Adaptation of iron absorption in men consuming diets with high or low iron bioavailability[1–4]

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ABSTRACT
Background: Short-term measurements of iron absorption are substantially influenced by dietary bioavailability of iron, yet bioavailability negligibly affects serum ferritin in longer, controlled trials.
Objective: Our objective was to test the hypothesis that in men fed diets with high or low iron bioavailability, iron absorption adapts to homeostatically maintain body iron stores.
Design: Heme- and nonheme-iron absorption from whole diets were measured in 31 healthy men at 0 and 10 wk while the men consumed weighed, 2-d repeating diets with either high or low iron bioavailability for 12 wk. The diets with high and low iron bioavailability contained, respectively, 14.4 and 15.3 mg nonheme Fe/d and 1.8 and 0.1 mg heme Fe/d and had different contents of meat, ascorbic acid, whole grains, legumes, and tea.
Results: Adaptation occurred with nonheme- but not with heme-iron absorption. Total iron absorption decreased from 0.96 to 0.69 mg/d ($P < 0.05$) and increased from 0.12 to 0.17 mg/d ($P < 0.05$) after 10 wk of the high- and low-bioavailability diets, respectively. This partial adaptation reduced the difference in iron bioavailability between the diets from 8- to 4-fold. Serum ferritin was insensitive to diet but fecal ferritin was substantially lower with the low- than the high-bioavailability diet. Erythrocyte incorporation of absorbed iron was inversely associated with serum ferritin.
Conclusions: Iron-replete men partially adapted to dietary iron bioavailability and iron absorption from a high-bioavailability diet was reduced to ≈0.7 mg Fe/d. Short-term measurements of absorption overestimate differences in iron bioavailability between diets. *Am J Clin Nutr* 2000;71:94–102.

KEY WORDS Gastrointestinal adaptation, nonheme-iron absorption, heme-iron absorption, dietary bioavailability, iron requirements, serum ferritin, fecal ferritin, ascorbic acid, meat, phytic acid, tea, men

INTRODUCTION

Cross-sectional inverse associations between serum ferritin, an indicator of iron stores, and both heme- and nonheme-iron absorption (1–4) suggest that humans biologically adapt their iron absorption in relation to iron stores. The adaptive response seems greater for nonheme iron than for heme iron (5). For instance, nonheme-iron absorption from a meal with high iron bioavailability varied 10–15 fold (≈1–15% absorbed) whereas heme-iron absorption varied only 2–3 fold (≈15–45% absorbed) as serum ferritin varied cross-sectionally from ≈10 to 200 μg/L (3). Blood donors with lower iron stores than nondonors absorbed much more nonheme iron than did nondonors, but similar amounts of or only slightly more heme iron (6, 7).

Cross-sectional data suggest that median serum ferritin values do not increase in men after 32 y of age or in women after 60 y of age (8). This is consistent with theories that iron stores are regulated by adaptation of iron absorption to maintain individual set points (9, 10).

Adaptive control of iron absorption may explain why controlled changes in dietary iron bioavailability have had negligible effects on serum ferritin. Dietary factors that influence iron bioavailability (from radiolabeled single meals) include the biochemical form of the iron (ie, heme or nonheme) and concurrently consumed enhancers (eg, ascorbic acid and an unidentified meat factor) or inhibitors (eg, phytic acid, polyphenols, phosphates, calcium, and eggs) (11–13). However, in controlled trials lasting weeks or months, serum ferritin was unresponsive to changes in ascorbic acid (14–17), calcium (18, 19), or meat (20) intakes. Women consuming controlled diets with different meat and phytic acid contents for 8 wk each had no change in serum ferritin despite a 6-fold difference in the amount of iron absorbed (21).

Extensive exposure does not seem to modify the degree of enhancement or inhibition by dietary factors that influence nonheme-iron absorption. In single-meal comparisons, dietary phytate inhibited nonheme-iron absorption to a similar degree in long-term vegetarians and control subjects (22). Ascorbic acid enhanced nonheme-iron absorption to a similar degree before and after 16 wk of ascorbic acid supplementation (14). In that study, 16 wk of ascorbic acid supplementation reduced nonheme-iron absorption by 25%

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[2] Mention of a trademark or proprietary product does not constitute a guarantee of or warranty for the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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(NS) in subjects given a test meal both with ($P = 0.08$) and without ($P = 0.17$) ascorbic acid. Perhaps the general efficiency of iron absorption was reduced by ascorbic acid supplementation without modification of its enhancing effect.

In this controlled-feeding trial comparing short-term measurements of iron absorption with longer-term measurements of iron status, we tested the hypothesis that in men fed diets with high or low iron bioavailability, iron absorption adapts to homeostatically maintain body iron stores. We also present data on the incorporation of absorbed iron into blood and on fecal ferritin excretion, an indicator of ferritin in the intestinal mucosa (23), which is sensitive to dietary iron bioavailability (21).

SUBJECTS AND METHODS

Subjects

The participants were 31 men with a mean (±SD) age of 44 ± 7 y (range: 32–56 y), a mean body weight of 89 ± 14 kg (range: 64–115 kg), and a mean body mass index (in kg/m^2) of 27 ± 3 (range: 21–33). The men were recruited through public advertising and were selected after an interview and blood analysis helped determine that they were ≥32 y of age, had no apparent underlying disease, had not donated blood or used iron supplements exceeding 20 mg/d for ≥6 mo before the study, and had serum ferritin values ≥20 and <450 μg/L. A minimum age of 32 y was chosen because serum ferritin, an indicator of iron stores, was shown to reach a stable equilibrium by this age in a large cross-sectional study (8). Serum ferritin values of the participants at the time of recruitment ranged from 22 to 336 μg/L (geometric $\bar{x}$: 112 μg/L). Applicants agreed to discontinue all nutrient supplements when their application was submitted, generally 6–12 wk before the beginning of the study. Only one participant had used iron supplements (18 mg/d) before his application was received; his serum ferritin and iron-absorption values were well within the range of values of the other participants. None of the men routinely used medications. Seven men regularly smoked tobacco; these men were evenly distributed between the 2 treatment groups (n = 3 or 4/treatment group) and their results were similar to those of the nonsmokers.

The participants gave 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.

Protocol

Subjects consumed weighed diets with either high or low iron bioavailability for 12 wk. The diets were randomly assigned and blocking was used to obtain similar serum ferritin values in both diet groups. The diets consisted of repeating 2-d menus. Dietary heme- and nonheme-iron absorption from the entire 2-d menu were measured initially and after 10 wk to test for adaptation with time. Blood iron indexes were measured at 0, 2, 10, and 12 wk. Fecal ferritin excretion was measured in feces collected for 12 d after each iron-absorption measurement.

Although a similar number of volunteers were assigned to consume each diet, 14 of those consuming the high-bioavailability diet and 17 consuming the low-bioavailability diet completed the study. Because of limited physical facilities, the men were studied at different times in 4 subgroups. In one subgroup of 8 men, participation was interrupted by a natural flood disaster after the first 2 wk of the study. After a delay of 4.5 mo, these 8 men began the 12-wk feeding period again; however, the initial iron-absorption measurements were not redone (to limit the use of radioactive tracers in these men). The initial iron-absorption measurements were compared with final measurements taken 10 wk after the feeding period was resumed. The diets were consumed for an additional 2 wk (12 wk total after the flood) to obtain final fecal and blood measurements. Statistical analyses yielded similar results with or without inclusion of data from this subgroup of 8 men; thus, data for these men are included in the data presented.

Diets

Two weighed, experimental diets in a 2-d menu cycle were planned by registered dietitians using ordinary foods, but food selections and serving sizes were varied to minimize or maximize iron bioavailability (**Tables 1** and **2**). The diet with high iron bioavailability provided generous quantities [394 g (≈14 oz)/d] of meat or poultry (two-thirds as beef or pork and one-third as chicken), refined cereal and grain products, no coffee or tea, and foods with ≥75 mg ascorbic acid with each meal. The low-bioavailability diet contained no meat, limited amounts [66 g (≈2.3 oz)/d] of poultry (chicken) and fish (shrimp), plenty of legumes and whole-grain cereal and bread products, tea (from 1 g dry, black instant) at each meal, and foods with an ascorbic acid content sufficient to just meet the recommended dietary allowance (27), distributed over several meals.

The 2 diets had similar calcium and total iron contents, but the high-bioavailability diet contained considerably more heme iron and ascorbic acid, slightly more vitamin A (calculated as retinol equivalents from retinol and β-carotene combined) (24), and considerably less phytic acid than did the low-bioavailability diet (Table 2) (25). The refined bread and cereal products in the menus were commercially enriched with iron to the extent common in the United States [20 mg per pound (460 g) flour]; iron-fortified breakfast cereals were not used. Coffee was excluded from the diets. City water, a low-energy carbonated water, and chewing gum were consumed as desired after analyses indicated a minimal trace element content. Limited amounts of salt, pepper, and selected low-energy carbonated beverages were individualized to volunteers' preferences and then served consistently throughout the study.

All diet ingredients except water were weighed, prepared, and provided to the volunteers by the research center. Volunteers ate one meal at the research center on weekdays and consumed the remaining foods away from the research center after minimal reheating. Foods were weighed to 1% accuracy and consumed quantitatively. So that individual body weights could be maintained, energy intakes were adjusted in increments of 1.13 MJ (270 kcal) by proportionally changing the amounts of all foods.

Iron-absorption measurements

Heme- and nonheme-iron absorption were measured by isotopically labeling the food items from the entire 2-d menu (3 meals/d for 2 d; evening snack foods were served with the third meal) with 37 kBq [^{55}Fe]hemoglobin and 37 kBq $^{59}FeCl_3$ at the beginning (days 1 and 2) and after 10 wk (days 70 and 71) of the 12-wk controlled-diet period. Radiolabeled hemoglobin was obtained by intravenously injecting 74 MBq ^{55}Fe into an iron-deficient, pathogen-free rabbit; bleeding the animal 2 wk later; and removing the stroma by lysis and centrifugation (28). The

TABLE 1
Menus for diets with high or low iron bioavailability

	High bioavailability		Low bioavailability	
	Day 1	Day 2	Day 1	Day 2
Breakfast	Orange juice Biscuits Cheese Ham	Orange juice Plain bagels Cream cheese Pork sausage	Instant tea Whole-wheat bread[1] Peanut butter[2] Strawberry jelly Red grapes	Instant tea Apple juice Wheat bagels[1] Cream cheese Blueberries Milk (2% fat)
Lunch	Hamburger White bun Ketchup Potato chips Lettuce salad Ranch dressing Cantaloupe Red grapes	Spaghetti with meat sauce Parmesan cheese Caesar salad Italian dressing White dinner roll Margarine Pineapple	Instant tea Bean and cheese burrito[2] Lettuce Ripe olives Taco sauce Sour cream Tortilla chips Apple crisp[1] Milk (2% fat)	Instant tea Spaghetti with tomato sauce Parmesan cheese Lettuce salad Ranch dressing Whole-wheat dinner roll[1] Margarine Frosted angel cake
Supper	Chicken pasta alfredo Broccoli White dinner roll Margarine Cheesecake Strawberries	Baked chicken Potatoes with gravy Corn Margarine Cabbage coleslaw Angel cake Mandarin oranges	Instant tea Shrimp pasta alfredo Peas Whole-wheat roll[1] Margarine Cheesecake Strawberries	Instant tea Baked chicken Potatoes with gravy Corn Baked beans[2] Whole-wheat bread[1] Margarine
Snack	Brownie Milk (2% fat)	Chocolate sundae Milk (2% fat)	Brownie	Chocolate sundae with peanuts[2]

[1] Contained whole-grain ingredients.
[2] Contained legumes (other than green peas).

isotopes were added to the diet in proportion to the heme- and nonheme-iron contents of the meals, yielding constant specific activities (ratios of ^{55}Fe to dietary heme iron and ^{59}Fe to nonheme iron) for all 6 meals. Accordingly, for the low-bioavailability diet, [^{55}Fe]hemoglobin was added only to the one meal daily that included heme iron (Table 1). The tracers were transferred with a pipette onto the foods that were the best sources of that form of iron in each meal. Meat, poultry, and fish dishes were precooked, cooled, radiolabeled, and then minimally reheated in the microwave just before being served.

Although dietary energy was occasionally adjusted over time to maintain body weights, the amount of energy served with the radiolabeled meals was consistent between dietary treatments for each participant. All labeled meals were consumed at the research center.

Absorption of nonheme iron was measured by whole-body scintillation counting, which detected only the gamma-emitting ^{59}Fe radioisotope. This custom-made whole-body counter uses 32 crystal NaI(Tl) detectors, each 10 × 10 × 41 cm, arranged in 2 planes above and below the participant, who lies supine. Initial total body activity was calculated from whole-body activity after 2 meals (measured ≥1 h after the second meal but before any unabsorbed isotope was excreted), divided by the fraction of the total activity contained in those 2 meals. The percentage of nonheme-iron absorption was measured as the portion of initial whole-body activity that remained after 2 wk (day 15), with correction for physical decay and background activity measured 1–2 d before the meals. In a previous study (21), the slopes of semi-logarithmic whole-body retention plots for 4 wk after isotope administration were not consistently different from zero; this indicates that iron excretion was minimal and that it was unnecessary to correct for endogenous excretion of iron during the 2 wk after isotope administration.

Radioisotope concentrations in blood (29) were also measured after 2 wk (day 15) and expressed as fractions of the administered radioisotope, determined from aliquots prepared when the foods were labeled. The blood retention of ^{59}Fe, expressed as a percentage of the administered dose, was measured from the blood radioisotope concentration together with an estimate of total blood volume based on body height and weight (30). The incorporation of iron into blood, expressed as a percentage of 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. Heme-iron absorption was determined by multiplying nonheme-iron absorption (measured by whole-body counting) by the ratio of ^{55}Fe to ^{59}Fe in the blood, with correction for physical decay and background activity measured before the meals. Absolute absorption of heme and nonheme iron (mg/d) was calculated by multiplying the observed percentage absorption by the analyzed dietary content of heme and nonheme iron, respectively. Total iron absorption (mg/d) was calculated as the sum of heme- and nonheme-iron absorption.

Chemical analyses

Fasting blood samples of 30 mL each were obtained at 0, 2, 10, and 12 wk. Duplicate diets were prepared for iron analyses.

TABLE 2
Calculated composition of the diets with high or low iron bioavailability[1]

	High bioavailability	Low bioavailability
Energy		
(MJ)	13.1 ± 1.3[2]	13.5 ± 1.5
(kcal)	3132 ± 303	3223 ± 348
Total iron (mg)	21.0 (16.2)	20.2 (15.4)
Nonheme iron (mg)	18.3 (14.4)	19.8 (15.3)
Heme iron (mg)	2.7 (1.8)	0.5 (0.1)
Ascorbic acid (mg)	284	61
Vitamin A (μg retinol equivalents)	1417	1160
Phytic acid (mg)	475	1851
Calcium (mg)	1062	1144

[1] Calculated from US Department of Agriculture food-composition data (24) and published data on phytic acid composition of foods as determined by a method of the Association of Official Analytical Chemists (25). For calculations of heme and nonheme iron, it is assumed that heme iron accounts for 40% of the total iron in meat, poultry, and fish (26); this fraction was verified by our analyses of total and heme iron. Actual values (determined by laboratory analysis) are in parentheses.

[2] $\bar{x}$ ± SD.

Feces were collected in 6-d composites for 12 d after each set of labeled meals (days 1–6, 6–12, 71–76, and 77–82). During sample collection, precautions were taken to avoid contamination by trace minerals.

Portions of the diet composites were digested with concentrated nitric acid and 70% perchloric acid by method (II)A of the Analytical Methods Committee (31). The iron content of the digestates was measured by inductively coupled argon plasma emission spectrophotometry. Analytic accuracy was monitored by assaying the typical diet (standard reference material 1548a) from the US National Institute of Standards and Technology (Gaithersburg, MD). Mean (±SD) measurements were 95 ± 9% of certified values for iron.

The same digestion and inductively coupled argon plasma emission methods were used to measure nonheme iron in meat-containing foods, after extraction by the procedure of Rhee and Ziprin (32). Heme iron in these foods was calculated as the difference between total and nonheme iron. By this method, heme iron was 42%, 39%, 45%, 35%, and 33% of the total iron in raw beef, raw chicken, raw pork, precooked ham, and precooked shrimp, respectively, consistent with the guideline that ≈40% of the iron in meat, poultry, and fish is heme iron (26). Our previous analyses indicated that cooking by our research procedures (generally, baking of individual dishes in closed containers) had negligible effects on the heme-iron content of beef and chicken dishes.

Hemoglobin, hematocrit, mean corpuscular volume, and erythrocyte distribution width were measured by using a Celldyne 3500 system (Abbott Laboratories, Abbott Park, IL). Serum iron was measured colorimetrically by using a Cobas Fara chemistry analyzer (Hoffmann-La Roche, Inc, Nutley, NJ) with a commercial chromogen (Ferene; Raichem Division of Hemagen Diagnostics, San Diego). Iron-binding capacity was similarly determined after a known amount of ferrous iron was added to the serum sample under alkaline conditions. Percentage transferrin saturation was calculated from serum iron and total iron-binding capacity. To reduce analytic variation, each volunteer's samples for either serum ferritin or fecal ferritin were stored frozen until they could be measured in a single analytic batch. Fecal ferritin was extracted from each lyophilized 6-d fecal composite by using the method described by Skikne et al (23) and filtered with 5-μm membrane filters. Serum and fecal ferritin concentrations were measured by an enzyme-linked immunosorbent assay using monoclonal antibodies (Abbott Laboratories) against human spleen ferritin, which mainly measure L-rich ferritin, the isoferritin primarily found in spleen and liver (33). The ferritin assay was calibrated against World Health Organization ferritin 80/602 First International Standard. Protein in fecal extracts was measured colorimetrically (34). C-reactive protein was measured by nephelometry (Behring Diagnostics Inc, Westwood, MA) to detect inflammation, which may be associated with increased serum ferritin, but this measurement was consistently within the normal range.

Statistics

Data on iron absorption, serum ferritin, and fecal ferritin 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 statistical relations were analyzed. Dietary treatment effects were measured by using repeated-measures analysis of variance (ANOVA) (35); Bonferroni contrasts were used to test for differences between high- and low-bioavailability diets with time and for differences between fecal ferritin concentrations at each time point. Absorption ratios (10 wk to 0 wk) were compared by using ANOVA. Simple linear and stepwise regression analyses were used to assess additional relations between variables (35).

RESULTS

Cross validation of iron absorption and erythrocyte incorporation

The 2 independent measures of ^{59}Fe retention (blood and whole body) were highly correlated on a logarithmic scale, despite retention of <1% of the administered dose by a considerable number of volunteers (**Figure 1**). Two weeks after isotope administration, 63% (±1 SD: 56–72%; range: 37–94%) of the absorbed ^{59}Fe (detectable by whole-body counting) had been incorporated into the blood. Incorporation was slightly but significantly lower (reduced to 58%; ± 1 SD: 44–72%; range: 27–84%) with the second isotope administration (a main effect of time) but was not affected by diet or a diet-by-time interaction. Blood incorporation of the absorbed iron was inversely associated with ln(serum ferritin) at both time points (initial measurement: R^2 = 0.20, P < 0.01, n = 31; final measurement: R^2 = 0.22, P < 0.01, n = 31) and was not associated with age.

Adaptation of iron absorption

The efficiency of nonheme-iron absorption adapted significantly to dietary iron bioavailability over time. A nearly 5-fold difference in nonheme-iron absorption (3.4% compared with 0.7%) between the 2 diets at the beginning of the study was significantly reduced to just over a 2-fold difference (2.1% compared with 0.9%; P < 0.01) after 10 wk (**Table 3**). Both a decrease in nonheme-iron absorption with time on the high-bioavailability diet (from 3.4% to 2.1%; P < 0.01) and an increase with time on the low-bioavailability diet (from 0.7% to

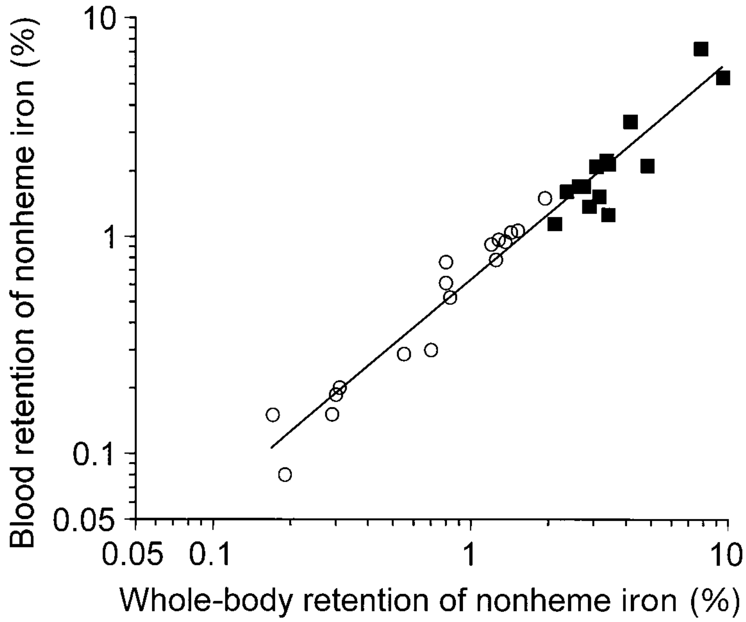

FIGURE 1. Correlation between ^{59}Fe in the blood and whole-body retention of the isotope [$\ln(y) = -0.47 + 1.00 \ln(x)$; $R^2 = 0.95$, $P < 0.0001$; $n = 31$] 2 wk after the isotope was first administered (weeks 0–2) in the high (■)- and low (○)-iron bioavailability diet groups. Results were comparable at weeks 10–12 [$\ln(y) = -059 + 1.04 \ln(x)$; $R^2 = 0.91$, $P < 0.0001$; $n = 31$ (data not shown)].

0.9%; $P < 0.05$) were significant. Adaptation was indicated both by a significant interaction between diet and time (ANOVA) and by significantly different absorption ratios (10 wk to 0 wk) between the 2 diets (Table 3). Because the 2 diets were similar in nonheme-iron content (Table 2), the results for absolute nonheme-iron absorption (mg/d) were similar to those for the absorptive efficiency (percentage absorption) (Table 3).

In contrast with nonheme-iron absorption, there was no significant difference in the efficiency of heme-iron absorption from the 2 diets nor any adaptation of heme-iron absorption with time (Table 3). However, because the high-bioavailability diet contained considerably more heme iron (Table 2), the absolute amount of heme iron absorbed from the 2 diets was substantially different (0.45 compared with 0.016 mg/d for the high- and low-bioavailability diets, respectively; $P < 0.01$) (Table 3), without changing significantly during the 10 wk between measurements.

The difference in the total amount of iron absorbed between the 2 diets was reduced from 8-fold (0.96 compared with 0.12 mg) to 4-fold (0.69 compared with 0.17 mg) in 10 wk (Table 3). The men consuming the high-bioavailability diet began the study absorbing nearly 1 mg total Fe/d but adapted to reduce their absorption to 0.69 mg/d (±1 SD: 0.52–0.92 mg/d) (Table 3), suggesting that these men needed no more than 0.7 mg/d, on average, to satisfy their requirement for absorbed iron.

Blood indexes of iron status

Despite considerable differences in iron absorption, blood indexes of iron status were unaffected by dietary treatment. Hemoglobin, erythrocyte distribution width, transferrin saturation, and serum ferritin were unaffected by time on the diet (the time-by-diet interaction was not significant). Although the diets were randomly assigned and blocking was used for serum ferritin, this assignment coincidentally resulted in slightly greater initial transferrin saturation for the group consuming the low-bioavailability diet (**Table 4**). It is unlikely that this difference confounded the iron-absorption results because it was slight, was within the normal range, was present initially and did not change with time on the diet, and was associated with a slight but opposite nonsignificant difference in serum ferritin.

Serum ferritin was unaffected by dietary treatment but declined significantly over time in both diet groups (Table 4), presumably because of blood sampling. The increased nonheme-iron absorption by volunteers consuming the low-bioavailability diet was probably not related to the reduction in ferritin with time. In a similar study (ZK Roughead and JR Hunt, unpublished observations, 1999), nonheme-iron absorption did not change significantly in the placebo group who consumed self-selected diets and had comparable amounts of blood drawn and reductions in serum ferritin. Furthermore, in the present study, the reduction in nonheme-iron absorption with time in the group consuming the high-bioavailability diet (Table 3) occurred despite the slight decrease in serum ferritin. Apparently, the adaptation observed in nonheme-iron absorption (Table 3) was independent of changes in serum ferritin.

Cross-sectional associations between serum ferritin and iron absorption

At the beginning of the study (week 0), nonheme-iron absorption was inversely related to serum ferritin in the high-bioavailability diet group but not in the low-bioavailability diet group (**Figure 2**). Interestingly, after 10 wk, this relation was no longer significant in the high-bioavailability diet group but had become significant in the low-bioavailability diet group. The change in percentage nonheme-iron absorption (10 wk/0 wk) tended to be more pronounced in volunteers with lower serum ferritin concentrations, especially for those consuming the low-bioavailability diet (high-bioavailability diet: $R^2 = 0.10$,

TABLE 3

Dietary heme- and nonheme-iron absorption in the subjects before (0 wk) and after 10 wk of consuming the diets with high or low iron bioavailability[1]

	High bioavailability (n = 14)	Low bioavailability (n = 17)	P Diet	P Time	P Diet × time
Nonheme-iron absorption (%)					
0 wk	3.4 (2.4–4.8)	0.7 (0.5–1.0)[2]	0.0001	NS	0.0003
10 wk	2.1 (1.5–3.0)[3]	0.9 (0.7–1.3)[2,3]			
Ratio (10 wk/0 wk)	0.6 (0.4–1.0)	1.4 (0.9–2.3)[2]	0.0001		
Nonheme-iron absorption (mg)					
0 wk	0.49 (0.35–0.69)	0.10 (0.07–0.15)[2]	0.0002	NS	0.0003
10 wk	0.30 (0.22–0.43)[3]	0.15 (0.10–0.20)[2,3]			
Heme-iron absorption (%)					
0 wk	26 (22–31)	22 (18–26)	NS	0.06	NS
10 wk	22 (18–26)	21 (18–26)			
Ratio (10 wk/0 wk)	0.8 (0.7–1.1)	0.9 (0.7–1.2)	NS		
Heme-iron absorption (mg)					
0 wk	0.45 (0.38–0.54)	0.016 (0.01–0.02)[2]	0.0001	0.06	NS
10 wk	0.38 (0.31–0.45)	0.016 (0.01–0.02)[2]			
Total iron absorption (mg)					
0 wk	0.96 (0.72–1.29)	0.12 (0.09–0.17)[2]	0.0001	NS	0.0008
10 wk	0.69 (0.52–0.92)[3]	0.17 (0.12–0.22)[2,3]			

[1] Geometric $\bar{x}$; ±1 SD in parentheses.
[2] Significantly different from high bioavailability, $P < 0.05$.
[3] Significantly different from 0 wk, $P < 0.05$.

NS; low-bioavailability diet: $R^2 = 0.25$, $P < 0.05$, data not shown). Heme-iron absorption was not significantly associated with serum ferritin in either diet group or in the 2 diet groups combined.

Fecal excretion of ferritin

Fecal ferritin excretion was significantly affected by dietary iron bioavailability and changed significantly with time, depending on the diet. Fecal ferritin excretion was significantly lower in the low-bioavailability diet group than in the high-bioavailability diet group, whether expressed as absolute daily excretion or in relation to the protein concentration of the fecal extract (Table 4). The difference between the 2 diets was apparent in the first 6-d stool sample and was nearly maximized with an 8-fold difference in the 7–12-d sample. A similar 8-fold difference persisted at the end of the study. The difference in fecal ferritin observed in the first 6-d stool sample probably was not a preexisting difference between groups because the diets were randomly assigned, the observed differences increased with time, and the difference was consistent with observations from previous work (21). However, future studies should collect fecal samples earlier (ie, at baseline) because fecal ferritin excretion adjusted to differences in dietary iron bioavailability within just a few days.

Fecal ferritin, expressed as absolute daily excretion, was directly associated with serum ferritin in both diet groups and at most of the 4 times that stool samples were collected. These associations were somewhat weaker when fecal ferritin was expressed in relation to the protein concentration of the extract (R^2 = 0.13–0.55, 4 of 8 correlations with $P < 0.05$), rather than as absolute daily excretion (R^2 = 0.21–0.62, 7 of 8 correlations with $P < 0.05$) (n = 14 or 17).

DISCUSSION

The results of the present study suggest that men with normal iron stores adapt to dietary iron bioavailability, increasing or decreasing nonheme-iron absorption to restore and maintain iron homeostasis. The initial values of 3.4% nonheme-iron absorption, 26% heme-iron absorption, and 0.96 mg total Fe absorption/d from the high-bioavailability diet in this study (Table 3) are comparable with the 4.5% nonheme-iron absorption, 23.2% heme-iron absorption, and 0.97 mg total Fe absorption/d from a high-bioavailability diet by men who were not blood donors (4). Although nonheme-iron absorption from the low-bioavailability diet (Table 3) was very low in these iron-replete men, the initial 5-fold difference between the high- and low-bioavailability diets (Table 3) was consistent with a 5-fold difference between high- and low-bioavailability meals reported by Cook et al (36).

The men in the present study had not maximized their ability to down-regulate iron absorption from a Western diet with high iron bioavailability. Although the initial absorption of ≈1 mg Fe/d was similar to that reported by Hallberg et al (4), the subsequent reduction in absorption (Table 3) suggests that men may need to absorb no more than 0.7 ± 0.2 mg/d. The estimation that men excrete 1 mg Fe/d (27), based on blood radioiron-retention plots for 2–5 y, is probably an overestimate of iron excretion because men whose radioiron tracer did not decrease significantly during the study were excluded (37). Earlier radiotracer work (38) indicated less excretion (0.33–0.52 mg/d). Adaptation data can contribute to estimates of dietary iron requirements.

Surprisingly, the decrease in absorption in the high-bioavailability diet group occurred despite the reduction in serum ferritin, which was unrelated to dietary treatment and was probably caused by procedural phlebotomy. This suggests that serum ferritin was not directly involved in the adaptation in iron absorption.

Unlike serum ferritin excretion, fecal ferritin excretion responded rapidly to dietary iron bioavailability. The greater fecal ferritin with the high-bioavailability diet than with the low-bioavailability diet (Table 4) was consistent with our previous report on vegetarian diets (21) and with increased fecal ferritin in response to oral or intravenous iron administration (23). These changes in fecal ferritin may reflect a passive response to the amount of iron entering the mucosal cell or may support the "mucosal block" theory that ferritin controls iron absorption by trapping unwanted iron and preventing its serosal transfer (39, 40). Consistent with the positive association between fecal and serum ferritin (21, 23), fecal ferritin excretion was greater in this study of iron-replete men than in our previous study of young women (21). However, the amount of ferritin excreted did not account for a substantial excretion of mucosal iron, as would be predicted by the mucosal block theory. This may reflect the nonquantitative nature of the assay (eg, partial recovery of mucosal ferritin because of intestinal digestion) or a minor contribution of mucosal ferritin to the control of iron absorption. Whether ferritin plays an active or a passive role, the rapid change in fecal ferritin suggests intestinal adaptation to the altered mucosal iron uptake resulting from the different luminal solubility of iron from the 2 diets.

The present results indicate that short-term studies overestimate differences in dietary iron bioavailability, even when bioavailability is determined from whole diets rather than from single meals. Studies of dietary iron bioavailability commonly tested absorption from single meals or a few days of meals without allowing for equilibration to the test diet. The results of such investigations are comparable with the initial measurements from the present study. After 10 wk of equilibration, differences in nonheme-iron absorption were reduced from 5-fold to >2-fold (Table 3) and differences in total iron absorption from 8-fold to 4-fold (Table 3). Presumably, the differences in absorption observed at 10 wk would in time (perhaps requiring months or years) affect body iron stores and serum ferritin, and this would likely cause iron absorption to adapt further. As reported previously, serum ferritin is inversely associated with a range of ≥15-fold in nonheme-iron absorption and 2–3-fold in heme-iron absorption (3). Thus, one can hypothesize that, as dietary iron bioavailability gradually changes body iron stores, absorptive efficiency is further modified to offset this change, tending to preserve the homeostatic status quo, or biological set point, for iron stores (9, 10).

Although the differences in bioavailability observed in short-term studies are reduced by biological adaptation, epidemiologic studies indicate that dietary iron bioavailability influences body iron stores over time. Consistent with the results of the present study (Table 3), heme iron appears to be more influential than nonheme iron. Meat consumption was positively related to iron status in 5 large studies (41–45), although the relation occurred only in women in 2 of those studies (41, 42) and did not occur in 1 other large study (46). In studies presenting regression analyses to predict serum ferritin, positive associations with meat intake accounted for only 3–6% of the total variance (42, 43). In a recent report (45), serum ferritin of an elderly population was positively associated with heme iron (but not with dietary nonheme iron), supplemental iron, dietary vitamin C, and alcohol, and negatively associated with caffeine (especially from coffee). However, dietary factors, including

TABLE 4
Blood indexes of iron status and fecal ferritin excretion in the subjects before (0 wk) and 2, 10, and 12 wk after consuming the diets with high or low iron bioavailability

	High bioavailability (*n* = 14)	Low bioavailability (*n* = 17)	*P* Diet	*P* Time	*P* Diet × time
Hemoglobin (g/L)					
0 wk	152 ± 3[1]	156 ± 3	NS	0.005	NS
2 wk	150 ± 3	155 ± 3			
10 wk	152 ± 3	158 ± 3			
12 wk	149 ± 3	155 ± 3			
Transferrin saturation (%)					
0 wk	23 ± 8	27 ± 8	0.05	NS	NS
2 wk	21 ± 8	28 ± 8			
10 wk	19 ± 8	29 ± 8			
12 wk	23 ± 8	27 ± 8			
Serum ferritin (μg/L)					
0 wk	118 (101–139)[2]	100 (86–118)	NS	0.005	NS
2 wk	108 (93–127)	93 (79–109)			
10 wk	110 (94–129)	86 (74–101)			
12 wk	105 (90–123)	82 (70–96)			
Fecal ferritin[3]					
(μg/d)					
Days 1–6	123 (71–211)	44 (26–76)	0.0001	0.0001	0.0006
Days 7–12	120 (70–207)	16 (9–27)			
Days 71–76	98 (57–169)	12 (7–20)			
Days 77–82	97 (56–167)	13 (7–22)			
(μg/g protein)					
Days 1–6	552 (329–926)	123 (73–206)	0.0001	0.0001	0.0002
Days 7–12	516 (307–865)	41 (24–69)			
Days 71–76	535 (319–897)	37 (22–61)			
Days 77–82	399 (238–669)	34 (20–57)			

[1] $\bar{x}$ ± SD.
[2] Geometric $\bar{x}$; ±1 SD in parentheses.
[3] Fecal ferritin values were significantly ($P < 0.01$) affected by diet at each sampling time and changed significantly ($P < 0.01$) with time after the first 6-d sample in the low-bioavailability diet group but not in the high-bioavailability diet group, as evaluated by Bonferroni contrasts.

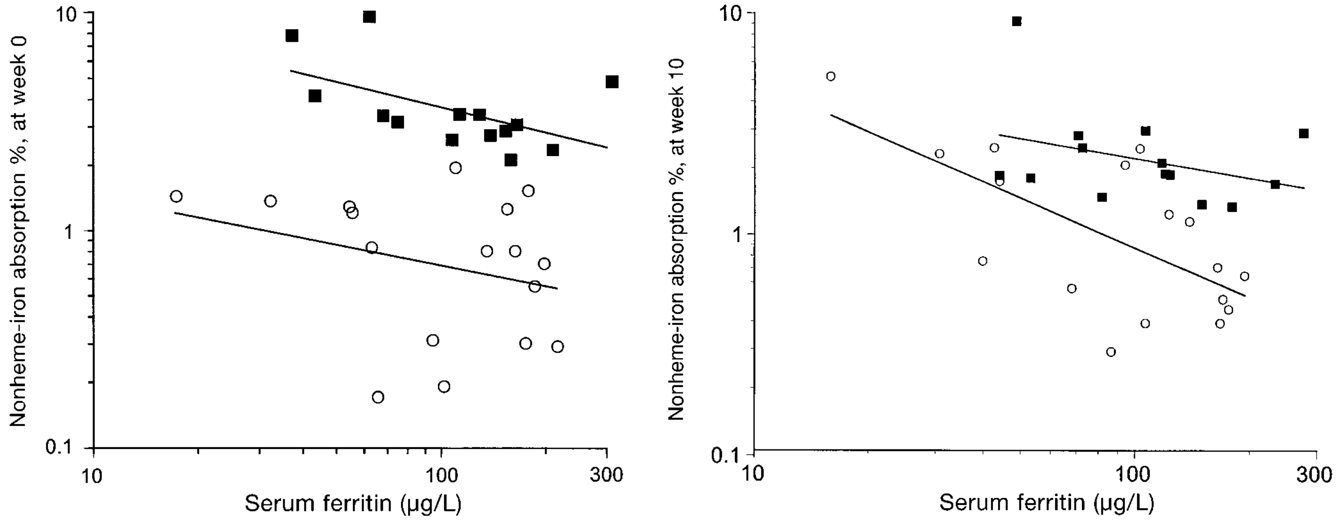

FIGURE 2. Comparison at weeks 0 and 10 of the association between serum ferritin and nonheme-iron absorption in subjects consuming the high (■)-iron-bioavailability diet or the low (○)-iron-bioavailability diet: week 0 (■: $R^2 = 0.29$, $P < 0.05$; $n = 14$. ○: $R^2 = 0.08$, NS; $n = 17$) and week 10 (■: $R^2 = 0.12$, NS; $n = 14$. ○: $R^2 = 0.44$, $P < 0.01$; $n = 17$).

iron supplements, accounted for only 17–18% of the variance in serum ferritin (45). Thus, although dietary bioavailability influences iron stores, the effects are long-term, are less than predicted from short-term absorption studies, and account for a minor portion of the variation in serum ferritin of a population.

Additional research is needed to determine whether women with low serum ferritin adapt to dietary iron bioavailability to the same extent as do men. The previous adaptation work by Cook et al (14) and Brune et al (47) suggests limited or no adaptation to specific enhancers or inhibitors of nonheme-iron absorption (*see* Introduction). However, further research is needed to determine whether the adaptation observed in the present study reflects a general reduction in the efficiency of nonheme-iron absorption or a more defined adaptation to specific enhancers and inhibitors of nonheme-iron absorption.

The 63% incorporation of absorbed iron into erythrocytes in these men, aged 32–56 y, is more similar to the 66% reported in older men (64–83 y) than to the 91% or 93% reported in younger men (19–33 y) (48, 49) and women (49). These differences are consistent with lower serum ferritin values in men aged <32 y and in women (8). The reduced incorporation observed with time in this study may be an unexplained effect of the controlled diet, given that this did not occur in placebo recipients consuming self-selected diets (ZK Roughead and JR Hunt, unpublished observations, 1999). If blood measurements only were used, the common assumption of 80% incorporation (29) would tend to produce an underestimate of true absorption by men with high serum ferritin.

In conclusion, there was an adaptive response in the absorption of nonheme but not heme iron in 10 wk in men consuming diets with either high or low iron bioavailability, resulting in reduced iron absorption from the high-bioavailability diet and increased iron absorption from the low-bioavailability diet. Differences in nonheme-iron bioavailability were reduced from 5-fold to >2-fold, and differences in total iron absorption were reduced from 8-fold to 4-fold. Serum ferritin and other blood measures of iron status were insensitive to dietary treatment, but fecal ferritin, an indicator of intestinal ferritin, changed within a few days in response to dietary iron bioavailability. The results indicate that men consuming Western diets have not maximized their ability to adapt their iron absorption to maintain homeostasis and that these men adapt to absorb an average of ≈0.7 mg Fe/d. This first longitudinal demonstration of adaptation to dietary iron bioavailability further indicates that short-term absorption measurements overestimate differences in iron bioavailability between diets.

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