

### Laboratory assessment of subjects

The laboratory screening included assessment of hemoglobin concentration, hematocrit, serum ferritin, total-iron-binding capacity, serum total cholesterol, serum high-density-lipoprotein cholesterol, serum low-density-lipoprotein cholesterol, serum triacylglycerols, serum glucose, serum insulin, serum bilirubin,

TABLE 1  
Characteristics of the subjects<sup>1</sup>

	Nondonors (n = 19)	Blood donors (n = 12)	All subjects (n = 31)
Age (y)	26.3 $\pm$ 7.67 (20–55)	34.3 $\pm$ 13.4 (22–59)	29.4 $\pm$ 10.8 (20–59)
Body weight (kg)	75.5 $\pm$ 9.17 (63–102)	79.8 $\pm$ 9.98 (66.6–98.1)	77.2 $\pm$ 9.6 (63–102)
Height (cm)	180 $\pm$ 5.5 (170–188)	183 $\pm$ 6.5 (174–196)	181 $\pm$ 6.1 (170–196)
BMI (kg/m <sup>2</sup> )	23.4 $\pm$ 2.6 (20–31.8)	2.43 $\pm$ 2.7 (20.9–28.7)	23.7 $\pm$ 2.6 (20–31.8)
Hematocrit	0.47 $\pm$ 0.02 (0.43–0.49)	0.46 $\pm$ 0.02 (0.43–0.51)	0.47 $\pm$ 0.02 (0.43–0.51)
Hemoglobin (g/L)	153 $\pm$ 7.0 (140–168)	151 $\pm$ 9.9 (133–169)	152 $\pm$ 8.2 (133–169)
Serum iron ( $\mu$ mol/L)	20.6 $\pm$ 3.7 (15–29.5)	19.9 $\pm$ 5.4 (12.5–29)	20.4 $\pm$ 4.4 (12.5–29.5)
Transferrin saturation (%)	34 $\pm$ 5.8 (24–45.5)	33 $\pm$ 9.2 (20.5–52.5)	33.4 $\pm$ 7.2 (20.5–52.5)
Serum ferritin ( $\mu$ g/L)	91.0 $\pm$ 36.9 (44–176)	36.8 $\pm$ 15.8 <sup>2</sup> (21–77)	70.0 $\pm$ 40.3 (21–176)
Erythrocyte sedimentation rate (mm/h)	2.9 $\pm$ 1.9 (1–9)	4.3 $\pm$ 4.1 (1.5–16)	3.3 $\pm$ 3 (1–16)
CDT (U/L)	15.9 $\pm$ 4.0 (9.6–23.3)	16.4 $\pm$ 4.7 (11.7–27.7)	16.0 $\pm$ 4.2 (9.6–27.7)
Serum cholesterol (mmol/L)	4.81 $\pm$ 0.95 (3.1–7.1)	4.75 $\pm$ 0.67 (0.67–3.1)	4.78 $\pm$ 0.84 (3.1–7.1)
Serum triacylglycerol (mmol/L)	1.06 $\pm$ 0.44 (0.49–1.9)	1.20 $\pm$ 0.67 (0.67–3.1)	1.10 $\pm$ 0.54 (0.49–3.1)
Serum LDL cholesterol (mmol/L)	3.02 $\pm$ 1.0 (1.3–5.6)	3.00 $\pm$ 0.60 (2–3.8)	3.01 $\pm$ 0.84 (1.3–5.6)
Serum HDL cholesterol (mmol/L)	1.38 $\pm$ 0.23 (1.1–1.9)	1.25 $\pm$ 0.20 (1–1.8)	1.31 $\pm$ 0.21 (1.0–1.9)
Serum ALAT ( $\mu$ kat/L)	0.40 $\pm$ 0.11 (0.27–0.69)	0.44 $\pm$ 0.14 (0.24–0.69)	0.41 $\pm$ 0.11 (0.24–0.69)
Serum ASAT ( $\mu$ kat/L)	0.38 $\pm$ 0.13 (0.27–0.85)	0.35 $\pm$ 0.07 (0.26–0.44)	0.37 $\pm$ 0.11 (0.26–0.85)
Serum ALP ( $\mu$ kat/L)	2.75 $\pm$ 0.44 (2–4.15)	2.53 $\pm$ 0.54 (1.55–3.3)	2.65 $\pm$ 0.49 (1.55–4.15)
Serum bilirubin ( $\mu$ mol/L)	13.5 $\pm$ 5.8 (6.3–33.5)	16.8 $\pm$ 12.9 (8.5–55)	15.4 $\pm$ 9.1 (8.5–55)
Serum $\gamma$ -glutamyltransferase ( $\mu$ kat/L)	0.33 $\pm$ 0.15 (0.13–0.69)	0.37 $\pm$ 0.13 (0.21–0.69)	0.33 $\pm$ 0.12 (0.13–0.69)
Serum insulin (mU/L)	9.22 $\pm$ 2.55 (3.5–26.3)	7.18 $\pm$ 1.82 (4.3–10.9)	8.35 $\pm$ 4.56 (3.5–26.3)
$\beta$ -glucose (mmol/L)	4.14 $\pm$ 0.76 (3.5–7.0)	4.29 $\pm$ 0.39 (3.6–4.8)	4.17 $\pm$ 0.65 (3.5–7)

<sup>1</sup>  $\bar{x}$   $\pm$  SD; range in parentheses. CDT, carbohydrate deficient transferrin; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase.

<sup>2</sup> Significantly different from nondonors.  $P < 0.0001$ .

serum  $\gamma$ -glutamyltransferase, CDT, and serum alanine aminotransferase, serum aspartate aminotransferase, and serum alkaline phosphatase activities.

Serum ferritin was determined with a double-antibody polyethyleneglycol radioimmunoassay (Diagnostic Products Corp. Los Angeles). The assay was calibrated against the World Health Organization's first international standard (IS 80/602).

CDT was determined with RIA CDTECT (Pharmacia-Upjohn CDT, Uppsala, Sweden). Serum iron and total-iron-binding capacity were measured by using methods described previously (10). The subjects' body weights were measured with a SECA Delta digital scale (Vogel and Halke, Hamburg, Germany).

### Experimental design

The subjects selected were carefully informed about the details of the study, the reasons for the selection procedure used, the use of radioactive isotopes, and the purpose of the study. The main purposes of the studies were to examine iron absorption in relation to iron status and to compare men and women in this respect. The iron status of women normally varies considerably because of marked differences in iron requirements as a result of varying menstrual iron losses. To ensure that the male participants had similarly varying iron requirements, about one-third of the men selected were non-anemic blood donors. To be able to compare iron absorption in men and women we served a diet with high iron bioavailability, which was identical to one of the diets served to women in a previous study (11).

The absorption of heme and nonheme iron was measured from all meals during one 5-d period from Monday through Friday. Nonheme iron in all meals was homogeneously labeled with an extrinsic  $^{59}\text{Fe}$  tracer to the same specific activity. Heme iron in the same meals was extrinsically labeled in the same way by using biosynthetically  $^{55}\text{Fe}$ -labeled rabbit hemoglobin. Each day, four meals were served: breakfast, lunch, dinner, and a light evening meal. A fruit snack (an apple or a banana) was served between breakfast and lunch and between lunch and dinner. The menu was different on all 5 d to make the diet as varied and realistic as possible. Further details of the composition and preparation of meals were described previously (9, 10).

All meals, except the evening meals, were served under supervision in the laboratory. The evening meals were prepared and labeled in the laboratory but were consumed in the participants' homes. The heme- and nonheme-iron content of all meals was known from chemical analyses or from food tables. A precise measure of the iron content of each meal was required to enable the homogeneous labeling of the heme and nonheme iron to the same specific activity in all meals with the two radioiron isotopes. Two weeks after the last serving, the total retention of  $^{59}\text{Fe}$  was measured in a whole-body counter. At the same time, a blood sample was drawn to determine the content of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  to determine the ratio of the absorption of the two tracers and the total retention of  $^{55}\text{Fe}$ . Body weight and height were measured in a standardized manner on the same morning as whole-body counting was performed.

After whole-body counting of the retention of  $^{59}\text{Fe}$ , a first oral reference dose containing 3 mg ferrous iron labeled with  $^{59}\text{Fe}$  (see below) was served to fasting subjects. A second reference dose was then given on the following morning, also to fasting subjects. Two weeks later, the retention of absorbed

iron from the reference doses was measured by whole-body counting.

### Meals

All meals were prepared in the laboratory kitchen as described previously (10, 11). The diet was designed to have high iron bioavailability and was described in detail previously (11). Fresh samples of raw and boiled vegetables and potatoes were analyzed for ascorbic acid on the same day as they were served according to a method described previously (13). For further details see the study by Gleerup et al (10).

Samples of rolls, meat dishes, and cheese were freeze-dried and ground to a powder in a porcelain mortar. Weighed amounts of this powder from the rolls were analyzed for total iron (14) and nonheme iron (15). Heme iron was calculated as the difference between total iron and nonheme iron. Phytic acid was determined by using the method of the Association of Official Analytical Chemists (16) but modified as described previously (10).

Duplicate portions of the meals were analyzed by homogenizing the food components in an Ultra Turrax homogenizer (Janke & Kunkel, IKA-Werk, Staufen, Germany) for 1 min in metaphosphoric acid (15% wt:vol and pH adjusted to 3.5–4.0 with 2 mol KOH/L). Samples were then freeze-dried from each portion. Methods to determine nitrogen, calcium, phosphorus, magnesium, iron, and zinc were described previously (10).

### Radioiron labeling of meals

#### *Nonheme iron*

An isotope solution,  $^{59}\text{Fe}$  as  $\text{FeCl}_3$  in 0.1 mol HCl/L, was prepared at the beginning of the study and, before use, checked for activity at the Radiation Physics Department, Sahlgrenska University Hospital, Göteborg. This radioiron solution was used throughout the study to label the nonheme iron of the meals, giving identical decay of the isotope over the time elapsed from the beginning to the end of the studies. Each subject received a total of 93 kBq  $^{59}\text{Fe}$ .

Each wheat-rye roll was labeled with the radioiron standard solution just before serving in amounts that gave exactly the same specific activity of nonheme iron in all meals. The roll was cut into halves and the radioiron solution was pipetted with precision pipettes (40–200  $\mu\text{L}$ , precision 0.8–0.4%; Finnpiette Digital Labsystems, Helsingfors, Finland). The radioiron solution was soaked into the roll. One person was responsible for all labeling during the study and all calculations of radioiron labeling were double-checked.

#### *Heme iron*

Labeled hemoglobin was prepared by intravenous administration of radioiron,  $^{55}\text{Fe}$ , into rabbits. Details of the procedure were described previously (14). Biosynthetically  $^{55}\text{Fe}$ -labeled rabbit hemoglobin was pipetted onto the meat in the meals in amounts directly related to the heme-iron content of the meal. Each subject received a total of 74 kBq  $^{55}\text{Fe}$ . The yield of the preparation of radioiron-labeled hemoglobin in the rabbit was lower than expected and was just enough for 23 of the 31 subjects. In the remaining eight subjects (three blood donors and five nondonors) the absorption of heme iron had to be calculated. We selected the log reference dose absorption to calculate the percentage heme-iron absorption ( $\gamma$ ) from log

reference dose absorption ( $x$ ) by using the equation  $y = 46.6x - 38.5$  ( $r^2 = 0.717$ ).

### Oral reference doses

A solution of 10 mL of 0.01 mol HCl/L containing 3 mg Fe as  $\text{FeSO}_4$  and 30 mg ascorbic acid labeled with  $^{59}\text{Fe}$  was used as a reference in the study. The subjects drank the solutions directly from the 10-mL vials containing the iron solution and then the vials were rinsed twice and this water was also consumed. Each subject received two reference doses on two consecutive mornings after an overnight fast. No food or drink was allowed for 3 h after the reference dose. Each subject received a total of 27 kBq  $^{59}\text{Fe}$  from two reference doses.

### Total radiation

The retention of  $^{59}\text{Fe}$  was based on measurements in a whole-body counter with high sensitivity. Total  $^{55}\text{Fe}$  retention could be calculated from the ratio of  $^{55}\text{Fe}$  to  $^{59}\text{Fe}$  in a blood sample. It was thus possible to determine the total retention of both radioiron isotopes in each subject with good accuracy and precision and thus also the total radiation. The mean and median total radiation received by the subjects were 0.23 and 0.19 mSv, respectively. The total range of radiation was 0.08–0.58 mSv. The natural background radiation in Sweden is  $\approx 4$  mSv and the median radiation thus constituted 4.8% of the background radiation, which is well within the normal geographic variation in radiation.

### Measurement of nonheme-iron, heme-iron, and total iron absorption

Analysis of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  in blood was made by using a modification of the method described by Eakins and Brown (17) with a liquid-scintillation spectrometer (Tri-Carb; Packard Instruments, Dallas). Relative absorption was calculated from samples of blood. The absolute absorption of  $^{59}\text{Fe}$  was measured by whole-body counting of  $^{59}\text{Fe}$  in a whole-body counter with high sensitivity. The absolute absorption of  $^{55}\text{Fe}$  was calculated from the absolute absorption of  $^{59}\text{Fe}$  in the whole-body counter and the relative absorption of the two tracers in blood samples.

All procedures and methods of calculation were described previously (14, 18). Based on measurements of iron absorption from identical meals labeled with two different isotopes served on different days, the CV for a single measurement of nonheme-iron absorption was 35% (9). Absorption is presented as

the mean from 15 meals. This implies that the CV of nonheme-iron absorption measurements was 8–9%. The method used to determine heme-iron absorption and total iron absorption is described in detail in a previous paper (10).

### Calculation of iron requirements

Total iron requirements were calculated as the sum of basal iron losses estimated from body weight ( $14 \mu\text{g Fe} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ ). Based on previous estimations, basal iron requirements had a CV of 15% (19).

### Statistical analysis

All calculations were made by using the Microsoft Excel 5.0 computer program (Redmont, WA). Statistical analyses were made with the STATVIEW 4.0 program (Abacus Concepts, Inc, Berkeley, CA).

## RESULTS

The results of the absorption measurements and the calculations of iron requirements are shown in **Table 2**. The results are given separately for the blood donors ( $n = 12$ ) and the non-donors ( $n = 19$ ). In the blood donors the mean ( $\pm$  SD) total daily iron absorption was  $3.00 \pm 0.97$  mg. Heme-iron absorption was  $0.67 \pm 0.13$  mg and constituted 22.3% of total iron absorption. The total basal iron requirement in the blood donors was  $1.13 \pm 0.13$  mg, which was not significantly different from that of the nonblood donor group ( $t = 0.85$ , NS).

In the 19 iron-replete men who had not served as blood donors, total daily iron absorption was  $0.97 \pm 0.30$  mg and heme-iron absorption was  $0.44 \pm 0.12$  mg. Heme iron thus constituted 45% of the total amount of iron absorbed. The calculated iron requirement in these 19 men was  $1.06 \pm 0.13$  mg based on total iron losses of  $14 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  (20). The mean difference between total iron absorption and iron requirements was not significantly different from zero ( $t = 0.88$ ,  $P = 0.39$ ). In the total sample the calculated total daily iron requirement was  $1.08 \pm 0.13$  mg. Total absorption was  $1.76 \pm 1.19$  mg and heme-iron constituted 30.6% of the total absorption.

None of the blood donors was anemic or had signs of iron-deficient erythropoiesis and none had given blood during the previous 2 mo. The absorption of heme iron, nonheme iron, and total iron was significantly different from that of the nondonors ( $P < 0.0001$ ). Serum ferritin in the blood donor-

**TABLE 2**  
Iron-absorption data<sup>a</sup>

	Nondonors ( $n = 19$ )	Blood donors ( $n = 12$ )	All subjects ( $n = 31$ )
Iron absorption			
Nonheme iron			
(%)	$4.5 \pm 1.96$ (2.5–9.4)	$17.4 \pm 8.4$ (2.6–33.9)	$10.3 \pm 8.8$ (2.5–33.9)
(mg)	$0.54 \pm 0.24$ (0.3–1.13)	$2.09 \pm 1.01$ (0.31–4.07)	$1.23 \pm 1.06$ (0.5–4.07)
Heme iron			
(%)	$23.2 \pm 7.0$ (14.9–36.1)	$34.9 \pm 8.1$ (15.7–43.5)	$28.6 \pm 9.45$ (14.9–43.5)
(mg)	$0.44 \pm 0.12$ (0.27–0.65)	$0.65 \pm 0.15$ (0.28–0.78)	$0.52 \pm 0.17$ (0.27–0.48)
Total iron absorption (mg)	$0.97 \pm 0.30$ (0.57–1.64)	$2.72 \pm 1.14$ (0.59–4.85)	$1.76 \pm 1.19$ (0.57–4.85)
Reference dose absorption (%)	$24.1 \pm 9.99$ (9.9–58.7)	$38.2 \pm 16.4$ (9.9–62.5)	$30.9 \pm 14.5$ (9.9–62.5)
Iron requirements (mg)	$1.06 \pm 0.13$ (0.88–1.453)	$1.13 \pm 0.13$ (0.91–1.37)	$1.08 \pm 0.13$ (0.88–1.43)

<sup>a</sup>  $\bar{x} \pm$  SD; range in parentheses.

was significantly lower than in nondonors. The comparison was made after log transformation of the serum ferritin values ( $P < 0.0001$ )

### Estimates and measurements of heme-iron absorption

In 23 subjects heme-iron absorption was measured directly by using biosynthetically labeled heme iron as described above. In the remaining eight subjects (three blood donors and five nondonors) heme-iron absorption had to be calculated from other measures of iron status by using the observed relations found in the 23 subjects. To find the best method to calculate percentage heme-iron absorption we examined its relation to different iron-status indexes and obtained the following correlation coefficients: log serum ferritin,  $r^2 = 0.36$ ; reference dose absorption,  $r^2 = 0.71$ ; percentage nonheme-iron absorption,  $r^2 = 0.69$ ; and log reference dose absorption,  $r^2 = 0.72$ . On the basis of these findings log reference dose absorption was chosen to calculate heme-iron absorption, in 8 of the 31 subjects in whom no direct measurements of heme-iron absorption were made, by using the formula given previously.

### Relation between log serum ferritin and iron absorption

The relation between the total amount of iron absorbed and log serum ferritin is shown in Figure 1. In 18 of the 19 men who had not served as blood donors iron absorption was lower than in the blood donors. In 17 of these 19 subjects the total amount of iron absorbed was below the upper calculated basal iron requirements, 1.4 mg/d ( $\bar{x} + 2$  SD).

As shown in Figure 1, iron absorption increased when log serum ferritin decreased. This was true in subjects with a log serum ferritin below  $\approx 1.8$  ( $\mu\text{g/L}$  serum ferritin 60–70  $\mu\text{g/L}$ ). In those having a log serum ferritin concentration greater than this value there was no systematic decrease in total iron absorption. The absorption in these normal subjects thus seemed

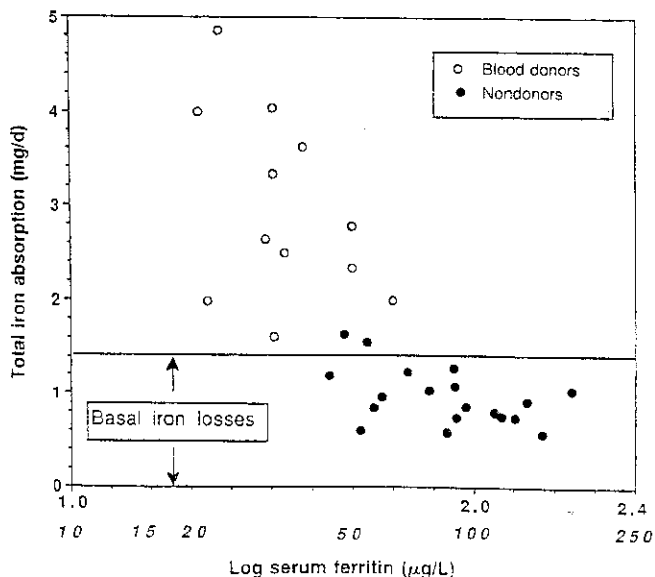


FIGURE 1. Total iron absorbed from the diet over 5 d in relation to log serum ferritin concentrations in 12 male blood donors and 19 male nondonors. The horizontal line indicates mean basal iron requirements in this group of men with a mean body weight of 77 kg and assuming a CV of 15% ( $77 \times 0.014 \times 1.30 = 1.4$  mg). On the abscissa, serum ferritin concentrations are also given in italics.

to form a "tail" in this graph. Six of the subjects had serum ferritin concentrations  $> 100$   $\mu\text{g/L}$  (log serum ferritin  $> 2.0$   $\mu\text{g/L}$ ). Note that the blood donor with the highest serum ferritin value (70  $\mu\text{g/L}$ ) was nearly excluded because of an unexplained elevated erythrocyte sedimentation rate (16 mm/h) and a high CDT concentration (27.7 U/L).

Relations between log serum ferritin and absorption of heme iron and nonheme iron were analyzed separately. As shown in Figure 2, both heme- and nonheme-iron absorption were influenced by iron status but the effect of iron status was more marked for nonheme iron. In a state of iron depletion corresponding to a serum ferritin concentration of  $\approx 10$   $\mu\text{g/L}$  the fractional absorption of the two kinds of iron was the same. At higher serum ferritin concentrations both decreased but nonheme iron decreased more than did heme iron. At serum ferritin concentrations of 15, 20, and 30  $\mu\text{g/L}$ , 40%, 80%, and 140% more iron was absorbed from heme iron, respectively.

### Total amounts of iron absorbed in men and women

In one of the two previous studies (10, 11) in women the same diet as in the present study was served to men and the same method was used to determine the total daily amounts of iron absorbed (11). There was one difference, however. In these two previous studies, heme-iron absorption was not measured directly but was estimated from the heme-iron content in the meals and the absorption of iron from the reference dose was calculated by using an equation derived from studies by Martinez-Torres and Layrisse (21). In that equation the squared

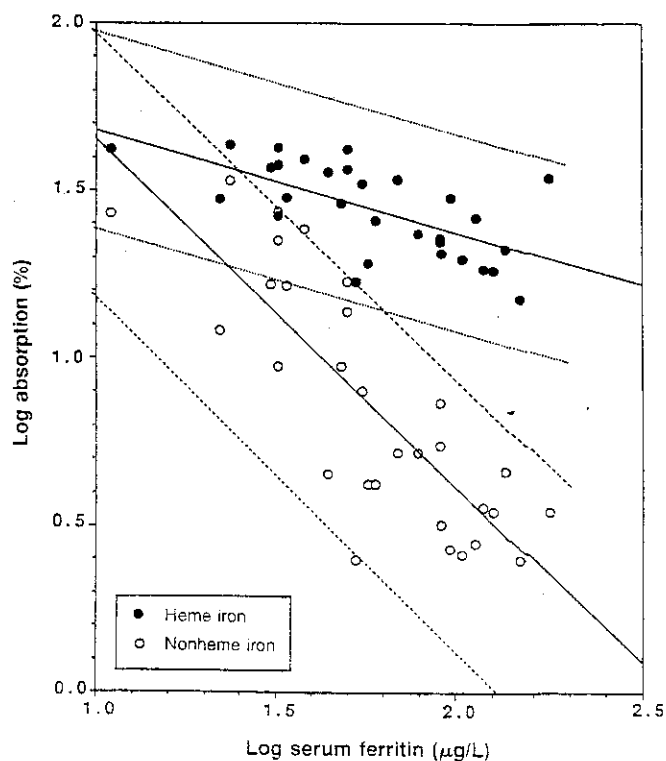


FIGURE 2. Relation between log absorption of heme and nonheme iron in relation to log serum ferritin concentrations. Dotted lines represent the 95% CIs of the regression lines. The regression line for heme-iron absorption was  $y = 1.9897 - 0.3092x$  ( $r^2 = 0.415$ ) and for nonheme iron was  $y = 2.6974 - 1.0432x$  ( $r^2 = 0.660$ ). The lines intersect at a serum ferritin concentration of 10  $\mu\text{g/L}$  and an absorption of  $\approx 50\%$ .

correlation coefficient between heme-iron absorption and log serum ferritin was only 0.36. In the present studies direct measurements of both heme- and nonheme-iron absorption from the same meals were made in 23 subjects under realistic conditions. This new equation, based on the use of log reference dose absorption (*see above*), gave a better prediction of heme-iron absorption ( $r^2 = 0.72$ ) and was therefore used in the eight men in whom no direct heme-iron absorption measurements were made. The new equation gave values for percentage heme-iron absorption that were  $28.4 \pm 12\%$  higher than those derived from the equation used previously. The previous equation was based on meals containing only veal. Therefore, in the present comparison of dietary iron absorption in women and men the same new equation was used in the women. Total iron absorption in women was thus recalculated to make the measurements in men and women directly comparable. Because men and women differ in body weight, iron absorption was calculated per kilogram body weight.

Another problem when men and women are compared is the "tail" in the relation between absorption and log serum ferritin seen in men but not in women. As discussed later there are reasons to believe that some of the high serum ferritin values seen in men were not true expressions of the size of their iron stores. In the sample of 21 women used in the comparison with men the highest serum ferritin value was  $70 \mu\text{g/L}$  (log serum ferritin  $1.85 \mu\text{g/L}$ ) and the highest serum ferritin value among the male blood donors was also  $70 \mu\text{g/L}$ . Therefore, when the regression line was calculated for the relation between iron absorption and log serum ferritin, only men with a serum ferritin concentration  $\leq 70 \mu\text{g/L}$  were included in the comparison so that the same range of serum ferritin values was used in men and women.

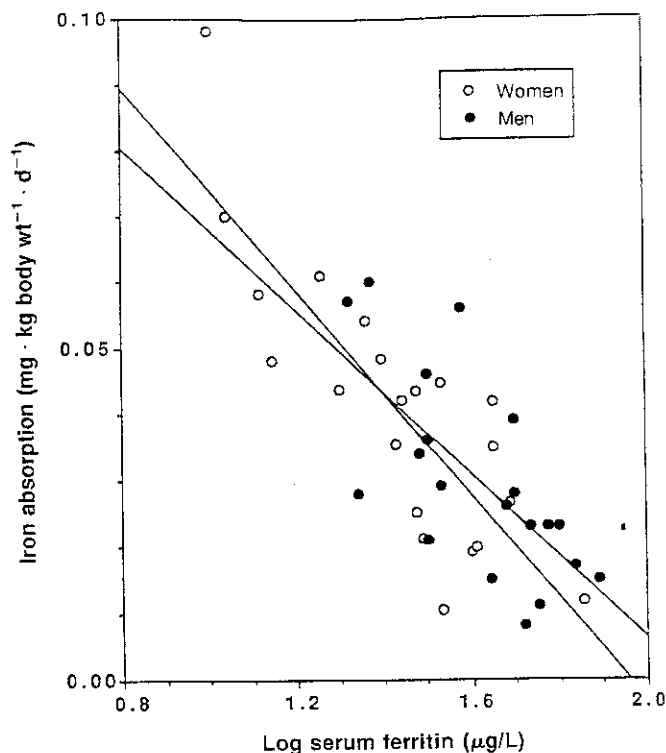
As shown in **Figure 3**, the regression lines for the relations between log serum ferritin and total iron absorption were very similar in men and women. The slopes of the regression lines were not significantly different ( $P > 0.30$ ,  $t$  test), which was also true for the  $y$  intercepts ( $P > 0.30$ ,  $t$  test). This means that men and women absorb the same amounts of iron from the same diet at the same iron status.

## DISCUSSION

### Validity of the method used to measure total daily amounts of dietary iron absorbed

In studies on the relation between iron absorption and iron status it is advantageous to be able to relate the absorption not just to a single meal but to the whole diet over a certain period of time. It is then possible to relate the absorption to individual iron requirements in validating the method and to study the effects of different iron statuses on iron absorption and iron balance.

The finding that the mean total amount of iron absorbed ( $0.98 \text{ mg}$ ) was not significantly different from the calculated basal iron requirement ( $1.06 \text{ mg}$ ) in those 19 men who had not served as blood donors, and who may be considered to be in iron balance, implies that the present method to measure the total amount of iron absorbed from the diet over several days is valid. This result is consistent with observations in the two previous studies (10, 11) in menstruating women when the same method was used to measure iron absorption. Good



**FIGURE 3.** Relation between total iron absorbed in 20 men with a log serum ferritin concentration  $< 1.9 \mu\text{g/L}$  and in 21 women who were served the same diet in another study (11). The regression line for the men was  $y = 0.13 - 0.062x$  ( $r^2 = 0.46$ ) and for the women was  $y = 0.152 - 0.078x$  ( $r^2 = 0.687$ ). The slopes were not significantly different nor were the intercepts ( $t$  test).

agreement was found between the total daily amount of iron absorbed from the diet and individual daily iron requirements. The latter was calculated from individual measurements of menstrual iron losses and basal iron losses calculated from body weight (11). There are thus three separate studies illustrating the validity of the present method to measure iron absorption from the whole diet over several days by using extrinsic labeling of all meals to the same specific activity.

### Validity of selection of subjects

The selection of subjects was made to obtain a sample of normal healthy men with greatly varying iron statuses, extending to about the same range as seen in healthy women. Therefore, clinical and laboratory examinations were conducted to exclude those who might have had any disorder or lifestyle factor (eg, smoking and alcohol abuse) known to influence iron-status indexes, especially the concentration of serum ferritin. Because increased erythropoiesis per se is known to increase iron absorption, those who had donated blood in the 2 mo before the study were excluded. As seen in Table 1, the distributions of hemoglobin concentrations and transferrin saturation values were within normal limits and not significantly different between blood donors and nondonors. It is also of interest that mean percentage nonheme-iron absorption values were not significantly different ( $P > 0.1$ ) between the blood donors and the sample of women served the same diet and used in the comparison of men and women (11). Thus, in the present

analyses of relations between dietary iron absorption and iron status in men we think it was justified to combine blood donors and nondonors in the present sample.

#### Absorption in relation to iron stores

As mentioned in the Introduction a relation between iron absorption and iron status, based on serum ferritin measurements, was observed in several studies (1-9). In a study on the relation between log absorption and log serum ferritin in 47 normal subjects the squared correlation coefficient was 0.67 (7). In another study, which used clinical data for 50 subjects, the squared correlation coefficient was 0.60 (4). This material, however, included patients with both iron deficiency and iron overload who had serum ferritin values up to 5000  $\mu\text{g/L}$ . A reexamination of the published data showed that limiting the range of serum ferritin to 250  $\mu\text{g/L}$  produced a squared correlation coefficient of only 0.21.

The balance of evidence suggests that serum ferritin is a good indicator of the amount of stored iron: low amounts indicate iron deficiency, high amounts indicate states of iron overload, and men have higher amounts than women. Moreover, a relation between the content of nonheme iron in the bone marrow and serum ferritin has also been shown (4). On the other hand it is also well-known that increased and often very high serum ferritin values are seen in inflammatory and infectious disorders, in liver disease, in starvation, and in association with a high alcohol intake.

Direct evidence of a relation between iron stores and serum ferritin concentrations was obtained in four studies by measuring the stores by quantitative phlebotomies (22-25). By pooling these results the squared correlation coefficient between the serum ferritin concentration and amounts of mobilizable iron stores was only  $\approx 0.5$ , indicating that only 50% of the variation in serum ferritin was related to variations in amounts of stored iron. All these data indicate that a considerable part of the variation in serum ferritin was not related to the size of the iron stores.

In our previous studies in women, significant relations were seen between total daily amounts of iron absorbed and log serum ferritin. The same was seen in the present studies in men with serum ferritin values  $\leq 70 \mu\text{g/L}$ . In men with serum ferritin values  $> 70 \mu\text{g/L}$ , however, no relation was noted (Figure 1). The results in these normal, iron-replete men thus formed a tail in the graph. A similar tail was also seen in the present data by relating reference dose absorption instead of food iron absorption to log serum ferritin (data not given here). Such a tail was not seen in the previous studies in women, who on the other hand had no serum ferritin values  $> 70 \mu\text{g/L}$ . Note that the frequency distribution of serum ferritin in the present sample of men was the same as in the extensive sample of men in the second National Health and Nutrition Examination Survey (NHANES II) in the United States (12). It should be remembered that 9 of the 40 men originally invited to participate in the present study were excluded after careful clinical and laboratory examinations. In the 31 men selected for the study we tried to exclude those men who might have had elevated concentrations of serum ferritin due to alcohol intake, smoking, liver disease, mild diabetes, and mild infections during the month preceding the study. Therefore, the probability is low that the tail was due to any known pathologic factor influencing serum ferritin.

To further examine the cause of the tail, the relation between dietary iron absorption and iron status was also examined by using the absorption from the reference dose instead of the concentration of serum ferritin as an independent measure of iron status. As shown in Figure 4 a straight line relation was seen over the whole range of dietary iron absorption. The squared correlation coefficient was high,  $r^2 = 0.832$ . This finding suggests that serum ferritin in the range seen in iron-replete men and  $> 70 \mu\text{g/L}$  is not a good measure of their iron stores.

In a critical review of the relation between serum ferritin and amounts of stored iron it was shown that the variation in serum ferritin was considerable, in turn making it difficult to predict amounts of stored iron from concentrations of serum ferritin. In two clinical studies the variation in liver iron concentration accounted for only 36% of the variation in serum ferritin (26). In an earlier study in healthy men no correlation was seen between iron stores measured by phlebotomies and serum ferritin concentration (24).

It was obvious in the present study that in normal subjects with a log serum ferritin concentration  $> 1.8 \mu\text{g/L}$  (serum ferritin  $> 60-70 \mu\text{g/L}$ ), iron absorption was within the range needed to meet basal iron requirements, which means that a further accumulation of iron in body iron stores will not take place by absorption of iron from the diet. Consequently, the high serum ferritin values observed in the iron-replete men above this critical range ( $> 60-70 \mu\text{g/L}$ ) were not due to the high iron stores derived from the diet but were probably related to other unknown factors (*see below*). This suggests that in men with higher than normal serum ferritin concentrations,

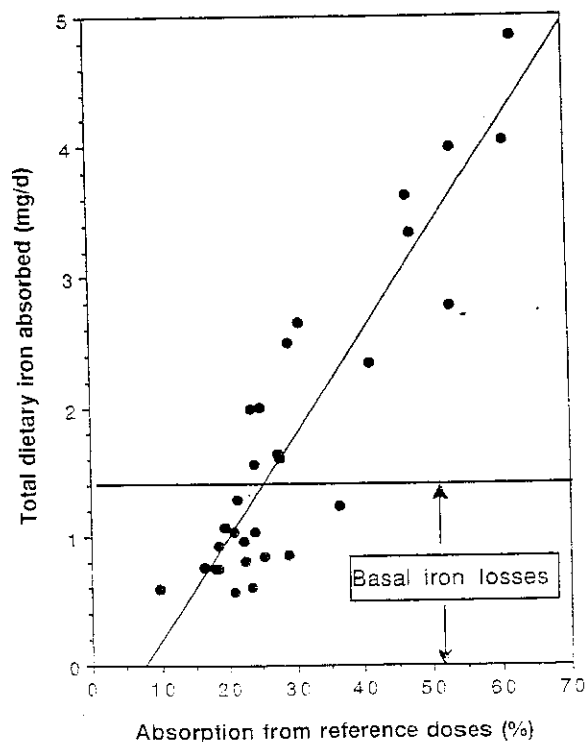


FIGURE 4. Relation between total dietary iron absorbed ( $y$ ) and absorption from reference doses ( $x$ ). The regression line was  $y = 0.079x - 0.606$  ( $r^2 = 0.832$ ). The horizontal line indicates mean basal iron requirements.

from all diets decreased to values below basal iron requirements, thus implying a cessation of any further increase in iron stores. This was also true for a diet with high iron bioavailability. It was estimated that in women with low iron requirements the maximal iron stores that might be attained by absorption of iron from the diet would be  $\approx 4$ –500 mg (11).

The decrease in iron absorption in men in the present study agrees with the findings in the women served the same diet. These results in both women and men thus strongly support the conclusion that in otherwise normal iron-replete subjects, iron will not accumulate in the body above certain upper physiologic limits.

There are several independent observations and studies that suggest that in normal subjects there is an effective control mechanism that will prevent accumulation of excess iron in the body. In epidemiologic studies it has been observed that serum ferritin values peak in the third to fourth decade in men (12, 33). In a carefully designed iron-fortification trial in which subjects were given 7.5 mg highly available extra iron daily for 2 y, iron status in women improved significantly whereas no effect on serum ferritin was noted in men (34). High doses of ascorbic acid (2 g/d) given with the main meals for 2 y to improve dietary iron bioavailability had no effect on serum ferritin in iron-replete men (35). In a recent study in an iron-replete man, 10 mg Fe as ferrous sulfate was mixed into one of the main meals for 500 d with no significant increase in serum ferritin (36).

Another example of the capacity of the mechanisms regulating iron absorption is from two extensive necropsy studies in which the content of nonheme storage iron in liver samples was measured. One study presented data from 3983 subjects in 18 countries (37) and the other study data from 277 subjects in the United States (38). The main result in both studies was that after the age of  $\approx 30$  y, there was no further increase in liver iron stores.

The variation in serum ferritin noted in the present sample of men was not significantly different from the variation reported in men of about the same age in the extensive NHANES II (12). This latter survey was made in 1976–1980, ie, at about the same time as the necropsy study in the United States. In the NHANES II, there was an increased skewness of the distribution of serum ferritin with a shift toward higher serum ferritin values after the age of 20 y, especially in men but also in women. Despite this, median values were only slightly changed with age.

Considering the present finding that there is an effective control mechanism of iron absorption and all the indirect evidence mentioned supporting this conclusion, there must be other factors related to age that are associated with an increase in the concentration of serum ferritin and responsible for the shift toward higher values.

The present conclusion that there was an effective down-regulation of iron absorption is important in the evaluation of a recent hypothesis (based on a statistical relation): that moderately elevated serum ferritin concentrations ( $> 200$   $\mu\text{g/L}$ ) increase morbidity in coronary artery disease in men (39). The conclusion in that study was that a high amount of stored iron is a risk for coronary artery disease. However, the causality of such a relation must be seriously questioned on the basis of the present conclusion: that moderately elevated concentrations of serum ferritin should not be translated into higher iron stores.

The findings from this Finnish study have not been confirmed by other studies.

Another important implication of our finding that there is an effective control mechanism for iron absorption and that in iron-replete subjects iron absorption is about the same from diets with greatly varying iron bioavailability is that studies of factors influencing iron absorption are less valid in iron-replete subjects. Even marked differences in bioavailability observed in iron-deficient subjects or in subjects with small or moderate iron stores will be hard or impossible to detect in iron-replete subjects and will thus give misleading results (11). In addition, the absorption of fortification iron will be regulated in the same effective way as all other dietary nonheme iron. Thus, there would be no reason why fortification iron would constitute a special risk for iron overload in otherwise normal and iron-replete subjects.

Because iron cannot be actively secreted from the body, an understanding of the regulation of iron absorption is key to understanding iron metabolism and iron balance. This was well illustrated in a recent review (40). From a teleologic perspective, the high iron content of our environment and the assumed high iron intake by early humans (41) make it reasonable to assume that for survival, both humans and animals must have developed effective control mechanisms to prevent both iron deficiency and iron overload. The present finding of an effective control of iron absorption is compatible with this perspective. ■

## REFERENCES

- Jacobs A, Miller F, Worwood M. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Br Med J* 1972;4:206–8.
- Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974;27:681–7.
- Heinrich HC, Brüggemann J, Gabbe EE, Gläser M. Correlation between diagnostic  $^{59}\text{Fe}^{2+}$ -absorption and serum ferritin concentration in man. *Z Naturforsch [C]* 1977;32:1023–5.
- Bezuda W, Bothwell TH, Torrance JD, et al. The relationship between marrow iron stores, plasma ferritin concentrations and iron absorption. *Scand J Haematol* 1979;22:113–20.
- Baynes RD, Bothwell TH, Bezuda WR, MacPhail AP, Derman DP. Relationship between absorption of inorganic and food iron in field studies. *Ann Nutr Metab* 1987;31:109–16.
- Taylor P, Martinez-Torres C, Leets I, Ramirez J, Garcia-Casal MN, Layrisse M. Relationships among iron absorption, percent saturation of plasma transferrin and serum ferritin concentration in humans. *J Nutr* 1988;118:1110–5.
- Lynch SR, Skikne BS, Cook JD. Food iron absorption in idiopathic hemochromatosis. *Blood* 1989;74:2187–93.
- Hallberg L, Hultén L, Bengtsson C, Lapidus L, Lindstedt G. Iron balance in menstruating women. *Eur J Clin Nutr* 1995;49:200–7.
- Magnusson B, Björn-Rasmussen E, Hallberg L, Rossander L. Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurements. *Scand J Haematol* 1981;27:201–8.
- Geerup A, Rossander-Hultén L, Gramatkovski E, Hallberg L. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. *Am J Clin Nutr* 1995;61:97–104.
- Hultén L, Gramatkovski E, Geerup A, Hallberg L. Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. *Eur J Clin Nutr* 1995;49:794–808.

12. Pilch SM, Senti FRE. 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, Federation of American Societies for Experimental Biology, 1984.
13. Deneke U, Michal G, Beutler HO. Neue Methode zur Bestimmung von Vitamin C in Lebensmitteln. (New method for determination of vitamin C in food-stuffs.) *Deutsche Lebensmittel-Rundschau* 1978;74:400-3 (in German).
14. Hallberg L. Food iron absorption. In: Cook JD, ed. *Methods in hematology*. London: Churchill, 1980:116-33.
15. Hallgren B. Determination of non-haem iron in tissues. *Acta Soc Med Ups* 1953;59:84-99.
16. Harland BF, Oberleas D. Anion-exchange method for determination of phytate in foods: collaborative study. *J Assoc Off Anal Chem* 1986;69:667-70.
17. Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. *Int J Appl Radiat Isot* 1966;17:391-7.
18. Björn-Rasmussen E, Hallberg L, Magnusson B, Rossander L, Svanberg B, Arvidsson B. Measurement of iron absorption from composite meals. *Am J Clin Nutr* 1976;29:772-8.
19. Hallberg L, Rossander-Hultén L. Iron requirements in menstruating women. *Am J Clin Nutr* 1991;54:1047-58.
20. Green R, Charlton RW, Seftel H, et al. Body iron excretion in man. A collaborative study. *Am J Med* 1968;45:336-53.
21. Martinez-Torres C, Layrisse M. Iron absorption from veal muscle. *Am J Clin Nutr* 1971;24:531-40.
22. Walters GO, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. *J Clin Pathol* 1973;26:770-2.
23. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* 1990;75:1870-6.
24. Birgegård G, Högman C, Killander A, Levander H, Simonsson B, Wide L. Serum ferritin and 2,3-DPG during quantitative phlebotomy and iron treatment. *Scand J Haematol* 1977;19:327-33.
25. Charlton RW, Derman D, Skikne B, et al. Iron stores, serum ferritin and iron absorption. In: Brown ED, ed. *Proteins of iron metabolism*. Philadelphia: Grune & Stratton, 1977:387-92.
26. Brittenham GM, Danish EH, Harris JW. Assessment of bone marrow and body iron stores: old techniques and new technologies. *Semin Hematol* 1981;18:194-221.
27. Pritchard JA, Mason RA. Iron stores of normal adults and replenishment with oral iron therapy. *J Am Med Assoc* 1964;190:897-901.
28. Hynes M. The iron reserve of a normal man. *J Clin Pathol* 1949;2:99-102.
29. Haskins D, Stevens AR, Finch S, Finch CA. Iron stores in man as measured by phlebotomy. *J Clin Invest* 1952;31:543-7.
30. Balcerzak SP, Westerman MP, Heinle EW, Taylor FH. Measurement of iron stores using desferrioxamine. *Ann Intern Med* 1968;68:518-25.
31. Conrad ME, Crosby WH. The natural history of iron deficiency induced by phlebotomy. *Blood* 1962;20:173-85.
32. Olsson KS. Iron stores in normal men and male blood donors. *Acta Med Scand* 1972;192:401-7.
33. Leggett BA, Brown NN, Bryant SJ, Duplock L, Powell LW, Halliday JW. Factors affecting the concentrations of ferritin in serum in a healthy Australian population. *Clin Chem* 1990;36:1350-5.
34. Ballot DE, MacPhail AP, Bothwell TH, Gillooly M, Mayet FG. Fortification of curry powder with NaFe(111)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. *Am J Clin Nutr* 1989;49:162-9.
35. Cook J, Watsson SS, Simpson KM, Lipschitz DA, Skikne BS. The effect of high ascorbic acid supplementation on body iron stores. *Blood* 1984;64:721-6.
36. Sayers MH, English G, Finch CA. Capacity of the store-regulator in maintaining iron balance. *Am J Hematol* 1994;47:194-7.
37. Charlton RW, Hawkins DM, Mavor WO, Bothwell TH. Hepatic storage concentrations in different population groups. *Am J Clin Nutr* 1970;23:358-71.
38. Sturgeon P, Shoden A. Total liver storage iron in normal populations of the USA. *Am J Clin Nutr* 1971;24:469-74.
39. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992;86:803-11.
40. Finch C. Regulators of iron balance in humans. *Blood* 1994;84:1697-702.
41. Eaton SB, Konner M. Paleolithic nutrition: a consideration of its nature and current implications. *N Engl J Med* 1985;312:283-9.

