Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk[1–3]

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See corresponding editorial on page 831.

ABSTRACT

Background: The characteristics of vegetarian diets suggest that these diets would have lower dietary iron bioavailability than nonvegetarian diets, but there is no evidence of iron deficiency in vegetarians.

Objective: We evaluated the responsiveness of serum and fecal ferritin to differences in iron absorption from controlled lactoovovegetarian and nonvegetarian diets.

Design: Twenty-one women aged 20–42 y with serum ferritin concentrations from 6 to 149 μg/L consumed lactoovovegetarian and nonvegetarian weighed diets for 8 wk each (crossover design). The diets differed substantially in meat and phytic acid contents. Nonheme-iron absorption was measured from the whole diets after 4 wk by using extrinsic ^{59}Fe and whole-body counting. Ferritin in extracts of fecal composites and in serum was measured by enzyme-linked immunosorbent assay the last 2 wk of each diet.

Results: Nonheme-iron absorption was less from the lactoovovegetarian diet than from the nonvegetarian diet (1.1% compared with 3.8%; $P < 0.01$; $n = 10$). Diet did not affect hemoglobin, transferrin saturation, erythrocyte protoporphyrin, or serum ferritin. Substantially less fecal ferritin was excreted with the lactoovovegetarian diet than with the nonvegetarian diet (1.1 compared with 6.0 μg/d, respectively; $P < 0.01$; $n = 21$).

Conclusions: This research indicates 1) 70% lower nonheme-iron absorption from a lactoovovegetarian diet than from a nonvegetarian diet; 2) an associated decrease in fecal ferritin excretion, suggesting partial physiologic adaptation to increase the efficiency of iron absorption; and 3) an insensitivity of blood iron indexes, including serum ferritin, to substantial differences in dietary iron absorption for 8 wk. Am J Clin Nutr 1999;69:944–52.

KEY WORDS Nonheme-iron absorption, bioavailability, iron status, serum ferritin, fecal ferritin, gastrointestinal adaptation, lactoovovegetarian diets, meat, phytic acid, hormonal contraceptives, women

INTRODUCTION

The Food and Nutrition Board of the National Research Council (1) has stated the following: "Iron deficiency anemia appears to be no more prevalent among vegetarian women than among nonvegetarian women, but further study of iron bioavailability in vegetarian diets is needed." Most studies of vegetarians in Western societies have not found poorer iron status in vegetarians than in omnivores on the basis of measurements of hemoglobin, hematocrit, serum iron, iron binding capacity, or transferrin saturation (2–5). However, several studies suggested that vegetarians, compared with omnivores, have a greater risk of low iron stores as indicated by lower concentrations of serum ferritin (5–11).

The iron bioavailability of vegetarian diets is a concern because these diets eliminate meat, which contains considerable amounts of highly absorbable iron, and because these diets commonly contain more inhibitors of iron absorption, such as phytic acid. Substantial research with single meals indicates excellent absorption of iron from meat, both because of highly bioavailable iron in the heme form (12–16) and because of unidentified factors in meat that promote heme-iron (12, 15) and nonheme-iron absorption (12, 14, 17). Inhibition of iron absorption by phytic acid (18) occurs in a dose-dependent manner (19), without apparent adaptation in persons who have consumed vegetarian diets for several years (20).

A primary objective of the present study was to measure nonheme-iron absorption from a whole lactoovovegetarian diet and to relate the absorption results to measures of iron status and excretion after an extended period (8 wk) of controlled diet. Results concerning zinc, other minerals, blood pressure, and plasma lipids are reported separately (21). Although nonheme-iron absorption from whole diets has been reported in a few other studies (22–26), this is the first study that allowed comparison of such absorption measurements with indexes of iron status after the same diets had been consumed for several weeks.

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TABLE 1
Calculated diet composition[1]

	Lactoovovegetarian	Nonvegetarian
Total iron (mg)	17.8 (12.6)[2]	17.3 (13.6)
Heme iron (mg)	0	1.5 (1.2)
Meat, 3/4 beef and 1/4 chicken (g)	0	184
Fiber (g)	40	16
Phytic acid (mg)	1656	542
Ascorbic acid (mg)	206	170
Vitamin A (μg RE)	1070	981
Calcium (mg)	983 (970)	986 (952)
Copper (mg)	2.5 (1.8)	1.5 (1.2)
Zinc (mg)	11.7 (9.4)	11.9 (10.9)

[1] Calculated from US Department of Agriculture food-composition data (33) and data on phytic acid in foods (32) with the assumption that heme iron is 40% of the total iron in meat, poultry, and fish (14) (this fraction was verified by our analyses of total and heme iron). Composition data are provided for the average energy of the diets, 9.6 MJ (2300 kcal). RE, retinol equivalents.

[2] Analyses in parentheses.

An additional objective of the present study was to determine the effect of differences in dietary iron bioavailability on fecal ferritin, an indicator of ferritin in the intestinal mucosa (27). Mucosal ferritin has been postulated to block the absorption of excess iron, preventing serosal transfer by retaining the iron in the mucosal cell until cell death and exfoliation into the intestinal lumen (28, 29). Mucosal ferritin (measured through intestinal biopsy) has been directly associated with serum ferritin (30) and inversely associated with heme-iron and nonheme-iron absorption (31).

SUBJECTS AND METHODS

Subjects

Study participants were 21 women aged ($\bar{x} \pm$ SD) 33.2 ± 7.0 y (range: 20–42 y), with a mean body weight of 62.1 ± 8.4 kg (range: 53–82 kg) and a mean body mass index (in kg/m^2) of 23.5 ± 2.8 (range: 19.0–29.0). Women were recruited through public advertisements and selected after an interview and blood analysis to establish that they had no apparent underlying disease and had not donated blood or used iron or zinc supplements providing >20 mg/d for ≥6 mo before the study. Applicants agreed to discontinue all nutrient supplements when their application was submitted, generally 6–12 wk before the start of the study. None of the women routinely used medications, except for 9 who routinely used hormonal contraceptives. The participants gave their informed consent and 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.

General protocol

Twenty-one women consumed both a lactoovovegetarian and a nonvegetarian diet for 8 wk each, with the diet order randomly assigned in a crossover design. The women changed dietary treatments after 8 wk without any delay. After 4 wk of each diet, nonheme-iron absorption was measured in a subsample of 10 women by labeling the entire 2-d menu cycle with ^{59}Fe. Zinc absorption, reported elsewhere (21), was determined by radiotracer in another subsample of 11 women. Because of limited physical facilities, these 2 subsamples were studied at different times, separated by a few weeks, and assignment into the 2 subsamples was determined by the chance order of volunteer recruitment into the study. Fecal ferritin excretion was measured in all women for the last 14 d of each diet, and blood measurements were made after 7 and 8 wk of each diet.

Diets

Registered dietitians planned 2 experimental diets containing ordinary foods in a 2-d menu cycle. Detailed menus are published elsewhere (21). The lactoovovegetarian and nonvegetarian diets contained 0 and 184 g meat (3 parts beef and 1 part chicken)/d (≈6.5 oz/d), respectively (Table 1). Refined bread and cereal products in the nonvegetarian diet were commercially enriched with iron to the extent common in the United States [43 mg Fe/kg flour (20 mg/lb)]; iron-fortified breakfast cereals were not used. In contrast with the nonvegetarian diet, the lactoovovegetarian diet contained legumes daily and used wholegrain (rather than refined) bread and cereal products, resulting in 2.5 times as much dietary fiber and 3 times as much phytic acid (Table 1). Dietary phytic acid was calculated from published data based on methods of the Association of Official Analytical Chemists (32). By HPLC analyses, the lactoovovegetarian diet contained 4 times as much total inositol phosphates as did the nonvegetarian diet (21). The lactoovovegetarian diet also contained somewhat greater amounts of fruit and vegetables and ≈21% more ascorbic acid than the nonvegetarian diet, as calculated from US Department of Agriculture food-composition data (33). Calcium contents of the 2 diets were not significantly different. Coffee and tea were excluded from the diets. City water, a low-energy carbonated water, and chewing gum were consumed by subjects as desired, after analyses indicated minimal trace element contents. Limited amounts of salt, pepper, and selected low-energy carbonated beverages were added to the diets according to each volunteer's preferences, and then served consistently throughout the study. Grain products were the main source of iron in both diets, followed by meat, poultry, and fish for the nonvegetarian diet and fruit and vegetables for the lactoovovegetarian diet (Figure 1).

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 some minimal reheating. Foods were weighed to 1% accuracy and consumed completely (dishes were scraped and then rinsed clean). To maintain each subject's body weight, we adjusted energy intakes in 0.84-MJ (200-kcal) increments by proportionally changing the amounts of all foods. Mean (±SD) daily energy consumption was 9.6 ± 0.9 MJ (2286 ± 222 kcal).

Measurement of nonheme-iron absorption

Nonheme-iron absorption was measured halfway through each diet period to allow time for equilibration and subsequent measurements and to presumably represent average absorption for the 8-wk period. After 4 wk of each diet, the entire menu (3 meals/d for 2 d; evening snack foods were served with the third meal) was labeled with 7.4 kBq (0.2 μCi) ^{59}Fe as an extrinsic radioisotopic tracer. For each meal, the tracer was pipetted onto the foods that were the best sources of nonheme iron and the

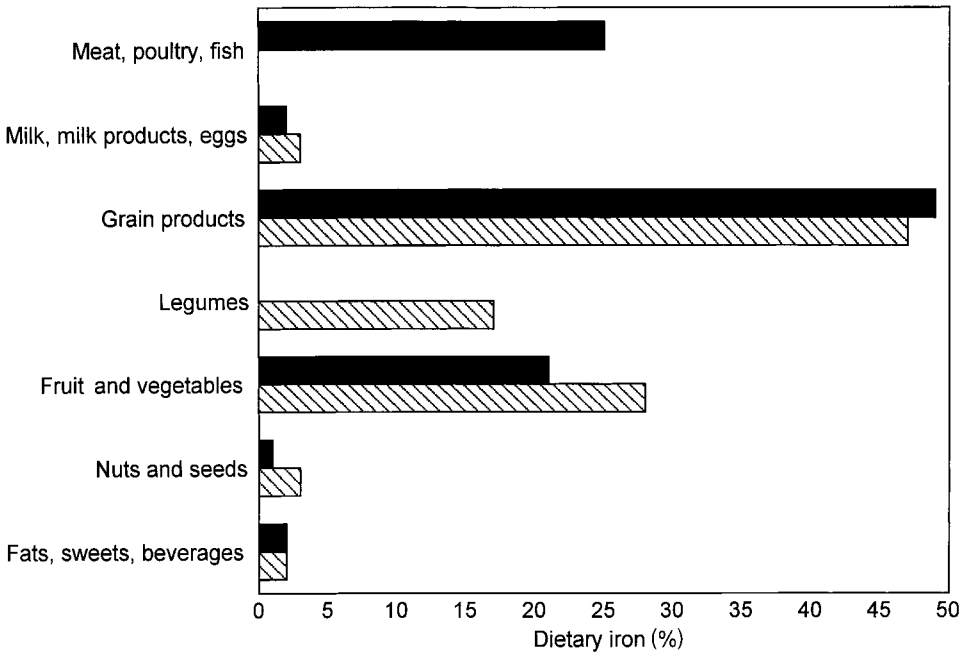

FIGURE 1. Distribution of iron in foods of the experimental diets (33). The nonvegetarian diet is indicated by the solid bars; the lactoovovegetarian diet is indicated by the hatched bars.

specific activity (ratio of ^{59}Fe to elemental nonheme iron) was constant for all meals. Although dietary energy was occasionally adjusted over time to maintain body weights, the amounts of energy served with the radiolabeled meals were consistent between dietary treatments for each participant. All labeled meals were consumed at the research center.

Absorption was determined by whole-body scintillation counting. Initial total-body activity was calculated from the whole-body activity after 2 meals (before any unabsorbed isotope was excreted), divided by the fraction of the total activity contained in those 2 meals. Percentage absorption was determined as the portion of initial whole-body activity that remained after 2 wk, with correction for physical decay and for background activity measured 1–2 d before the meals. The slopes of semilogarithmic whole-body retention plots for the final 4 wk of the diet period were not consistently different from zero, indicating that iron excretion was minimal and that it was unnecessary to correct for endogenous excretion of iron during the 2 wk after isotope administration.

The minimal amount of radioisotopic tracer used in the present study was sufficient for whole-body counting, but precluded comparison of the whole-body counting results with those from a more commonly reported method of measuring radioisotope concentrations in blood after 2 wk (34). Subsequent comparison of these 2 independent methods in our laboratory indicated that they were highly correlated ($r^2 = 0.95$, $n = 31$) and measured iron absorption with similar magnitude (JR Hunt and ZK Roughead, unpublished observations, 1998). In addition, the magnitude of nonheme-iron absorption from a hamburger meal administered under fasting conditions was similar in our laboratory to that reported by others (3.3% for healthy men and 7.1% for healthy women with ferritin concentrations >12 μg/L in our laboratory compared with 2.5% and 7.7%, respectively) (Hunt and Roughead, unpublished observations, 1998; 16). Thus, measurement of nonheme-iron absorption by whole-body counting was comparable with the more commonly used erythrocyte isotope-incorporation method (34) and with the results of other investigators using the same conditions (16).

To allow comparison of our results with the work of others and to eliminate the effect of differences in iron status of the volunteers, nonheme-iron absorption was normalized to that expected if the serum ferritin concentration of all volunteers was 40 μg/L. The following equation was used (22):

$$\text{Log } A_n = \log A_o + \log F_o - \log 40 \quad (1)$$

where A_n is normalized absorption, A_o is observed absorption, and F_o is serum ferritin (because serum ferritin was not affected by dietary treatment in this study, F_o was taken as the mean of all serum ferritin measurements during the study for each volunteer).

Absorption of nonheme iron (mg/d) was calculated by multiplying the observed percentage absorption by the analyzed dietary nonheme-iron content. Total iron absorption (mg/d) was calculated by adding the estimated heme-iron absorption to the nonheme-iron absorption. Heme-iron absorption from the nonvegetarian diet was estimated for each volunteer by using the analyzed heme-iron content of the diet and the following logarithmic relation between serum ferritin and percentage heme-iron absorption (25):

$$\text{Log (percentage heme-iron absorption)} = 1.9897 - 0.3092 \times \log (\text{serum ferritin in } \mu\text{g/L}) \quad (2)$$

Chemical analyses

Blood taken by phlebotomy was limited to 30 mL per dietary period and was obtained after 7 and 8 wk of each diet after subjects had fasted overnight. Analyses from these 2 samples were

averaged. Feces were collected completely for the last 14 d of each dietary treatment. Samples were collected with precautions to avoid trace mineral contamination.

Duplicate diets were prepared for iron analyses. Portions of the diet composites were digested with concentrated nitric acid and 70% perchloric acid by method (II)A of the Analytical Methods Committee (35). The iron content of the digestates was determined by inductively coupled argon plasma emission spectrophotometry (ICAP). Analytic accuracy was monitored by assaying bovine liver samples (Standard Reference Material 1577b) from the National Institute of Standards and Technology (Gaithersburg, MD). Mean (±SD) measurements were 99 ± 4% of certified values for iron.

The same digestion and ICAP method was used to measure nonheme iron in meat-containing foods after nonheme iron was extracted by the procedure of Rhee and Ziprin (36). Heme iron in these foods was calculated as the difference between total and nonheme iron. By this method, heme iron was 39.6% and 40.7% of the total iron in raw beef and chicken, respectively, which is consistent with the guideline that ≈40% of the iron in meat, poultry, and fish is in the heme form (14). Heme iron was also measured in the cooked foods (chicken burrito, beef lasagna, beef patty, and beef au gratin casserole) and there was no evidence that the amount of heme iron decreased with cooking.

Hemoglobin was measured with a Coulter counter (S+4; Coulter Electronics, Hialeah, FL). Serum iron was measured by Zeeman graphite furnace atomic-absorption spectrophotometry with prior precipitation by trichloroacetic acid (37). Iron binding capacity was measured by saturation with iron followed by adsorption of excess iron with magnesium carbonate. Percentage transferrin saturation was calculated from serum iron and total iron binding capacity. Zinc protoporphyrin was measured by hematofluorometry (38), C-reactive protein was measured by nephelometry (Behring Diagnostics Inc, Westwood, MA), and serum transferrin was measured by radioimmunodiffusion (Calbiochem-Behring, La Jolla, CA). Fecal ferritin was extracted from each lyophilized 14-d fecal composite by the method described by Skikne et al (27), filtered through 5-μm membrane filters, and measured. Serum and fecal ferritin were measured by an enzyme-linked immunosorbent assay with monoclonal antibodies against human spleen ferritin (Abbott Laboratories, Abbott Park, IL), which mainly measure L-rich ferritin, the isoferritin found primarily in spleen and liver (39). This assay is calibrated against World Health Organization ferritin 80/602 First International Standard. Protein in fecal extracts was determined colorimetrically (40).

To examine the possibility that the ferritin in the stools was from dietary sources, lyophilized diet composites were analyzed. No cross-reactivity was found. No testing was done for possible blood contamination of feces (attributable to gastrointestinal bleeding or menstruation); however, any such contamination could be expected to be small in these healthy women and to contribute to random variability. The stability of ferritin to digestive enzymes was tested in vitro by using the digestive method of Gangloff et al (41). Briefly, different quantities of ferritin standards were incubated at 37°C with a mixture of pancreatin and bile extract (both from Sigma, St Louis) suspended in 0.1 mol $NaHCO_3$/L, pH 7.1, for 2 h. This incubation resulted in a <5% reduction in ferritin as measured by enzyme-linked immunosorbent assay.

Statistics

Iron absorption, serum and fecal ferritin concentrations, and erythrocyte zinc protoporphyrin data 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. Dietary treatment effects were determined by using repeated-measures analysis of variance, with individual volunteers serving as their own controls (42). Pearson's correlation coefficients (42) were used to assess additional relations between variables.

RESULTS

Nonheme-iron absorption from the lactoovovegetarian diet was 70% less than from the nonvegetarian diet (1.1% compared with 3.8%; Table 2). The sequence in which the diets were fed to the volunteers did not significantly affect the results, suggesting that the results would not have been altered by a break or washout period between diets. Normalizing the observed absorption measurements to an arbitrary serum ferritin concentration of 40 μg/L decreased these values slightly (0.9% absorption from the lactoovovegetarian diet compared with 3.0% from the nonvegetarian diet; $P < 0.01$; $n = 10$) because the volunteers who participated in iron absorption measurements had serum ferritin values slightly <40 μg/L (geometric $\bar{x}$: 34 μg/L; $n = 10$). These normalized data are provided for comparison with other studies. Because the present study design used volunteers as their own controls, normalization to a similar serum ferritin concentration did not change the treatment effect and the normalized data were not used further. The observed amount of nonheme iron absorbed from the whole diet was 0.14 and 0.48 mg/d from the lactoovovegetarian and nonvegetarian diets, respectively (Table 2). Because heme-iron absorption contributed to iron absorption only for the nonvegetarian diet, total iron absorption was 0.14 and 0.89 mg/d from the lactoovovegetarian and nonvegetarian diets, respectively.

Despite this 6-fold difference in dietary iron bioavailability, none of the blood indexes of iron nutriture were affected by consuming these diets for 8 wk (Table 2 and Table 3), including serum ferritin, an indicator of iron stores. C-reactive protein, an indicator of inflammation that may influence serum ferritin concentrations, was not elevated in any volunteer and was unaffected by diet. An expected difference in serum ferritin can be estimated by using the general guideline that 1 μg ferritin/L blood serum corresponds to either 8–10 mg stored iron or 120 μg storage iron/kg body wt (43). According to this guidelines, a difference of 42 mg (0.75 mg/d for 8 wk) absorbed iron in this study would result in an estimated difference in serum ferritin of 4–5 μg/L. Although this difference is small, there was considerable statistical power to detect such a difference (>90% for a difference of 4 μg/L, $\alpha = 0.05$).

Serum ferritin was logarithmically and inversely associated with nonheme-iron absorption (Figure 2). The strength of this relation was similar for both diets, with diet influencing the intercept but not the slope of the association.

About one-sixth as much fecal ferritin was excreted with the lactoovovegetarian compared with the nonvegetarian diet (1.1 compared with 6.0 μg/d; Table 2). Results were similar when fecal ferritin was expressed as ng/mg protein in the stools (0.5 compared with 3.1 μg/d, respectively; $P < 0.001$; $n = 21$). Fecal ferritin was not correlated with nonheme-iron absorption from

TABLE 2
Effect of consuming lactoovovegetarian and nonvegetarian diets for 8 wk each on hemoglobin, serum ferritin, fecal ferritin, and iron absorption in women[1]

Subject, age, and hormonal contraceptive status[2]	Hemoglobin		Serum ferritin		Fecal ferritin		Nonheme-iron absorption, observed[3]		Nonheme-iron absorption, observed		Total iron absorption, nonveg[4]
	Veg	Nonveg	Veg	Nonveg	Veg	Nonveg	Veg	Nonveg	Veg	Nonveg	
	g/L		μg/L		μg/d		%		mg		mg
A, 37 y	129	135	6	6	0.1	1.1	—	—	—	—	—
B, 38 y	130	126	8	7	3.4	1.7	—	—	—	—	—
C, 31 y	128	127	8	9	0.4	0.5	—	—	—	—	—
D, 36 y	130	124	9	10	0.5	0.9	—	—	—	—	—
E, 39 y	132	129	10	9	0	0	5.9	12.9	0.75	1.71	2.27
F, 32 y	131	134	10	12	0.3	16.0	—	—	—	—	—
G, 42 y	132	137	12	11	0.3	0.8	—	—	—	—	—
H, 36 y	132	136	12	16	0.5	10.4	—	—	—	—	—
I, 33 y	148	152	18	20	0.3	10.2	2.4	6.7	0.36	0.90	1.34
J, 24 y, H	138	131	21	19	0.9	0.9	0.8	3.7	0.08	0.45	0.86
K, 25 y, H	127	134	23	30	3.5	76.7	4.3	10	0.59	1.13	1.48
L, 21 y	138	139	26	25	0.7	21.0	1.4	3.4	0.17	0.41	0.79
M, 31 y, H	139	129	34	33	4.9	45.0	—	—	—	—	—
N, 38 y, H	139	141	38	54	3.3	26.1	—	—	—	—	—
O, 24 y, H	131	140	44	42	0.9	34.6	1.5	4.4	0.20	0.50	0.80
P, 41 y	129	141	63	42	10.0	39.9	1.2	4.3	0.17	0.57	0.90
Q, 42 y, H	135	143	65	61	4.2	37.5	0.7	1.3	0.09	0.16	0.45
R, 20 y, H	132	134	80	65	1.3	10.0	0.1	2.7	0.01	0.41	0.76
S, 36 y, H	122	127	86	70	4.9	53.2	0.6	0.6	0.08	0.07	0.32
T, 42 y	131	128	144	155	0.5	4.9	—	—	—	—	—
U, 29 y, H	148	142	179[5]	117[5]	1.9	8.7	—	—	—	—	—
$\bar{x}$[6]	133	135	22	22	1.1	6.0[7]	1.1	3.8[7]	0.14	0.48[7]	0.89[8]
−1 SD	130	131	19	19	0.5	2.6	0.6	2.1	0.08	0.26	0.50
+1 SD	137	138	24	24	2.5	14.0	2.1	6.7	0.26	0.87	1.61

[1] Veg, lactoovovegetarian diet; Nonveg, nonvegetarian diet.
[2] H indicates volunteers who used hormonal contraceptives.
[3] Nonheme-iron absorption was measured in a subsample of 10 women chosen by the chance order of recruitment into the study.
[4] Total iron absorption from the vegetarian diet was the same as nonheme-iron absorption. For the nonvegetarian diet, total iron absorption was calculated from the heme- and nonheme-iron analyses of the diet, the observed nonheme-iron absorption (uncorrected for ferritin), and the estimated heme-iron absorption for each volunteer, based on the logarithmic relation between serum ferritin and heme-iron absorption (25).
[5] Serum ferritin analyses from one volunteer were eliminated post hoc from further analyses, with no effect on the nonsignificance of the dietary comparison. This volunteer's serum ferritin concentration varied considerably over the course of the experiment (consecutive values of 66, 198, 160, 130, and 103 μg/L during weeks 0, 7, 8, 15, and 16, respectively), despite consistent and normal C-reactive protein concentrations. Her fecal ferritin values did not appear to be affected and were retained in all analyses.
[6] Except for hemoglobin, values are geometric means. Serum ferritin values for each volunteer represent mean values from weeks 7 and 8 of each diet.
[7,8] Significantly different from lactoovovegetarian diet: [7] $P < 0.01$, [8] $P < 0.001$.

either diet. However, it was logarithmically and directly associated with serum ferritin (Figure 3). No significant correlation was found between fecal ferritin and any other iron status index (ie, transferrin saturation, iron binding capacity, plasma iron, or erythrocyte zinc protoporphyrin).

Hormonal contraceptive use was associated with greater serum ferritin concentrations (42 compared with 17 μg/L for contraceptive users and nonusers, respectively; $P < 0.01$; $n = 8$ and 12). Contraceptive use was also associated with greater fecal ferritin excretion (7.4 compared with 1.5 μg/d; $P < 0.01$; $n = 9$ and 12) and fecal ferritin expressed per total protein (3.0 compared with 0.8 ng/mg protein; $P < 0.01$). The women using contraceptives also absorbed iron less efficiently than did those who did not use contraceptives (1.4% compared with 3.6%; $P < 0.01$; $n = 6$ and 4). The effects of dietary iron bioavailability on ferritin, fecal ferritin, and iron absorption (lactoovovegetarian compared with nonvegetarian diets) were independent of the effects of hormonal contraceptive use.

Two of the 21 women (designated as S and T in Table 2) had self-supplemented with 18 mg Fe/d before applying to enter the study. These women tended to have higher serum ferritin concentrations and lower iron absorption (iron absorption was measured in only one of the women) than the other women.

DISCUSSION

Our results indicate substantially lower dietary iron bioavailability from a lactoovovegetarian diet characteristic of vegetarian diets in Western societies than from a nonvegetarian diet. Although it has been proposed that differences in nonheme-iron absorption may be less when measured from whole diets than when measured from single meals under fasting conditions (22), the present findings showed a 3.5-fold difference in nonheme-iron absorption with whole diets. The difference in nonheme-iron absorption between the 2 diets (1.1% compared with 3.8% for the lactoovovegetarian and nonvegetarian diets, respectively)

TABLE 3
Additional blood iron indexes and C-reactive protein concentrations of volunteers after consumption of lactoovovegetarian and nonvegetarian diets for 8 wk each[1]

	Lactoovovegetarian	Nonvegetarian
Serum transferrin (g/L)	262 (235, 289)	275 (248, 302)
Serum iron (μmol/L)	18 (15, 20)	17 (14, 19)
Iron binding capacity (μmol/L)	63 (60, 66)	64 (60, 67)
Transferrin saturation (%)	29 (23, 34)	28 (22, 34)
RBC zinc protoporphyrin (μmol/mol Hb)	17 (12, 24)	21 (15, 30)
C-reactive protein (mg/L)	0.32 (0.23, 0.42)	0.37 (0.28, 0.47)

[1]Least-squares means (−1 SD, +1 SD); n = 21; geometric mean for zinc protoporphyrin. RBC, red blood cell; Hb, hemoglobin. There were no significant differences between diets.

was greater than predicted (1.6% compared with 2.6%, respectively) by using a recently published bioavailability algorithm that adjusts for meat, poultry, fish, ascorbic acid, tea, and phytic acid in diets (44).

We know of one other study in which nonheme-iron absorption from whole diets was measured in women by using radioisotopic tracers with constant specific activity in meals (23). Women in that study absorbed 8.6% and 11.4% of nonheme iron from diets differing in distribution of calcium, with phytate and meat contents in both diets similar to the present nonvegetarian diet. These investigators described some of their subjects as being iron deficient; 8 of 21 (compared with 1 of 10 in the present study) had a serum ferritin concentration <15 μg/L (23). Thus, the 2–3 times greater iron absorption that Gleerup et al (23) observed probably reflected the lower iron status of their volunteers.

The present nonvegetarian diet met the recommended dietary allowance of 15 mg Fe/d (45) and was generally similar in composition to typical American diets, except for a greater calcium content (Table 1). We have no reason to believe that the nonvegetarian diet was inadequate in absorbable iron. The total iron absorbed from the nonvegetarian diet (0.89 mg/d) was slightly less than the estimated 1 mg Fe/d excreted by men (45). This estimate is also applied to women, after allowing for additional menstrual iron excretion (45). The 1-mg estimate, which was based on blood radioiron retention plots in men for 2–5 y, probably overestimated iron excretion because of exclusion of men whose blood radioiron tracer did not decrease significantly during the study (46). Greater iron excretion observed in Bantu men with higher iron stores (46) suggests that women, with lower iron stores, may excrete less (nonmenstrual) iron. Other radioiron tracer work indicated excretion of 0.33–0.52 mg Fe/d in 3 men and 1 woman aged 48 y (47). The distribution of women's menstrual excretion of iron is highly skewed, with some large values; the median amount of iron excreted throughout the menstrual cycle is 0.44 mg/d (48). The above excretion data suggest that many women of childbearing age may replace iron losses by absorbing 0.8–1.0 mg Fe/d, depending on menstrual loss. It is notable that the volunteer with the lowest iron stores (by serum ferritin) absorbed considerably more iron, 2.27 mg/d from the nonvegetarian diet, than did the other volunteers (Table 2).

The lower total iron absorption from the vegetarian diet than from the nonvegetarian diet (0.14 compared with 0.89 mg/d, respectively) is less likely to provide adequate absorbable iron to maintain iron stores for an extended period. Serum ferritin did not change in the 8-wk periods of the present study (Table 2); however, cross-sectional studies indicating lower serum ferritin concentrations in vegetarians than in omnivores suggest that differences would likely be detected after many months or perhaps years (5–11). Biological adaptation is likely to mitigate any change. Although vegetarians do not appear to adapt to inhibitors of iron absorption such as high-phytate wheat bran (20), this does not preclude adaptation through increases in the overall efficiency of iron absorption in response to lower iron

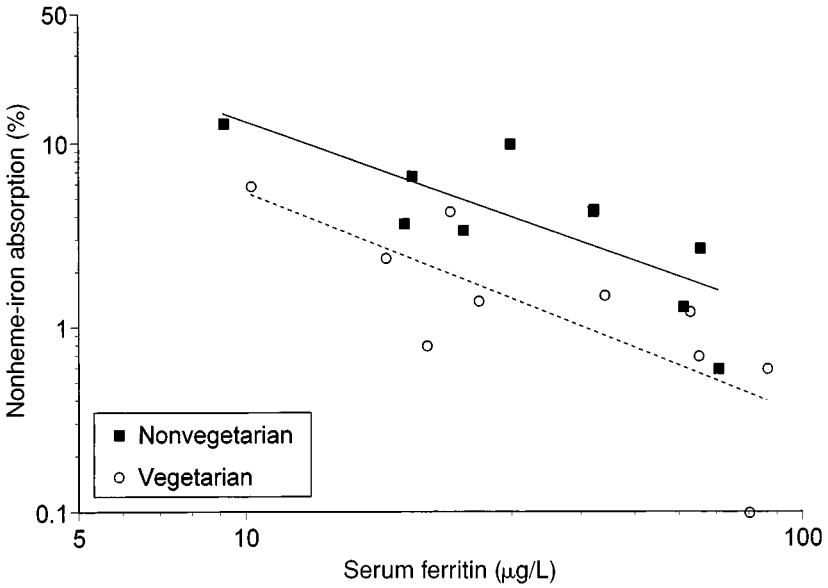

FIGURE 2. Relation between nonheme-iron absorption and serum ferritin. Data were log transformed. Nonvegetarian diet: $R^2 = 0.60$, $P < 0.01$; lactoovovegetarian diet: $R^2 = 0.59$, $P < 0.01$.

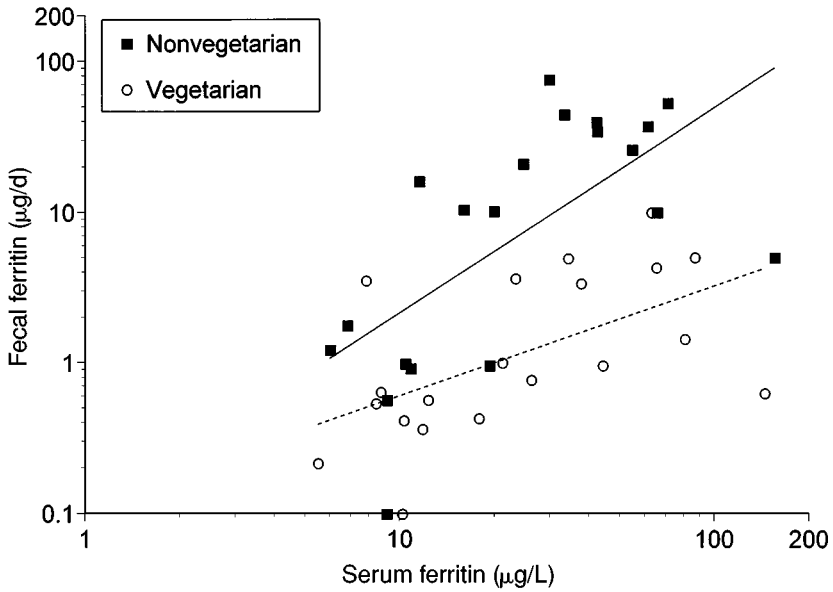

FIGURE 3. Relation between fecal ferritin and serum ferritin. Data were log transformed. Nonvegetarian diet: $R^2 = 0.43$, $P < 0.01$; lactoovovegetarian diet: $R^2 = 0.32$, $P < 0.01$.

stores (Figure 2). In this study, dietary iron bioavailability resulted in a 3.5-fold difference in nonheme-iron absorption, whereas, consistent with the report of Lynch et al (16), individual variation in serum ferritin was associated with a ≥10-fold difference (Figure 2). Biological control was apparently more influential than dietary iron bioavailability in determining nonheme-iron absorption from these diets. Adaptive control of absorption may explain why vegetarians often have lower iron stores than nonvegetarians (5–11) but not iron deficiency (2–5). Although the current study indicates much less iron absorption from a lactoovovegetarian diet than from a nonvegetarian diet, the serum ferritin concentrations and other iron indexes do not justify concern about the iron status of vegetarians without evidence of a greater incidence of iron deficiency.

The lack of change in serum ferritin was not because of insufficient statistical power. Intraindividual variation in serum ferritin concentrations of women consuming self-selected diets is relatively high (49), but is reduced by half when women consume controlled diets (50). In addition to logarithmic data transformation, the statistical power of the present study was probably also increased by having the volunteers serve as their own controls, having a diet period that was a multiple of a 4-wk menstrual cycle, and subsampling at 7 and 8 wk.

This research adds to a growing list of reports indicating that in controlled trials of several weeks' or months' duration, serum ferritin is unresponsive to changes in dietary iron bioavailability, whether through supplementation of meals with ascorbic acid (51–54) or calcium (26, 55), controlled meat intake (56), or a combination of factors such as meat and phytic acid contents (as in the present study). Although it has been suggested that there is less adaptive control of heme- than of nonheme-iron absorption (57), it has not been possible to show a positive response of serum ferritin to meat intake under controlled feeding conditions (Table 2) (56). Studies in which the relation between changes in serum ferritin and changes in body iron were quantified used phlebotomy (58, 59). The present study allowed a direct comparison of iron absorption with serum ferritin response and indicated that serum ferritin was not as responsive to changes in dietary iron absorption as was predicted from iron depletion by phlebotomy. As indicated above, years may be required for dietary changes to influence serum ferritin.

In contrast with serum ferritin, fecal ferritin excretion responded positively to dietary iron bioavailability (Table 2). The lactoovovegetarian diet contained slightly more ascorbic acid and vitamin A than the nonvegetarian diet (Table 1), both of which are enhancers of nonheme-iron absorption (14, 60). However, the lack of meat and increased phytic acid content reduced nonheme-iron absorption (14, 20), probably by reducing iron solubility in the intestinal lumen and entry into the intestinal mucosa. The lower fecal ferritin excretion observed with the lactoovovegetarian diet suggests reduced mucosal ferritin concentrations (27), which may have been a passive response to reduced mucosal iron, but is also consistent with the mucosal block hypothesis for the partial control of iron absorption (28, 29). According to this hypothesis, less mucosal ferritin would enhance serosal transfer of iron to the body by reducing mucosal cell blocking of iron, which is held as ferritin until cell exfoliation. However, if serosal transfer of mucosal iron was greater with the lactoovovegetarian diet, this did not fully offset the reduced luminal solubility of iron because iron absorption remained lower (Table 2).

The fecal ferritin measurements in this study did not account for a substantial excretion of mucosal iron. Powell et al (61) showed that in normal subjects only one-third of the iron initially taken up by the mucosal cell is retained by the body; the remaining two-thirds is excreted in the feces within days, presumably as ferritin iron. In the present study, if the excreted fecal ferritin was fully saturated with iron [4500 atoms Fe per molecule (62)], it would account for only 1.8–4.3 μg/d, compared with the 0.14 and 0.89 mg Fe/d absorbed from the lactoovovegetarian and nonvegetarian diets, respectively. This negligible amount of ferritin excreted may indicate nonquantitative recovery of exfoli-

ated mucosal ferritin because of partial intestinal digestion or may indicate a minor contribution of mucosal ferritin to control of total iron absorption in volunteers with relatively low iron stores. This is the first observation of increased fecal ferritin with increased dietary iron bioavailability (Table 2). This observation is consistent with a report by Skikne et al (27) of increased fecal ferritin associated with supplemental iron. Skikne et al (27) also reported a positive correlation between fecal and serum ferritin, as shown in the present study (Figure 3).

In conclusion, the present study of iron absorption and status of women consuming controlled lactoovovegetarian and nonvegetarian diets indicated the following: 1) 70% lower nonheme-iron absorption from a lactoovovegetarian diet than from a nonvegetarian diet, probably because of a lack of enhanced iron absorption from meat and lower intestinal iron solubility associated with substantial dietary phytic acid; 2) an associated decrease in fecal ferritin excretion, indicating intestinal responsiveness to dietary iron bioavailability; and 3) an insensitivity of blood iron indexes, including serum ferritin, to substantial differences in dietary iron absorption for 8 wk.

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