

Adaptation in iron absorption: iron supplementation reduces nonheme-iron but not heme-iron absorption from food¹⁻⁴

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ABSTRACT

Background: Results of cross-sectional studies suggest that in healthy people, iron absorption adapts to meet physiologic needs and stabilize iron stores, but this has not been adequately tested in longitudinal studies.

Objective: We tested whether heme- and nonheme-iron absorption decrease in response to increased iron intake and whether iron stores reach a steady state.

Design: In a randomized, placebo-controlled trial, heme- and nonheme-iron absorption by healthy men and women ($n = 57$) were measured before and after 12 wk of supplementation with 50 mg Fe/d as ferrous sulfate. Serum and fecal ferritin were measured during supplementation and for 6 mo thereafter.

Results: Initially, both heme- and nonheme-iron absorption were inversely associated with serum ferritin concentration. Volunteers who took iron supplements, even those with serum ferritin $< 21 \mu\text{g/L}$ ($n = 5$), adapted to absorb less nonheme iron (3.2% at week 12 compared with 5.0% at week 0, $P < 0.001$) but not less heme iron from a beef-based meal. Serum ferritin concentration was slightly but significantly higher after iron supplementation than after placebo (difference = $13 \mu\text{g/L}$). This higher ferritin concentration persisted for ≥ 6 mo after supplementation, except in subjects with low iron stores, whose serum ferritin returned to baseline within 3 mo. Fecal ferritin excretion increased 2.5-fold ($P < 0.05$) during supplementation.

Conclusions: Healthy individuals, even those with low iron stores, had reduced nonheme-iron absorption from food in response to iron supplementation. Despite this partial adaptation, iron stores were greater after iron supplementation than after placebo and this difference was sustained, except in individuals with the lowest iron stores. *Am J Clin Nutr* 2000;72:982-9.

KEY WORDS Iron supplementation, heme iron, nonheme iron, iron absorption, fecal ferritin, serum ferritin, adaptation, meat, erythrocyte incorporation, humans, anemia, iron deficiency, hemoglobin, hematocrit

INTRODUCTION

Body iron content is controlled largely through absorption rather than excretion (1). In healthy individuals, 4 major factors determine iron absorption: physiologic need for iron, dietary iron intake, bioavailability of dietary iron, and adaptation. Adaptation is the ability of the mucosal cell to adjust iron absorption to phys-

ologic demands and to the amount and bioavailability of dietary iron (2). Physiologic demands are the most important determinant of iron absorption. For example, nonanemic women who did not use supplemental iron absorbed 7%, 36%, and 66% of nonheme iron from a meal at 12, 24, and 36 wk of normal pregnancy, respectively (3). Thus, absorption increased as physiologic need rose during pregnancy. The theory that iron absorption responds to physiologic requirements is also supported by the inverse correlation between iron stores, as indicated by serum ferritin concentration, and heme- and nonheme-iron absorption (4-7). Absorption of both heme and nonheme iron is adaptively enhanced in male blood donors compared with nondonors (8).

Longitudinal studies of human adaptation of iron absorption have been limited. Nonheme-iron absorption was not clearly reduced after iron loading with intramuscular injections of iron dextran in healthy men (9). Absorption of supplemental nonheme iron by healthy women was nonsignificantly reduced, by 13%, in each of 2 separate comparisons after preconditioning with iron supplementation for 6 consecutive days (10). Men consuming controlled experimental diets for 10 wk showed significant adaptation in their absorption of nonheme iron. Absorption from a high-bioavailability diet was reduced (by 38%), whereas absorption from a low-bioavailability diet was increased (by 29%), without affecting heme-iron absorption (11).

Ferritin in the intestinal mucosal cells may help regulate iron absorption by blocking the serosal transfer of unwanted iron (12). Fecal ferritin, an indicator of mucosal ferritin, is responsive to iron supplementation (13) and to the bioavailability of dietary iron (11, 14). Changes in mucosal ferritin could affect the absorption of

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both heme and nonheme iron, which are believed to enter a common intracellular iron pool after passage into mucosal cells (2).

Cross-sectional data indicate that median serum ferritin concentrations stabilize in men after 32 y and in women after 60 y of age (15). This suggests that the iron absorption of individuals adapts to maintain a set point in iron stores (16, 17). However, these observations need to be tested further in longitudinal studies.

The specific objectives of this study were to test the effects of increased iron intake on heme- and nonheme-iron absorption, on iron stores as indicated by serum ferritin, and on fecal ferritin excretion in healthy men and women.

SUBJECTS AND METHODS

General protocol

To test for adaptation, heme- and nonheme-iron absorption were measured in 57 healthy men and women before and after 12 wk of supplementation with iron. The volunteers were randomly assigned (by blocks to obtain similar serum ferritin concentrations in both groups) to receive either an iron supplement (50 mg elemental Fe as ferrous sulfate) or a placebo capsule daily. Blood indexes of iron status were measured at 0, 2, 12, and 14 wk. In addition, serum ferritin concentrations were monitored periodically after supplementation was discontinued (during weeks 14, 18, 24, and 36 of the study). Fecal ferritin excretion was determined from single stool samples collected weekly for the first 14 wk and during weeks 18, 24, and 36 of the study.

Subjects

Volunteers were recruited through public advertising and were selected after we determined their eligibility on the basis of an interview and blood analysis. The eligibility requirements were age ≥ 21 y, no apparent underlying disease, normal hemoglobin (≥ 12 or 14 g/L for women and men, respectively), serum ferritin concentration < 450 $\mu\text{g/L}$, no blood donation in the past 2 y, and no use of iron supplements for ≥ 6 mo. Participants agreed to discontinue all nutrient supplements when their applications were submitted, generally 6–12 wk before the beginning of the study. None of the participants routinely used medication. Women were excluded if they had been pregnant within the previous 2 y. All participants gave their informed consent. The study was approved for human subjects by the University of North Dakota's Radioactive Drug Research Committee and Institutional Review Board and by the US Department of Agriculture's Human Studies Review and Radiological Safety Committees.

The study participants were 30 women and 27 men with the following characteristics ($\bar{x} \pm \text{SD}$): age, 38 ± 10 y (range: 22–58 y); admission body weight, 80 ± 17 kg (range: 54–131 kg); body mass index (in kg/m^2), 27 ± 5 (range: 21–42); and serum ferritin concentration, 57 $\mu\text{g/L}$ (range: 11–230 $\mu\text{g/L}$). Seven volunteers (all men) smoked tobacco regularly. Ten women used oral contraceptives (3 women in the iron group and 7 in the placebo group) and 4 used estrogen replacement therapy (3 women in the iron group and 1 in the placebo group). An equal number of participants in the iron and placebo groups (3 in each) withdrew before supplementation was completed for reasons unrelated to the study; their data were not included in the analyses. For the follow-up measurements after supplementation was discontinued (weeks 12–36 of the study), 3 participants withdrew for reasons unrelated to the study (eg, relocation and medication use).

Supplements

The 50-mg Fe supplements were purchased as gelatin capsules from Rugby Laboratories (Norcross, GA) and contained 43.8 ± 1.6 mg elemental Fe (by analysis) in the form of ferrous sulfate plus negligible amounts of other trace elements. The placebo capsules, which contained lactose, were purchased from Gallipot, St Paul. The iron and placebo capsules were not identical in appearance, but the participants were not told which treatment they were receiving. The participants were instructed to take the supplements every evening with food and to take a missed capsule as soon as they remembered, allowing ≥ 8 h between the doses. To enhance and monitor compliance, a monthly supply of the capsules was provided in blister packs with each capsule dated. Participants returned these packs to the Grand Forks Human Nutrition Research Center before a new pack was issued to them. Also, participants responded to questionnaires that monitored compliance, medication use, and any side effects. No side effects or missed doses were reported with either the iron or placebo capsules (perhaps doses were not missed because each capsule was dated) in the weekly questionnaires or in the monthly interviews, which were conducted by a registered dietitian.

Test meal

The test meal, patterned after that described by Lynch et al (6), consisted of ground beef (113 g), a bun (53 g), French fries (68 g), a vanilla milk shake (150 mL), and tomato ketchup (20 g); the meal contained 1.2 mg heme Fe and 5.1 mg total Fe by analysis. The radiotracers ^{55}Fe (9.3 kBq as rabbit hemoglobin) and ^{59}Fe (18.5 kBq as FeCl_3) were added to the cooked hamburger patty, which was briefly reheated in a microwave oven before being served. Because 4 test meals were served (at the beginning of the study and again after 12 wk, on 2 consecutive days each time), the total radioisotope administered to each individual was 37 kBq ^{55}Fe and 74 kBq ^{59}Fe . The test meals were weighed to 1% accuracy and were consumed at the research center.

The ^{59}Fe and ^{55}Fe isotopes were purchased from NEN Life Science Products, Boston. Radiolabeled hemoglobin was obtained by intravenously injecting 74 MBq (2 mCi) of ^{59}Fe into an iron-deficient, pathogen-free rabbit, exsanguinating the animal 2 wk later, and removing the stroma by lysing and centrifugation (18). The specific activity of the final preparation was 0.585 kBq/ μg Fe. The amounts of iron added to each test meal as a result of labeling with ^{55}Fe and ^{59}Fe were ≤ 19 μg and ≤ 0.2 μg , respectively.

Heme- and nonheme-iron absorption measurements

Absorption of heme and nonheme iron from the test meal was measured at the beginning of the study and again after 12 wk of daily supplementation with iron or the placebo. The participants fasted for ≥ 10 h before and 4 h after the test meal (water was allowed). Absorption of nonheme iron was determined by whole-body scintillation counting, which detected only the gamma-emitting ^{59}Fe radioisotope. The custom-made whole-body counter used 32 crystal NaI(Tl) detectors ($10 \times 10 \times 41$ cm each) arranged in 2 planes above and below a bed. The initial total-body activity from the 2 meals was calculated as twice the whole-body activity measured 1–3 h after the first test meal (before any unabsorbed isotope was excreted). The percentage of nonheme iron absorbed was determined as the portion of initial whole-body activity that remained after 2 wk (day 15), with correction for physical decay and for background activity

measured 1–2 d before the test meals. In a previous study, the slopes of semilogarithmic whole-body retention plots for 4 wk after isotope administration were not different from zero, which indicates that iron excretion was minimal and that it was not necessary to correct for endogenous excretion of iron during the 2 wk after isotope administration (14).

Radioisotope concentrations in blood (19) were also measured after 2 wk (day 15) by using a liquid scintillation analyzer (Tri-Carb 1600 TR; Packard, Meriden, CT) and were expressed as fractions of the administered radioisotope, measured from dose aliquots prepared when the hamburgers were labeled. The blood retention of ^{59}Fe , expressed as a percentage of the administered dose, was determined from the blood radioisotope concentration together with an estimate of total blood volume that was derived from body weight and height (20, 21). The blood incorporation of iron, expressed as a percentage of the absorbed nonheme iron, was determined by dividing the fractional blood retention of ^{59}Fe by the fractional absorption of ^{59}Fe as measured by whole-body counting. Because the whole-body counter was unable to detect ^{55}Fe , erythrocyte incorporation could only be measured for nonheme iron (^{59}Fe), but was assumed to also apply to heme iron, which is believed to form a common iron pool with nonheme iron in intestinal mucosal cells. Heme-iron absorption was determined by multiplying the nonheme-iron absorption (determined by whole-body counting) by the ratio of ^{55}Fe to ^{59}Fe in the blood, with correction for radioactive decay and for background activity measured before the meals.

Chemical analyses

Blood samples obtained by phlebotomy after an overnight fast were limited to 30 mL each at weeks 0, 2, 12, and 14 and to 10 mL each at weeks 18, 24, and 36 of the study. The first stool sample of each week was collected weekly during the first 14 wk and during weeks 18, 24, and 36.

Duplicate aliquots of the test meals were digested with concentrated nitric and 70% perchloric acids by using method (II)A of the Analytical Methods Committee (22). The iron contents of the digestates and of the supplements dissolved in nitric acid were determined by inductively coupled argon plasma emission spectrophotometry. Analytic accuracy was monitored by performing periodic analyses of certified standard reference materials from the National Institute of Standards and Technology. The measurements were $95 \pm 9\%$ ($\bar{x} \pm \text{SD}$) of certified values for iron.

The same methods of digestion and inductively coupled argon plasma emission spectrophotometry were used to measure nonheme iron in the test meal after extraction (23). Heme iron in the test meal was calculated as the difference between total and nonheme iron. Our previous analyses indicated that cooking procedures (baking and briefly reheating in the microwave) had negligible effects on the heme-iron content of beef.

Hemoglobin and hematocrit were measured with a CellDyne 3500 System (Abbott Laboratories, Abbott Park, IL). Serum iron concentration was measured colorimetrically with a Cobas Fara Chemistry Analyzer (Hoffmann-LaRoche Inc, Nutley, NJ) by using a commercial chromagen (Ferene; Raichem Division of Hemagen Diagnostics, San Diego). Iron binding capacity was similarly determined after adding a known amount of ferrous iron to the serum sample under alkaline conditions. Percentage transferrin saturation was calculated from serum iron concentration and total-iron-binding capacity. To reduce analytic variation, each participant's samples for serum ferritin or fecal ferritin

were frozen and measured in a single analytic batch. Fecal ferritin was extracted from individually lyophilized samples (13) and filtered through 5- μm membrane filters. Serum and fecal ferritin were measured by enzyme-linked immunosorbent assay with monoclonal antibodies against human spleen ferritin (Abbott Laboratories). This assay, which mainly measures L-rich ferritin, the isoform found primarily in spleen and liver (24), was calibrated against World Health Organization ferritin 80/602 First International Standard. Protein in fecal extracts was determined colorimetrically (25). In a previous study, no cross-reactivity was found with dietary sources of ferritin (14). Transferrin receptors were measured by enzyme-linked immunosorbent assay with monoclonal antibodies specific to transferrin receptors (Quantikin Human Transferrin Receptor Immunoassay; R&D Systems Inc, Minneapolis). C-reactive protein was measured by nephelometry (Behring Diagnostics Inc, Westwood, MA) to help detect increases in serum ferritin concentration related to inflammation. Because serum ferritin is an acute-phase reactant, if a serum C-reactive protein concentration was >5 mg/L and the corresponding serum ferritin value was 50% greater than the preceding measurement, then the serum ferritin value was considered artificially high because of inflammation and was omitted from the analyses.

Statistical analyses

The data for heme- and nonheme-iron absorption, serum and fecal ferritin concentrations, and transferrin receptors were logarithmically transformed and geometric means are reported. All fecal ferritin data were increased by a negligible 0.1 $\mu\text{g/d}$ to forgo transformation of some zero values when analyzing statistical relations. In contrast with the serum ferritin values, which required logarithmic transformation because of a skewed distribution, the distribution of changes in serum ferritin was not skewed and was therefore not transformed; arithmetic means are reported for these changes. Supplement effects were determined by using repeated-measures analysis of variance (26). Pearson's product-moment correlation coefficients (26) were used to assess additional relations between variables. Bonferroni contrasts were used to test for differences between treatments with time. Absorption ratios (week 12:week 0) were compared by using Student's *t* test. Simple linear and stepwise regression analyses (26) were used to assess additional relations between variables.

RESULTS

Iron absorption and incorporation into blood

Supplementation with ≈ 50 mg Fe/d for 12 wk significantly reduced nonheme-iron absorption from the test meal by 36% (3.2% compared with 5.0%; $P < 0.01$; **Table 1**). Nonheme-iron absorption was unaffected by the placebo. The adaptation in nonheme-iron absorption was also indicated by the significant difference between treatment groups in the ratio of nonheme-iron absorption at the 2 time points, ie, week 12:week 0 ($P < 0.01$; **Table 1**). Although heme-iron absorption decreased over time ($P = 0.05$), this change was observed in both groups and was not affected by iron supplementation (**Table 1**). Iron supplementation decreased the total amount of iron absorbed from the meal (served without the supplement) by $\approx 25\%$ ($P < 0.05$; **Table 1**).

For all volunteers combined, 2 wk after the first labeled meal, an average of $\approx 77\%$ of the newly absorbed iron was incorporated



TABLE 1

Dietary heme- and nonheme-iron absorption and erythrocyte incorporation of absorbed iron before (week 0) and after (week 12) daily supplementation with 50 mg Fe as ferrous sulfate for 12 wk¹

	Iron supplement (n = 30)	Placebo (n = 27)	P ²		
			Supplement	Time	Supplement × time
Nonheme iron absorption (%)					
Week 0	5.0 (3.4–7.4)	5.2 (3.5–7.6)	NS	0.01	0.01
Week 12	3.2 (2.2–4.7) ³	5.0 (3.4–7.4)			
Week 12:week 0	0.6 (0.4–1.0)	1.0 (0.5–1.8)	0.01		
Heme iron absorption (%)					
Week 0	22 (16–31)	25 (18–34)	NS	0.05	NS
Week 12	19 (14–26)	23 (17–32)			
Week 12:week 0	0.9 (0.5–1.4)	0.9 (0.6–1.4)	NS		
Total iron absorption (mg)					
Week 0	0.49 (0.37–0.64)	0.52 (0.40–0.69)	NS	0.01	0.05
Week 12	0.37 (0.28–0.49) ³	0.50 (0.38–0.65)			
Erythrocyte incorporation of absorbed iron (%)					
Week 0	80 (71–89)	75 (67–84)	NS	0.01	0.01
Week 12	71 (64–80) ³	75 (67–84)			

¹Geometric means (minus and plus pooled SD; these numbers are not evenly distributed around the mean because of the logarithmic transformation).

²ANOVA for all analyses except for comparisons between ratios, which were done with Student's *t* test.

³Significantly different from week 0, *P* < 0.05 (contrast between pairs of means).

into the blood. Iron supplementation significantly decreased the incorporation of absorbed iron tracer into the blood, from 80% at week 0 to 71% at week 12 (Table 1; *P* < 0.05). This is consistent with an increased pool of newly absorbed iron, an unchanged rate of erythropoiesis, and greater incorporation of iron into body storage pools. The incorporation of the absorbed iron into the blood did not change with placebo (Table 1).

Iron-status indicators

Iron supplementation for 12 wk did not affect most indexes of iron status, such as blood hemoglobin concentration, transferrin saturation, or transferrin receptors (Table 2). However, mean serum ferritin concentration increased slightly with supplementation and decreased with placebo, from 60 to 48 µg ferritin/L, pre-

sumably because of procedural phlebotomy (*P* < 0.001; Table 2). This observed reduction was only slightly more than an estimate of ≈5–6 µg ferritin/L derived from the 90-mL blood loss reflected at 14 wk and the guidelines that 1 µg ferritin/L serum corresponds to 8–10 mg stored iron (17) and whole blood contains ≈500 mg Fe/L.

Because serum ferritin concentrations were slightly, but not significantly, greater for the placebo group than the iron group at baseline (Table 2), significant differences between the iron and placebo groups at specific time points were detectable only when the data were expressed as changes from baseline (Figure 1). At the end of supplementation (week 12), serum ferritin concentration was 13 µg/L greater in the iron group than in the placebo group (*P* < 0.05), and this difference tended to persist for ≥6 mo after iron supplementation was discontinued (*P* < 0.05 at 36 wk, Figure 1).

TABLE 2

Initial blood indexes of iron status and time-specific serum ferritin concentrations during 12 wk of daily iron supplementation (50 mg Fe/d as ferrous sulfate) and after supplementation was discontinued

	Iron supplement (n = 30)	Placebo (n = 27)	P (ANOVA)		
			Supplement	Time	Supplement × time
Hemoglobin (g/L) ¹	146 ± 14 ²	145 ± 14	NS	0.002	NS
Serum iron (µmol/L)	15.0 ± 4.1 ²	14.2 ± 4.1	NS	NS	NS
Transferrin saturation	0.306 ± 0.087 ²	0.268 ± 0.087	NS	NS	NS
Transferrin receptors (nmol/L)	16.9 (14.9–19.3) ³	16.9 (14.9–19.3)	NS	NS	NS
Serum ferritin (µg/L)					
Week 0	51 (43–61) ³	60 (51–71)	NS	0.01	0.0001
Week 2	52 (44–61)	53 (45–64)			
Week 12	60 (50–71) ⁴	52 (44–62)			
Week 14	54 (46–64)	48 (41–57) ⁴			
Week 18	52 (44–62)	49 (41–58) ⁴			
Week 24	55 (46–65)	51 (43–60) ⁴			
Week 36	56 (47–67)	53 (45–63)			

¹Hemoglobin was significantly affected by time, such that values were ≈1–2% lower than baseline (week 0) at 14, 18, and 24 wk (data not shown for specific time points); these changes were independent of dietary treatment.

²Least-squares means ± pooled SD.

³Geometric least-squares means (minus and plus pooled SD).

⁴Significantly different from baseline in each supplement group, *P* < 0.05 (Bonferroni contrasts). There were no significant differences between supplement groups at specific time points until data were expressed as a change from baseline (see Figure 1).

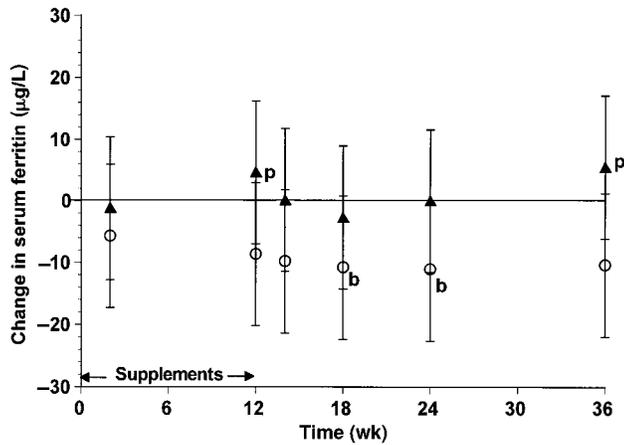


FIGURE 1. Changes in serum ferritin concentration during and after iron supplementation: for the entire study, changes in serum ferritin were significantly ($P < 0.0001$) different in volunteers receiving iron supplements (\blacktriangle ; $n = 30$) than in those receiving placebo (\circ ; $n = 27$), which partly reflects protection from depletion resulting from procedural phlebotomy; these differences persisted for ≥ 6 mo after supplementation ended. Individual mean values with p were significantly different from the placebo group at that time point and those with b were significantly different from baseline within the same group ($P < 0.05$). Data are arithmetic means \pm pooled SDs.

Thus, iron supplementation protected serum ferritin concentration from a decline associated with moderate experimental phlebotomy, and this difference in serum ferritin persisted for as long as observation continued.

Correlations between serum ferritin, iron absorption, and iron incorporation

At week 0, both heme- and nonheme-iron absorption were inversely correlated with serum ferritin concentration (ln-transformed data: $R^2 = 0.20$ and 0.43 , respectively; $P < 0.001$ for both, $n = 57$; **Figure 2**). After 12 wk of supplementation, however, this relation persisted for only heme-iron absorption ($R^2 = 0.20$, $P < 0.05$, $n = 30$). The relation with serum ferritin also persisted for nonheme-iron absorption in the placebo group ($R^2 = 0.27$, $P < 0.01$, $n = 27$). At week 0, incorporation of the absorbed iron into blood was inversely and logarithmically related to serum ferritin concentration ($y = 125x^{-0.12}$; $R^2 = 0.34$, $P < 0.001$, $n = 57$). The strength of this association decreased after supplementation with iron ($y = 120x^{-0.13}$; $R^2 = 0.14$, $P < 0.05$) but not after placebo ($y = 133x^{-0.14}$; $R^2 = 0.49$, $P < 0.0001$). Blood incorporation of absorbed iron was not related to age, body mass index, hemoglobin, or transferrin saturation.

When whole-body counting is not possible, it is commonly assumed that 80% of absorbed iron is incorporated into red blood cells. This assumed average is consistent with the 77% average incorporation at week 0 in the present study. However, this assumption alters the apparent relation between serum ferritin concentration and iron absorption determined from blood measurements (Figure 2) because serum ferritin is inversely correlated with both iron absorption and iron incorporation into blood. Nonheme-iron absorption was determined by using whole-body counting or by using blood measurements only (and assuming 80% blood incorporation), and these 2 independent

methods were highly correlated for both the first ($R^2 = 0.97$) and second ($R^2 = 0.91$) absorption measurements. At week 0, heme- and nonheme-iron absorption measurements by either method were inversely correlated with serum ferritin concentration (Figure 2). However, absorption measurements obtained by using only blood measurements exaggerated the relation between iron absorption and serum ferritin, especially for heme-iron absorption; this exaggeration would underestimate iron absorption by those with high iron stores and overestimate iron absorption by those with low iron stores (Figure 2).

Fecal ferritin excretion

Fecal ferritin excretion, which did not differ significantly between the 2 groups at baseline, increased rapidly with iron supplementation and remained significantly higher than baseline until 1 wk after supplementation was discontinued (**Figure 3**). At week 12, fecal ferritin excretion was 2.5-fold higher in the iron-supplemented group than in the placebo group ($P < 0.05$). Fecal ferritin excretion was inversely correlated with nonheme-iron absorption ($R^2 = 0.20$, $P < 0.001$, $n = 57$) at week 0, but not at week 12 of iron supplementation ($R^2 = 0.02$, NS, $n = 30$) and was not correlated with heme-iron absorption at either time point. At week 0, serum ferritin concentration accounted for $\approx 25\%$ of the variation in fecal ferritin excretion ($R^2 = 0.25$, $P < 0.001$, $n = 57$). This direct association between serum and fecal ferritin remained significant at week 12 in the placebo group ($R^2 = 0.40$, $P < 0.001$, $n = 27$), but not in the iron-supplemented group.

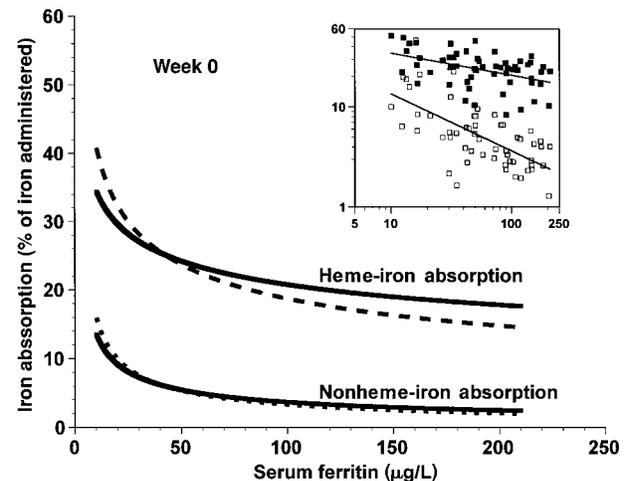


FIGURE 2. Cross-sectional relation between serum ferritin and heme- and nonheme-iron absorption, as influenced by assumptions about blood incorporation of absorbed iron, at week 0 (before supplementation): absorption of both heme and nonheme iron was inversely associated with serum ferritin ($n = 57$): $y = 57.4x^{-0.22}$; $R^2 = 0.20$, $P < 0.001$ for heme iron; $y = 49.0x^{-0.56}$; $R^2 = 0.43$, $P < 0.0001$ for nonheme iron (absorption measured by using whole-body counting, solid lines). Because the blood incorporation of absorbed iron is also related to serum ferritin (see text), the commonly used assumption that 80% of the absorbed iron is incorporated into blood results in an exaggeration of these associations: $y = 89.7x^{-0.34}$; $R^2 = 0.34$, $P < 0.0001$ for heme iron; $y = 76.5x^{-0.68}$; $R^2 = 0.52$, $P < 0.0001$ for nonheme iron (absorption measured by using blood measurements only, dashed lines). Inset: data for heme- (\blacksquare) and nonheme- (\square) iron absorption and models (absorption measured by using whole-body counting, solid lines) are shown on a logarithmic scale, $n = 57$.

The placebo-group data provided an opportunity to examine variability in the fecal ferritin excretion of healthy people consuming self-selected Western diets. In the placebo group, the intra- and interindividual variation in weekly fecal ferritin excretion was $\approx 17\%$ and 30% , respectively.

Subjects with low iron status

As expected, the subjects with low iron stores (serum ferritin $< 21 \mu\text{g/L}$, $n = 5$, all women) absorbed considerably more nonheme iron from the meal than those with higher iron stores, both before and after iron supplementation (Figure 4). However, in response to iron supplementation, these volunteers with low iron stores, the subjects who would most benefit from high iron absorption, had nonheme-iron absorption that was reduced at least as substantially as that of subjects with higher iron stores [from 11.8% to 4.5% with iron supplements and low iron stores, $n = 5$ (Figure 4) and from 16.8% to 12.8% with placebo and low iron stores, $n = 4$]. Adaptation in nonheme-iron absorption was significantly different between the iron-supplemented ($n = 5$) and the placebo ($n = 4$) groups, as determined by absorption ratios (week 12:week 0, 0.38 compared with 0.76 , respectively, $P < 0.05$). Heme-iron absorption was also somewhat higher in individuals with low iron stores and was unaffected by iron supplementation (Figure 4).

In contrast with the subjects with higher iron stores, those with low iron stores did not sustain a greater serum ferritin concentration than the placebo group after iron supplementation was discontinued (Figure 5). Serum ferritin in these women was $\approx 9 \mu\text{g/L}$ greater than that of subjects in the placebo group ($P < 0.05$) during supplementation; however, serum ferritin concentrations returned to baseline within 3 mo after iron supplements were discontinued (Figure 5).

DISCUSSION

With daily iron supplementation, the iron absorption of healthy people adapted by decreasing nonheme-iron but not heme-iron absorption from food. Adaptation was incomplete, as evidenced by the greater serum ferritin concentrations in the iron-supplemented group than in the placebo group.

Adaptation reduced, but did not completely offset, the effect of iron supplementation. The increase in the amount of iron absorbed with supplementation can be estimated by assuming that subjects consumed $\approx 15 \text{ mg Fe}$ (1.5 mg heme Fe) from food (with bioavailability similar to that of the test meal) and that absorption of supplemental iron was 2.3% initially (10) and was then reduced by 36% . Initial daily absorption of 2.2 mg Fe (1 mg from food and 1.2 mg from the supplement) would be reduced to $\approx 1.5 \text{ mg}$ after 12 wk. Thus, despite partial adaptation, at 12 wk subjects who took iron supplements would have absorbed $\approx 0.5 \text{ mg}$ more iron daily than subjects who took placebo, assuming that the placebo group absorbed $\approx 1 \text{ mg Fe/d}$ from food. Further adaptation would be likely if iron stores continued to increase.

Although heme-iron absorption did not adapt longitudinally to iron supplementation for 12 wk, long-term adaptation was suggested by the cross-sectional association between heme-iron absorption and serum ferritin concentration (Figure 2). This is consistent with other cross-sectional observations (4–8). It can be argued (2) that adaptation is less complete for heme-iron than for nonheme-iron absorption, because absorption of heme iron varies only 2–3 fold (range of 15–40%) compared with 10–15 fold

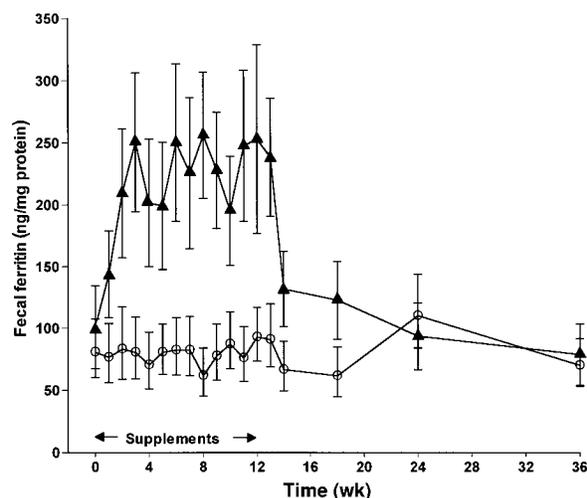


FIGURE 3. Fecal ferritin excretion during and after iron supplementation: fecal ferritin excretion in those receiving iron supplements (\blacktriangle ; $n = 30$) was significantly ($P < 0.05$) increased, by 2.5-fold, compared with those receiving placebo (\circ , $n = 27$) within 2 wk after supplementation began and until 1 wk after supplementation was discontinued (which in part reflects gastrointestinal transit time). The data are geometric means \pm pooled SDs.

(range of 1–15%) for nonheme iron across a range of serum ferritin concentrations (Figure 2). However, the shapes of the 2 curves relating heme-iron or nonheme-iron absorption to serum ferritin with linear axes (Figure 2) indicate a similar average difference (10–15 percentage points) in heme-iron or nonheme-iron absorption as serum ferritin increases from 10 to $210 \mu\text{g/L}$. Whereas the ability to reduce nonheme-iron absorption to an efficiency of $\leq 1\%$ shows an impressive degree of control, heme-iron absorption is not without biological control.

The 2.5-fold increase in fecal ferritin observed with supplementation (Figure 3) is consistent with other reports of greater fecal ferritin with comparable supplementation (13) or with diets differing in iron bioavailability (11, 14). It is also consistent with the finding that intracellular iron induces ferritin synthesis through the dissociation of iron-responsive proteins from ferritin mRNA (27, 28). It is not known whether a change in mucosal ferritin represents only localized control of intracellular iron, or if it is important in the control of nonheme-iron absorption. The lack of adaptation in heme-iron absorption despite increased fecal ferritin suggests that mucosal ferritin may not be involved in the control of heme-iron absorption (although heme induces ferritin synthesis) (27). The associations of ferritin excretion with nonheme-iron absorption (inverse) and serum ferritin (positive) were eliminated by iron supplementation; this implies that mucosal ferritin may have reached a similar concentration regardless of iron status.

Our finding that there was no short-term adaptation in heme-iron absorption with increased nonheme-iron intake (Table 1) is consistent with data indicating that the specific mucosal receptors for heme are inhibited by heme iron, but not by nonheme iron (29). Yet no adaptation in heme-iron absorption was seen, even when men consumed diets differing in heme-iron and nonheme-iron content (11).

So why did short-term absorptive adaptation occur for nonheme iron [even in subjects with low iron stores (Figure 5)] and

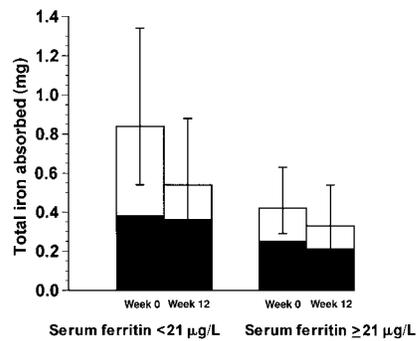


FIGURE 4. Adaptation in iron absorption with iron supplementation in individuals with low or adequate iron stores: subjects with low iron stores ($n = 5$) absorbed more nonheme (□) and heme (■) iron than did those with higher iron stores ($n = 25$) both before and after supplementation with iron. However, with iron supplementation, the reduction in the efficiency of nonheme-iron absorption by those with low iron stores was at least as substantial as that observed in the individuals with higher iron stores. Nonheme-iron absorption was significantly ($P < 0.05$) reduced between 0 and 12 wk in both subjects with low and with high serum ferritin, but did not change in those receiving the placebo (placebo data not shown). The data are geometric means \pm pooled SDs. SDs are for total iron absorbed.

not for heme iron? Perhaps it is because iron bound in heme is not as oxidatively reactive as free iron (30), and the intestinal mucosa may not need to defend against ingested heme iron to the same degree as it does with nonheme iron. Mucosal ferritin may provide a localized cytosolic defense against oxidative generation of potentially toxic free radicals by sequestering and storing excess nonheme iron, and reducing iron catalysis of Fenton-type reactions producing free radicals (27) both inside mucosal cells and in the intestinal lumen. Consistent with this, iron supplementation increased the free radical-producing capacity of feces in healthy volunteers (31) and increased breath ethane, an indicator of oxidative damage in vivo, in women with low iron stores (32). In epidemiologic studies, iron intake was associated with risk of colon cancer (33, 34). Further studies are needed to test whether short-term adaptation in iron absorption with iron supplementation represents, in part, a localized protective mechanism.

The adaptation in nonheme-iron absorption was not strongly related to changes in body iron stores; iron supplementation reduced nonheme-iron absorption with little change in serum ferritin concentration, and nonheme-iron absorption was unaffected in the placebo group despite reduced serum ferritin secondary to phlebotomy (Figure 1). Also, iron supplementation interfered with the inverse relation between serum ferritin and nonheme-iron absorption. The weak association between changes in serum ferritin and nonheme-iron absorption in this study suggests that the adaptation observed may be related to recent iron intake rather than long-term iron stores.

It has been postulated that iron absorption is controlled to maintain an individualized homeostatic set point in iron stores (16, 17). The sustained difference in serum ferritin observed after discontinuing iron supplementation in the present study suggests that people with adequate iron stores do not fully adapt and iron supplementation may have lasting effects on iron stores. This finding is of practical importance because increased body iron has been implicated in the etiology of chronic illnesses such as heart disease (35) and cancer (36). The amount

of iron in US diets increased during the 1980s and 1990s, mainly because of commercial fortification of breads and cereals (37). In addition, self-supplementation is not uncommon; in 1980, 17% of US males aged 25–64 y and 21% of US females >65 y of age regularly used nutrient supplements containing iron, with a median dose of 18 mg (38). Men and postmenopausal women should be discouraged from using iron supplements without clinical assessment of iron status.

The downward adaptation in iron absorption, even by the participants with low iron stores, raises a question about the upward adaptation reported during pregnancy: would pregnant women, supplemented with iron, adapt longitudinally to increase their iron absorption from food as well as was observed in women who were not supplemented (3)? Further research is needed to determine the effect of iron supplementation on total iron retention throughout pregnancy.

Not only did the iron-supplemented participants with low iron stores show decreased nonheme-iron absorption from food (Figure 4), their serum ferritin values returned to baseline within 3 mo after supplementation ended (Figure 5). These results agree with those in adolescent Indonesian females, whose serum ferritin concentrations declined toward baseline within 6 mo after iron supplementation ended (39). These findings imply that people with low iron stores are unable to sustain an increase in serum ferritin, possibly because of higher iron excretion. Thus, continued supplementation or other dietary changes would be necessary to sustain an increase in iron stores in these populations. However, if hemoglobin and transferrin saturation are normal, increasing serum ferritin concentration may not provide any functional benefit.

The present study confirms our previous observation that the incorporation of absorbed iron into erythrocytes is inversely related to iron status, as indicated by serum ferritin (11). Decreased erythrocyte incorporation of absorbed iron associated with iron supplementation (compared with placebo) was also reported in pregnant women (40).

In conclusion, healthy people responded to supplemental iron with decreased absorption of nonheme iron, but not heme

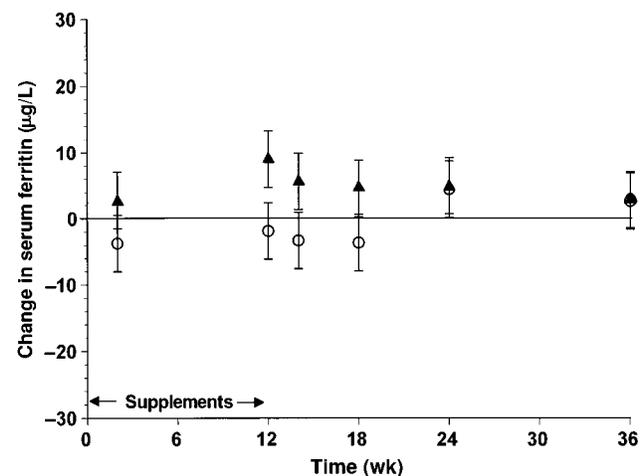


FIGURE 5. Changes in serum ferritin concentration in subjects with low iron stores (serum ferritin $<21 \mu\text{g/L}$): serum ferritin increased slightly but significantly ($P < 0.05$) with iron supplementation (▲; $n = 5$) compared with placebo (○; $n = 4$) but returned to baseline within 3 mo after supplementation was discontinued. Data are arithmetic means \pm pooled SDs.

iron, from food. Adaptation was at least as great in subjects with low serum ferritin as in other subjects. Adaptation disturbed the inverse relation between iron stores and nonheme-iron absorption, without altering a similar relation for heme-iron absorption. However, adaptation was incomplete; in subjects with adequate-to-high iron stores, iron supplementation (compared with placebo) resulted in a greater serum ferritin concentration that persisted after supplementation ended. In contrast, the effects of iron supplementation were short-lived in subjects with the lowest iron stores. 

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REFERENCES

- McCance R, Widdowson E. Absorption and excretion of iron. *Lancet* 1937;2:680-4.
- Cook JD. Adaptation in iron metabolism. *Am J Clin Nutr* 1990; 51:301-8.
- Barrett JF, Whittaker PG, Williams JG, Lind T. Absorption of non-haem iron from food during normal pregnancy. *BMJ* 1994;309:79-82.
- Cook JD, Lipschitz DA, Miles LE, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974; 27:681-7.
- 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, Hulthen L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr* 1997;66:347-56.
- Hallberg L, Björn-Rasmussen E. Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as haem and non-haem iron. *Scand J Haematol* 1972;9:193-7.
- Pirzio-Biroli G, Finch CA, Loden B. Iron absorption III. The influence of iron stores on iron absorption in the normal subject. *J Lab Clin Med* 1960;55:216-20.
- Cook JD, Reddy MB. Efficacy of weekly compared with daily iron supplementation. *Am J Clin Nutr* 1995;62:117-20.
- Hunt JR, Roughead ZK. Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. *Am J Clin Nutr* 2000;71:94-102.
- Hahn PF, Bale WF, Ross JF, Balfour WM, Whipple GH. Radioactive iron absorption by gastro-intestinal tract: influence of anaemia, anoxia and antecedent feeding distribution in growing dogs. *J Exp Med* 1943;78:166-88.
- Skikne BS, Whittaker P, Cooke A, Cook JD. Ferritin excretion and iron balance in humans. *Br J Haematol* 1995;90:681-7.
- Hunt JR, Roughead ZK. Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovo vegetarian diets for 8 wk. *Am J Clin Nutr* 1999; 69:944-52.
- Custer EM, Finch CA, Sobel RE, Zettner A. Population norms for serum ferritin. *J Lab Clin Med* 1995;126:88-94.
- Gavin MW, McCarthy DM, Garry PJ. Evidence that iron stores regulate iron absorption—a setpoint theory. *Am J Clin Nutr* 1994; 59:1376-80.
- Sayers MH, English G, Finch C. Capacity of the store-regulator in maintaining iron balance. *Am J Hematol* 1994;47:194-7.
- Dawson RB, Rafal S, Weintraub LR. Absorption of hemoglobin iron: the role of xanthine oxidase in the intestinal heme-splitting reaction. *Blood* 1970;35:94-103.
- Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron metabolism in man. London: Blackwell Scientific Publications, 1979.
- Wennesland R, Brown E, Hopper JL, et al. Red cell, plasma and blood volume in healthy men measured by radiochromium (⁵¹Cr) cell tagging and hematocrit: influence of age, somatotype and habits of physical activity on variance after regression of volumes to height and weight combined. *J Clin Invest* 1959;38:1065-77.
- Brown E, Hopper J, Hodges JL, Bradley B, Wennesland R, Yamauchi H. Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. *J Clin Invest* 1962;41:2182-90.
- Analytical Methods Committee. Methods of destruction of organic matter. *Analyst* 1960;85:643-56.
- Rhee KS, Ziprin YA. Modification of the Schriker nonheme iron method to minimize pigment effects for red meats. *J Food Sci* 1987;52:1174-6.
- Wagstaff M, Worwood M, Jacobs A. Properties of human tissue iso-ferritins. *Biochem J* 1978;173:969-77.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.
- SAS Institute Inc. SAS/STAT user's guide, version 6. 4th ed. Cary, NC: SAS Institute Inc, 1990.
- Mascotti DP, Rup D, Thach RE. Regulation of iron metabolism: translational effects mediated by iron, heme, and cytokines. *Annu Rev Nutr* 1995;15:239-61.
- Eisenstein RS, Blemings KP. Iron regulatory proteins, iron responsive elements and iron homeostasis. *J Nutr* 1998;128:2295-8.
- Grasbeck R, Kouvenen I, Lundberg M, Tenhunen R. An intestinal receptor for heme. *Scand J Haematol* 1979;23:5-9.
- Hallaway PE. Iron and damage to biomolecules. In: Lauffer RB, ed. Iron and human disease. Boca Raton, FL: CRC Press, 1992:209-30.
- Lund EK, Wharf SG, Fairweather-Tait SJ, Johnson IT. Oral ferrous sulfate supplements increase the free radical-generating capacity of feces from healthy volunteers. *Am J Clin Nutr* 1999;69:250-5.
- Mertz SD, Woodhouse LR, Donangelo CM, et al. Breath ethane excretion rate in young women is increased by daily iron but not by daily zinc supplementation. *FASEB J* 1999;13:A241 (abstr).
- Wurzelmann JI, Silver A, Schreinemachers DM, Sandler RS, Everson RB. Iron intake and the risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 1996;5:503-7.
- Kato I, Dnistrian AM, Schwartz M, et al. Iron intake, body iron stores and colorectal cancer risk in women: a nested case-control study. *Int J Cancer* 1999;80:693-8.
- Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Sapanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. *Circulation* 1992; 86:803-11.
- Stevens RG, Graubard BI, Micozzi MS, Neriishi K, Blumberg BS. Moderate elevation of body iron level and increased risk of cancer occurrence and death. *Int J Cancer* 1994;56:364-9.
- Gerrior S, Bente L. Nutrient content of the US food supply, 1909-1994. US Department of Agriculture, Center for Nutrition Policy and Promotion, Home Economics Research Report no. 53, 1997.
- Stewart ML, McDonald JT, Levy AS, Schucker RE, Henderson DP. Vitamin/mineral supplement use: a telephone survey of adults in the United States. *J Am Diet Assoc* 1985;85:1585-90.
- Angeles-Agdeppa I, Schultink W, Sastroamidjojo S, Gross R, Karyadi D. Weekly micronutrient supplementation to build iron stores in female Indonesian adolescents. *Am J Clin Nutr* 1997;66:177-83.
- O'Brien KO, Zavaleta N, Caulfield LE, Yang DX, Abrams SA. Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant Peruvian women. *Am J Clin Nutr* 1999;69:509-15.