Adaptation in iron absorption: iron supplementation reduces nonheme-iron but not heme-iron absorption from food

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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 µg/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 µg/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.


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

Cross-sectional data indicate that median serum ferritin con-
centrations 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 ran-
domly assigned (by blocks to obtain similar serum ferritin con-
centrations 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 measured in 57 healthy men and women before and after
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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 μg/L, no blood donation in the past 2 y, and
no use of iron supplements for ≥6 mo. Participants agreed to dis-
continue 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 Institu-
tional 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 (± SD): age, 38 ± 10 y (range: 22–58 y);
admission body weight, 80 ± 17 kg (range: 54–131 kg); body
mass index (in kg/m²), 27 ± 5 (range: 21–42); and serum
ferritin concentration, 57 μg/L (range: 11–230 μg/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 par-
ticipants 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 cap-
sules 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 identi-
cal in appearance, but the participants were not told which treat-
ment 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 mon-
tored 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 analy-
isis. The radiotracers 55Fe (9.3 kBq as rabbit hemoglobin) and 59Fe
(18.5 kBq as FeCl₃) were added to the cooked hamburger patty,
which was briefly reheatened 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
55Fe and 74 kBq 59Fe. The test meals were weighed to 1% accu-
racity and were consumed at the research center.

The 59Fe and 55Fe isotopes were purchased from NEN Life Sci-
ence Products, Boston. Radiolabeled hemoglobin was obtained by
intravenously injecting 74 MBq (2 mCi) of 59Fe into an iron-defi-
cient, 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
55Fe and 59Fe 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 59Fe radioisotope. The custom-made whole-
body counter used 32 crystal NaI(Tl) detectors (10 × 10 × 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 ini-
tial 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 $^{59}$Fe, erythrocyte incorporation could only be measured for nonheme iron ($^{55}$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 ± 9% (x ± 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 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 µg/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 $\approx$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, i.e., 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...
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, presumably 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 1

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

1 Geometric means (minus and plus pooled SD; these numbers are not evenly distributed around the mean because of the logarithmic transformation).
2 ANOVA for all analyses except for comparisons between ratios, which were done with Student’s t test.
3 Significantly different from week 0, P < 0.05 (contrast between pairs of means).

### 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

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

1 Hemoglobin 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.
2 Least-squares means ± pooled SD.
3 Geometric least-squares means (minus and plus pooled SD).
4 Significantly 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).
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.
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 ≈17% and 30%, respectively.

**Subjects with low iron status**

As expected, the subjects with low iron stores (serum ferritin <21 μg/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 ≈9 μg/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 ≈15 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 ≈1.5 mg after 12 wk. Thus, despite partial adaptation, at 12 wk subjects who took iron supplements would have absorbed ≈0.5 mg more iron daily than subjects who took placebo, assuming that the placebo group absorbed ≈1 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 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 (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 μg/L. Whereas the ability to reduce nonheme-iron absorption to an efficiency of 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 hemeiron 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
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 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

![Figure 4](https://academic.oup.com/ajcn/article-abstract/72/4/982/4729438)

**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 ± pooled SDs. SDs are for total iron absorbed.

**FIGURE 5.** Changes in serum ferritin concentration in subjects with low iron stores (serum ferritin <21 µg/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 ± 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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