Dietary determinants of iron stores in a free-living elderly population: The Framingham Heart Study[1–4]

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See corresponding editorial on page 593.

ABSTRACT Epidemiologic studies have found a relation between body iron stores and risk of chronic disease. Iron-absorption studies from single meals have shown that many dietary factors can influence nonheme-iron bioavailability. However, little is known about the association of these dietary factors with iron stores in free-living elderly populations. To address this question, we investigated the consumption of various dietary components and iron stores in an elderly sample of The Framingham Heart Study participants. Serum ferritin was used as a measure of body iron stores in 634 free-living elderly (67–93 y of age), and dietary intake during the previous year was assessed by a food-frequency questionnaire. The relation between serum ferritin and various dietary factors was assessed by multiple regression analysis. Subjects whose ferritin concentrations might be pathologically elevated because of infection, inflammation, liver disease, or genetic hemochromatosis were excluded from the analysis. After we controlled for sex, age, body mass index, total energy intake, smoking, and use of aspirin and other medications known to affect blood loss, we found five significant dietary factors associated with iron stores. Heme iron, supplemental iron, dietary vitamin C, and alcohol were positively associated with serum ferritin, whereas coffee intake had a negative association. As expected, sex was a strong predictor of serum ferritin—women having significantly lower mean concentrations than men. However, age was not related to serum ferritin in our elderly population. Our results suggest that in typical Western-style diets, a small number of dietary factors probably modulate the bioavailability of dietary iron and influence the accumulation of iron stores. *Am J Clin Nutr* 1998;67:722–33.

KEY WORDS Serum ferritin, iron bioavailability, heme, meat intake, aging, iron absorption, dietary pattern, the elderly, vitamin C

INTRODUCTION

Iron is the nutrient most commonly deficient in the world (1–4). Its universal sex- and age specificity is well known. The most susceptible segments of the population are infants, children (5, 6), and women during their reproductive years (2, 5, 7). The major functional impairment in adults is decreased work performance and productivity (8–10; PR Dallman, unpublished observations, 1994). In infants and children, iron deficiency is associated with impaired psychomotor development, decreased cognitive function, and negative behavioral changes (4, 8, 9, 11). In both adults and children, these impairments have serious adverse developmental and economic implications (10, 12; PR Dallman, unpublished observations, 1994). Therefore, it is important to understand the factors involved in maintaining positive iron balance.

Because the focus of much research has been the prevention of iron deficiency in vulnerable groups, much less is known about iron nutriture in the elderly. They are generally not perceived as a high-risk group, and iron deficiency is less common among healthy elderly individuals (5; PR Dallman, unpublished observations, 1994). On the other hand, there is an age-associated increase in iron stores (1, 9, 13–17), and there is accumulating evidence, albeit controversial, suggesting that increased body iron stores are associated with such adverse health outcomes as heart disease (18–20), cancer (21), diabetes (22, 23), and perhaps other metabolic disorders associated with the insulin resistance syndrome (24). Consequently, the liability in iron nutriture for the elderly may not be one of negative iron balance and deficiency as found in the young, but rather one of positive iron balance and progressive iron loading.

About 60 y ago McCance and Widdowson (25, 26) showed a lack of a major excretory route for iron in humans because total body iron is regulated at the point of absorption. Consequently, it became clear that understanding the process of iron absorption

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was critical to understanding iron balance. In the historical development of iron-absorption techniques, the most important discovery was the extrinsic tag method with use of two different radioisotopes (27). The application of this method elucidated the well-known and widely held two-pool concept of iron absorption (28–34).

Two classes of dietary iron, heme and nonheme, form two different iron pools in the lumen of the gut, from which iron is absorbed by different mechanisms. The concern in regard to the bioavailability of dietary iron arose from this fundamental concept, and an extensive literature spanning the past 35 y has described the dietary factors affecting iron absorption. From these experimental studies, performed primarily with single foods or meals, it is now well understood that the absorption of heme iron is relatively unaffected by other dietary factors (28, 33, 35–41) compared with the absorption of nonheme iron. Although meat has been shown to enhance heme absorption (35, 42, 43) and calcium to inhibit it (43, 44), the absorption of nonheme iron can be enhanced or inhibited by various dietary components. The major enhancers of nonheme are vitamin C (41, 45–47) and meat (35, 37, 48, 49); the major inhibitors are phytate (50–53), fiber (54–56), various polyphenols (41, 57–61), and calcium (43, 44, 62), although the inhibitory effect of calcium on both heme and nonheme absorption is controversial (63, 64).

Because most dietary iron is nonheme, the absolute amount of dietary iron becomes less important than the kind of iron and the type of meal in which it is found. This helps to explain "the paradox of iron metabolism" (4) seen in developing nations where, after taking into account pathologic blood loss due to hookworm infestation, there is still a high prevalence of iron deficiency anemia in the face of high dietary iron intakes. The primarily plant-based diets found in developing countries are considered to have low iron bioavailability (65–67) because of their almost exclusively nonheme iron content, combined with reduced or negligible amounts of dietary enhancers of nonheme-iron absorption and large amounts of dietary inhibitors found in staples such as beans, cereals, beverages, and spices. However, in the context of a varied Western diet containing highly bioavailable heme iron, Cook et al (68) showed that the influence of dietary factors on nonheme-iron absorption appears to be less critical than results from single-meal absorption studies would suggest.

An important challenge remaining in bioavailability research is to document the dietary determinants of iron status in free-living populations. Compared with the massive amount of information discussing the bioavailability of dietary iron from single foods and meals, there is clearly a paucity of data addressing this question. We know of four studies that have assessed the relation between iron status, as measured by serum ferritin, and various dietary factors known to affect iron absorption (69–72), only one of which involved elderly subjects (72). Therefore, in this paper we address the question of whether normal variations in dietary pattern are associated with differences in iron stores (serum ferritin) in a large population-based US elderly sample participating in The Framingham Heart Study.

SUBJECTS AND METHODS

Study population

Initiated in 1948–1950, The Framingham Heart Study is a longitudinal study of heart disease risk factors and is described in detail elsewhere (73). The procedures and protocols of the study were approved by the Institutional Review Board for Human Research at Boston Medical Center. The study population originally consisted of 5127 men and women aged 30–62 y, selected largely at random from residents in Framingham, MA. Information collected included demographics, medical history, height, weight, cigarette smoking habit, and various clinical and biochemical measures. Subjects were followed in 2-y cycles to ascertain the development of disease and changes in clinical, biochemical, and behavioral variables.

One thousand four hundred one surviving members of the original cohort, aged 67–95 y, participated in the 20th cycle of data collection (cycle 20) between February 1988 and January 1990. During cycle 20, nonfasting blood samples were collected by venipuncture into evacuated tubes containing EDTA. The samples were received at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging (HNRCA) at Tufts University in Boston 1 d after collection for the clinical chemistry assessments. Plasma aliquots were stored in trace mineral–free Nunc vials (Fisher Scientific, Pittsburgh) at −20 °C. Of the 1401 surviving cohort members at cycle 20, 634 made up the subpopulation used for the analyses in this paper. The rationale and sequence for exclusions are described below. All materials and data used for these analyses were collected at the cycle 20 examination. Consequently, cycle 20 measurements of weight (kg) divided by height (m) squared were used to calculate the body mass index (BMI).

Dietary data

Dietary intake was estimated with the Willett 126-item semiquantitative food-frequency questionnaire, which has been validated for iron intake (74). It was mailed to subjects before their cycle 20 examination for completion at home. The completed forms were collected and checked at the examination, then forwarded to the HNRCA, where they were reviewed, coded, and sent to the Harvard School of Public Health for nutrient analysis.

Biochemical measures

Serum ferritin was measured by the Magic Ferritin ^{125}I radioimmunoassay (Ciba Corning, Norwood, MA). This two-site immunoradiometric sandwich assay involves constant amounts of two antibodies, one covalently coupled to paramagnetic particles and the other labeled with ^{125}I. The labeled and immobilized antibodies are mixed and incubated with the subject's sample. Separation of the bound and free radioactivity is by magnetic separation and decantation of the supernate. In our laboratory, assay of the WHO international ferritin standard 80/578 with the Magic Ferritin radioimmunoassay yielded mean ferritin values that were within 5–10% of the stated concentrations of this quality control.

The Iron Panel of the International Committee for Standardization in Hematology has suggested that the rate of degradation of ferritin in specimens stored at −20 °C is < 0.3%/y (75). Cycle 20 sera from The Framingham Study were stored at −20 °C for 3–5 y before being assayed for ferritin. This translates into a small practical effect (0.9–1.5%) in terms of the true original values.

C-reactive protein (CRP) was measured by an immunoturbidimetric method using the CRP SPQ Test System Antibody Reagent Set II (INCSTAR, Stillwater, MN) on a Cobas Fara II centrifugal analyzer (Roche, Nutley, NJ).

Anemia of chronic disease

Anemia of chronic disease is a mild-to-moderate anemia that is associated with chronic infection, inflammation, liver disease, and malignancy (9, 76–78). It mimics the biochemical profile of iron deficiency except for a normal or disproportionately elevated serum ferritin concentration (77–80). There is increased prevalence of chronic disease with age (76, 81–83), and the evidence suggests that anemia of chronic disease is a major cause of anemia in the elderly (9). We attempted to control for its possible confounding effect on serum ferritin concentration by establishing the following exclusion criteria. Inflammation was defined as an elevated CRP concentration (≥6 mg/L); infection was defined as a white blood cell count above or below the normal range for men and women (men: >10.6 or <3.9 $\times$ 10^9/L; women: >11.0 or <3.5 $\times$ 10^9/L); possible liver disease was defined as an abnormal elevation of any one of the following three liver enzymes: alanine aminotransferase more than two times the upper limit of normal [>1.23 μkat/L (>74 U/L)], aspartate aminotransferase more than two times the upper limit for normal [>1.13 μkat/L (>68 U/L)], alkaline phosphatase >1.5 times the upper limit for normal [>2.58 μkat/L (>154.5 U/L)]. The white blood cell count and liver enzymes were measured at the Nutrition Evaluation Laboratory of the HNRCA the day after the blood was drawn at the cycle 20 exam. Fourteen percent (n = 103) of the population met at least one of the above criteria.

Genetic hemochromatosis

Serum ferritin can be pathologically elevated in conditions of genetic iron overload. The most common inherited form of iron overload in whites is genetic hemochromatosis, an autosomal recessive disorder characterized by increased iron absorption that affects ≈1 in 300 in populations of northern European descent (84). We attempted to control for this by defining those with the highest probability of being homozygous for genetic hemochromatosis as having all three of the following abnormal iron indexes: serum ferritin >300 μg/L, serum iron >32 μmol/L (>180 μg/dL), and transferrin saturation >0.50 (85, 86). Three subjects met these criteria. The serum iron, iron-binding capacity, and total-iron-binding capacity were measured at the same time as was CRP, and will be used in an evaluation of the iron status of the Framingham cohort that will be published in a separate report.

Sample exclusions

Food-frequency questionnaires were completed by 1068 cycle 20 participants. Those with missing data for >12 food items, or with total estimated energy intakes <2510.4 kJ (<600 kcal) or >16 736 kJ (>4000 kcal), were excluded, leaving 974 cohort members with valid questionnaires. For 234 of these 974 subjects, there was insufficient serum available to determine either CRP or iron indexes, resulting in a reduction of the sample to 740. We excluded the 105 individuals who may have had pathologically altered serum ferritin concentrations due to infection, inflammation, liver disease, and genetic hemochromatosis because the relation with dietary determinants could be affected in these individuals. An additional female subject with a serum ferritin concentration of 934 μg/L was also excluded because she was an extreme outlier with the potential for undue influence. Furthermore, because she had an unusually elevated hemoglobin concentration (203 g/L, or 20.3 g/dL) and hematocrit value (0.59), as well as an abnormally elevated red blood cell count (6.7 $\times$ 10^{12}/L, or 6.7 $\times$ 10^6/mm^3), she may have had polycythemia vera, a neoplastic stem cell disorder of unknown cause. Associated primarily with excessive proliferation of red blood cell precursors, it is typically characterized by elevated hemoglobin and hematocrit values with or without an abnormal red blood cell count (87). The geometric mean serum ferritin of the 106 subjects who were excluded was 106 μg/L, which was significantly greater (P < 0.05) than the mean for the remaining 634 individuals (85 μg/L), who constituted the sample for these analyses. (Note that there were 106 exclusions instead of 107 because one subject met the criteria for an elevated CRP value as well as for genetic hemochromatosis).

Data analysis

All statistical procedures were performed by using SAS, version 6 (88). Three least-squares regression analyses were carried out by using PROC REG to investigate the relation between iron stores as measured by serum ferritin and various dietary factors shown previously in the literature to affect iron bioavailability. Because serum ferritin concentration was positively skewed, a natural logarithmic transformation was applied to the measurements before the regression analyses.

There were two categories of independent variables in all models: covariates and dietary factors (**Table 1**). Covariates represent nonnutritional determinants of serum ferritin and were included to control for their possible confounding effects on ferritin concentrations. Covariates shown previously to be associated with serum ferritin include sex (1, 7, 14, 16, 17, 81, 83, 89–91), age (1, 9, 13–15, 90–93), and alcohol (94–101). Serum ferritin concentrations are strongly correlated with body iron stores (1, 7, 102–104), which are markedly influenced by body size and excess blood loss (84). The analyses adjusted for these potential effects by including BMI and the use of aspirin and various medications known to increase the possibility of bleeding. Smoking status was also included, although it has not been clearly established as a determinant of serum ferritin concentration (22, 90). Pipe and cigar smokers were included in the smoking group. Total energy intake was included in the regression models to adjust for possible systematic over- or underreporting of dietary intake (74). The aspirin, medication, and smoking variables were created as bivariate ìyes, noî variables on the basis of cycle 20 examination questionnaire responses. The medications variable indicated use of one or more of the following four categories of drugs known to affect blood loss: antiplatelet medications, anticoagulants, nonsteroidal antiinflammatory agents, and antiulcer medications.

Dietary factors included in model 1 were total (dietary plus supplemental) intake of iron, vitamin C, and calcium, plus intake of dietary fiber and caffeine. Model 2 decomposed total intakes of iron, vitamin C, and calcium into dietary and supplemental components, and further characterized dietary iron intake as heme or nonheme. Nonheme iron was determined as the difference between dietary iron and heme iron. Supplemental intakes of iron, vitamin C, and calcium were created as bivariate "yes, no" variables: yes for supplemental intakes greater than two-thirds of the recommended dietary allowance (RDA; 105) for older adults, no for supplemental intakes less than or equal to two-thirds of the RDA. The specific cutpoints were as follows: 6.6 mg Fe, 39.6 mg vitamin C, and 528 mg Ca.

Model 3 included food and beverage groups rather than nutrients. The related food items collapsed from the Willett food-frequency questionnaire that make up these food and beverage groups are listed in **Table 2**. Supplemental sources of iron, vitamin C, and calcium were included as covariates in model 3, but in this case as continuous variables.

TABLE 1
Variables included in three multiple regression models[1]

Variables	Model 1	Model 2	Model 3
Dependent	Serum ferritin	Serum ferritin	Serum ferritin
Independent			
Covariates	Age	Age	Age
	Sex	Sex	Sex
	BMI	BMI	BMI
	Alcohol	Alcohol	Alcohol
	Smoking	Smoking	Smoking
	Aspirin	Aspirin	Aspirin
	Medications	Medications	Medications
	Total energy	Total energy	Total energy
			Supplemental iron
			Supplemental vitamin C
			Supplemental calcium
Dietary factors	Total iron	Heme iron	Milk
		Nonheme iron	Fruit
		Supplemental iron	Dark green and orange vegetables
	Total vitamin C	Dietary vitamin C	Other vegetables
		Supplemental vitamin C	Beans and legumes
	Total calcium	Dietary calcium	Poultry
		Supplemental calcium	Meat
	Dietary fiber	Dietary fiber	Processed meats
	Caffeine	Caffeine	Fish
			Sweet baked goods
			Breads
			Cold cereals
			Tea
			Coffee

[1] Age in y, BMI in kg/m^2, alcohol in g, total energy in kJ, total iron in mg, total vitamin C in mg, total calcium in mg, dietary fiber in g, caffeine in mg, and all food and beverage variables in servings/wk. Supplemental intakes were in mg. *See* text for descriptions of supplemental intake variables used in models 2 and 3. *See* text for descriptions of smoking, aspirin, and medications variables. Serum ferritin was natural log transformed.

The following model-building procedure was used for all three analyses. Collinearity diagnostics revealed no redundant variables in the models. Sex interactions with each independent variable were tested. Only one was significant, sex-by–dietary nonheme iron intake, and is discussed below. Interactions described previously between dietary iron and other dietary factors (eg, iron and calcium and iron and fiber) were tested in models 1 and 2. Because the regression line is sensitive to the influence of aberrant data points, the validity of least-squares regression assumptions was met empirically by checking residual plots of crude (simple) regressions of each independent variable with serum ferritin. Individuals with influential values were excluded from a model if their removal resulted in a ≥ 33% change in the regression coefficient estimate. This resulted in 7, 8, and 18 exclusions from models 1, 2, and 3, respectively (1%, 1%, and 2.8% of the sample). However, these data points were not excluded from the calculation of descriptive statistics.

Because a logarithmic transformation was applied to serum ferritin, the antilog of regression coefficients gives the multiplicative change in serum ferritin for every unit difference in predictor. CIs for the coefficients are computed as the antilog of the geometric mean ± 1.96 × SE.

RESULTS

Characteristics of the study population for serum ferritin and covariates

The sample was composed of 254 men (40%) and 380 women, whose mean (± SD) age was 75.3 ± 5.0 y. The geometric mean serum ferritin of the population and its 95% CI was 85 μg/L (79, 92 μg/L). There was a significant sex difference in geometric mean ferritin concentrations between men and women (P = 0.0001): men, 108 μg/L (95% CI: 95, 122); women, 72 μg/L (95% CI: 66, 79). The mean (±SD) BMI was 26.4 ± 4.4 with a range of 16 to 51 (5th percentile = 20.5, 95th percentile = 33.8). The mean (±SD) energy intake for the population was 7368 ± 2573 kJ/d (1761 ± 614.9 kcal/d). Twelve percent of our elderly population smoked. Thirty-four percent of the elderly subjects used aspirin, whereas 28% took at least one of the medications discussed in the previous section known to increase the risk of bleeding. Fifty percent of this elderly population drank alcohol, the average intake among drinkers being 17 g/d, which is approximately equivalent to the ethanol content of one regular beer or a 2-oz (57 g) Manhattan cocktail.

Sample characteristics for dietary factors included in models 1 and 2

The mean (±SD) intake of total iron was 17.8 ± 15.1 mg/d. The mean (±SD) daily intakes of dietary iron without supplements, heme iron, and nonheme iron were 13.5 ± 7.6, 0.90 ± 0.53, and 12.6 ± 7.4 mg/d, respectively. Sixteen percent of subjects used iron supplements (n = 103), the mean intake among users being 26 mg supplemental Fe/d (5th percentile = 6 mg/d, 95th percentile = 60 mg/d). There were no significant differences between iron supplement users and nonusers in average intake of heme, nonheme, or total dietary iron.

The mean (±SD) daily intake of total vitamin C was 251 ± 282 mg. Mean (±SD) intake of dietary vitamin C without sup-

TABLE 2
Related food items that make up the food variables included in model 3

Food variable	Food items	Serving size[1]
Milk	Skim, low-fat, whole	240 mL
Fruit	Banana, apple pear, orange, peach, apricot, plum	1
	Fresh or frozen berries, prunes	120 mL
	Grapefruit	1/2
	Watermelon	1 slice
	Cantaloupe	1/4
	Raisins or grapes	28.4 g
	Fruit juices	small glass
Dark green and orange vegetables	Cooked broccoli, kale, collards, spinach, mustard or chard greens, carrots, yellow winter squash	120 mL
	Raw spinach, romaine or leaf lettuce	470 mL
Other vegetables	Cabbage, cole slaw, cauliflower, brussels sprouts, corn, mixed vegetables, eggplant, zucchini, summer squash, string beans, beets, alfalfa sprouts	120 mL
	Tomatoes	1
	Celery	10-cm stick
	Iceberg or head lettuce	470 mL
Beans and legumes	Lentils, peas, limas, other beans	470 mL
	Tofu, soybeans	85.2–113.6 g
Poultry	Chicken and turkey, with or without skin	113.6–170.4 g
Meat	Liver	85.2–113.6 g
	Hamburger patty	1
	Beef, pork, lamb in sandwich or mixed dish	< 113.6 g
	Beef, pork, lamb as main dish	113.6–170.4 g
Processed meats	Bacon	2 slices
	Hot dogs	1
	Sausage, salami, bologna, and similar products	1 piece or slice
Fish	Canned tuna	85.2–113.6 g
	Salmon, mackerel, sardines, other fish; shrimp, lobster, scallops as main dish	85.2–142 g
Sweet baked goods	Cookies, brownies, doughnuts, muffins, biscuits, sweet rolls, coffee cake, pastries, cakes, pies	1 serving or slice
Breads	White or dark bread, English muffins, bagels, rolls, pancakes, waffles	1 serving or slice

[1] Serving size for cold cereals, tea, and coffee, which are also included in model 3, was 236 mL (1 cup).

plements was 153 ± 79 mg/d. Thirty-one percent of the elderly subjects used vitamin C supplements (n = 195), the mean intake being 318 mg/d (5th percentile = 11 mg/d, 95th percentile = 120 mg/d). Supplement users had a significantly greater (P = 0.03) average intake of dietary vitamin C (164 mg/d) than nonusers (148 mg/d).

The mean (±SD) total daily calcium intake was 807 ± 437 mg. The mean (±SD) intake of dietary calcium without supplements was 720 ± 351 mg/d. Sixteen percent of the elderly took calcium supplements (n = 113), the mean intake being 483 mg/d (5th percentile = 93 mg/d, 95th percentile = 1500 mg/d). Calcium supplement users did not have a significantly greater average intake of dietary calcium than nonusers. The mean (±SD) daily intake of dietary fiber was 19.3 ± 8 g. The mean (±SD) daily caffeine intake was 192 ± 162 mg.

Sample characteristics for food variables included in model 3

Food intake characteristics of the study population for food and beverage groups included in model 3 are shown in **Table 3**. Approximately 80% of these elderly subjects used milk, with an average intake of 1.65 L/wk (7 cups/wk) or 236 mL/d (1 cup/d) among users. Ninety-nine percent of the sample ate fruit or drank fruit juices, the average was ≈19 servings/wk or almost 3 servings/d. In terms of fresh fruit eaten, that is ≈2.5 pieces of fruit/d. Ninety-seven percent of these elderly subjects consumed dark green and orange vegetables, the users averaging ≈6 servings/wk or almost 1 serving/d. Although 85% of the sample ate beans, the average weekly serving for users was a little less than 236 mL (1 cup). Ninety-six percent of these elderly subjects ate chicken or turkey, with a mean intake among consumers of 2.5 servings/wk, or 284–426 g (10–15 oz). Ninety-six percent ate meat, the users averaging 3 servings/wk or 3 hamburgers/wk, for example. Approximately 80% of subjects ate processed meats at 2.5 servings/wk or the equivalent of 2.5 hot dogs or 5 slices of bacon weekly. Ninety-five percent of the sample using sweet baked goods (eg, doughnuts, muffins, and pie) averaged 10 servings or units per week or ≈1.5 muffins per day, for example. The 73% who ate cold breakfast cereals had a mean intake of 940 mL/wk (4 cups/wk) or ≈160 mL/d (two-thirds cup/d). About two-thirds of the study population drank coffee, the users averaging 2.36 L/wk (10 cups/wk), or ≈354 mL/d (1.5 cups/d). The 58% who were tea drinkers averaged ≈1.77 L/wk (7.5 cups/wk), or 236 mL/d (1 cup/d).

Nutrients associated with serum ferritin: regression models 1 and 2

The regression of serum ferritin on dietary factors is shown in **Table 4**.

Model 1

After the other variables present in the model were controlled for, total iron intake was positively associated with serum ferritin

TABLE 3
Dietary intake characteristics for food items in model 3: percentage users and mean intake of users[1]

	Percentage users	Intake
	%	*servings/wk*[2]
Milk	79.7	6.9 ± 6.0
Fruit	99.1	18.5 ± 10.9
Dark green and orange vegetables	97.6	5.5 ± 4.8
Other vegetables	99.4	11.7 ± 7.3
Beans and legumes	84.5	1.6 ± 1.2
Poultry	96.4	2.3 ± 1.7
Meat	96.2	3.1 ± 2.4
Processed meats	78.9	2.6 ± 3.2
Fish	92.7	2.4 ± 1.9
Sweet baked goods	95.0	10.3 ± 10.4
Breads	98.6	12.2 ± 8.4
Cold cereals	72.9	3.9 ± 3.0
Tea	55.1	7.5 ± 7.8
Coffee	67.4	10.2 ± 8.0

[1] For a detailed delineation of food variables and serving sizes *see* Table 2.
[2] $\bar{x}$ ± SD.

(P = 0.007), whereas caffeine was negatively associated (P = 0.001). Each milligram of total iron consumed was associated with a 0.9% greater serum ferritin concentration (95% CI: 0.2%, 2%) or a difference of 19% per 20 mg difference in total iron intake. Each milligram of caffeine was associated with a 0.08% lower serum ferritin concentration (95% CI: 0.04%, 0.12%) or a difference of 8% per 100-mg difference in caffeine intake. Dietary fiber, total vitamin C, and total calcium were not associated with serum ferritin. Of the covariates, significant positive associations were seen for BMI (P = 0.001) and alcohol intake (P = 0.008). Total energy intake was marginally negatively associated with serum ferritin (P = 0.08). Women had significantly (P < 0.0001) lower mean serum ferritin concentrations than men after considering the influence of diet and other covariates.

Model 2

When expanding the nutrients in model 1 into their component parts, serum ferritin was positively associated with heme iron (P = 0.0001), supplemental iron (P = 0.0001), and dietary vitamin C without supplements (P = 0.04), and negatively associated with caffeine (P = 0.0009). Each milligram of heme iron intake was associated with 46% greater serum ferritin (95% CI: 22%, 74%). Use of iron supplements, previously defined as those with intakes greater than two-thirds of the RDA for adults, was associated with 63% greater serum ferritin (95% CI: 28%, 109%). A 10-mg difference in dietary vitamin C was associated with 1% greater serum ferritin (95% CI: 0.02%, 2%) or a difference of 13% per 100-mg difference in dietary vitamin C intake. The coefficient for caffeine was unchanged from model 1.

There was a significant sex-by–dietary nonheme iron intake interaction (P = 0.0033). For women, ingestion of greater amounts of nonheme iron did not relate to serum ferritin concentrations, whereas for elderly men, intake of greater quantities of nonheme iron was associated with lower serum ferritin concentrations.

Supplemental vitamin C, dietary and supplemental calcium, and dietary fiber were not related to serum ferritin. Of the covariates, BMI (P = 0.007) and alcohol (P = 0.0001) were again positively associated, whereas total energy (P = 0.0003) was negatively associated with serum ferritin. Each gram of alcohol was associated with 1% greater serum ferritin (95% CI: 0.6%, 2%) or a difference of 12% per 10-g difference in alcohol intake. (One 12-oz light beer or one glass of table wine contains ≈10 g alcohol.)

Foods associated with serum ferritin: regression model 3

Results of regressing serum ferritin on food group variables included in model 3 are shown in **Table 5**. After the other variables in the model were controlled for, the significant positive predictors of serum ferritin were fruit (P = 0.04), meat (P = 0.0015), and processed meats (P = 0.02). The one significant negative predictor was coffee (P = 0.006). Each serving of meat per week was associated with 6% greater serum ferritin (95% CI: 2%, 11%) or a difference of 20% per 3 servings meat/wk. Each serving of processed meat per week was associated with 4% greater serum ferritin (95% CI: 0.6%, 7%) or a difference of 12% for a difference of 3 servings/wk in processed meat intake. Each serving of fruit per week was associated with 0.9% greater serum ferritin (95% CI: 0.7%, 2%) or a difference of 4% for a difference in fruit intake of 5 servings/wk. Each 236 mL/wk (1 cup/wk) of coffee consumed was associated with 1% lower serum ferritin (95% CI: 0.3%, 2%) or a difference of 6% with a 1.18-L/wk (5 cups/wk) difference in coffee intake. Vegetables, beans, fish, baked goods, cold cereal, milk, poultry, and tea were not significantly related to serum ferritin. Of the covariates, BMI (P = 0.007), supplemental iron (P = 0.001), and alcohol (P = 0.0003) were positively associated with serum ferritin, whereas total energy intake (P = 0.004) was negatively associated.

DISCUSSION

The vast majority of dietary iron is in the form of nonheme iron. It has been known for some time that the amount of nonheme iron absorbed from a meal is the net effect of various dietary factors present that either enhance or inhibit its absorption. In contrast, heme-iron absorption is generally not influenced by dietary factors. It is less certain what the long-term effects are of the ingestion of these factors on the iron status of a free-living population. A population or epidemiologic approach can provide added insight into the practical effect of various dietary factors on iron availability. Our study makes a unique contribution to iron bioavailability research because it is the first epidemiologic study to describe the dietary determinants of iron stores in a large elderly US population. In addition, there were methodologic problems in previous papers (69–72) that we attempted to address. First, we estimated usual dietary intake over the year preceding the cycle 20 examination by means of a food-frequency questionnaire. Second, we attempted to rigorously control for the many possible confounding effects on serum ferritin concentrations of various nondietary factors, especially its disproportionate elevation due to chronic disease (76, 79, 81, 89, 90, 106).

Positive predictors of serum ferritin

We found that total iron intake (model 1) was a significant positive predictor of serum ferritin. When we divided total iron intake into its component parts (model 2) of heme iron, nonheme iron, and supplemental iron, we found that the intakes of heme and supplemental iron were significant after all other factors in the model were controlled for.

Our observation that total iron intake was associated with serum ferritin in elderly men and women is not consistent with

TABLE 4
Results of regression of serum ferritin on dietary components in cycle 20 of The Framingham Heart Study cohort (models 1 and 2)[1]

	Model 1[2]		Model 2[3]	
Dietary factors	Regression coefficients ± SE[4]	*P*	Regression coefficients ± SE[4]	*P*
Total iron (mg)	0.0087 ± 0.0032	0.007		
Heme iron			0.377 ± 0.091	0.0001
Nonheme iron			−0.014 ± 0.008	0.08
Supplemental iron[5]			0.49 ± 0.13	0.0001
Total vitamin C (mg)	0.00018 ± 0.00016	0.28	0.3	
Dietary vitamin C			0.0012 ± 0.0006	0.04
Supplemental vitamin C[5]			−0.08 ± 0.10	0.46
Total calcium (mg)	0.00003 ± 0.0001	0.81		
Dietary calcium			0.00011 ± 0.00016	0.46
Supplemental calcium[5]			−0.018 ± 0.20	0.93
Dietary fiber (g)	0.0036 ± 0.007	0.60	0.012 ± 0.007	0.10
Caffeine (mg)	-0.0008 ± 0.0002	0.0012	−0.0008 ± 0.00024	0.0009

[1] Serum ferritin was natural log transformed to normalize the distribution and equalize the variance. The covariates considered for these models include age, sex, BMI, alcohol intake, smoking status, use of aspirin and medications, and energy intake.
[2] $R^2 = 0.12$.
[3] $R^2 = 0.17$.
[4] Difference in log-transformed serum ferritin for each indicated amount of nutrient intake per day.
[5] Two-thirds of the recommended dietary allowance (105).

that of others who have found no significant correlation between iron status, as measured by serum ferritin, and total iron intake in both the young (69, 107–109) and the old (14, 110–113). There are three possible reasons why our results differ from previous findings. First, many studies failed to consider possible confounders of serum ferritin (9, 14, 69, 107, 109–112) or other dietary factors that might influence iron absorption and thus modify iron stores (14, 107, 109–112). We performed a crude or unadjusted regression of total iron intake on serum ferritin, which also resulted in no association between the two variables ($P = 0.17$). Consequently, we conclude that the lack of association between total iron intake and serum ferritin reported in previous studies may be due to a lack of control for possible confounders of serum ferritin or dietary factors potentially affecting iron bioavailability. Second, several studies did not include supplemental iron in total iron intake (44, 69, 107, 111). We performed another crude or unadjusted regression, dividing total iron into dietary and supplemental iron intakes. There was no association between serum ferritin and dietary iron ($P = 0.16$), whereas supplemental iron was positively associated with ferritin ($P = 0.0007$). Thus, exclusion of supplemental iron from total iron intake may be another reason for the previously reported lack of association between total iron intake and serum ferritin. Third, some studies (109, 110, 112, 113) may have lacked the power to detect the relation between total iron intake and serum ferritin because of a small sample size.

As mentioned previously, heme iron was a significant positive predictor of serum ferritin in model 2. The importance of heme iron intake for iron stores is further supported by our findings of significant food predictors of serum ferritin in model 3: the meat and processed meat variables were positively associated with serum ferritin. Both of these food groups contain meat items rich in heme iron (Table 2). Our results in this elderly American cohort confirm previous findings of heme (70) and meat intake (69, 71) as significant positive predictors of iron stores. Thus, we corroborate, on a population basis, the well-known superiority of meat in iron nutriture (35, 42, 114). Heme iron in meat is highly bioavailable, with efficient absorption of ≈20–25% (115–118), which does not change with increasing dose (116, 118), is less dramatically affected by iron status than nonheme iron (117, 119, 120), and is not greatly influenced by other dietary components in the meal (28, 33, 35–38, 40, 41, 114, 115). In contrast, despite the fact that nonheme iron was the major source (mean of 93%) of dietary iron, its intake was not significantly related to serum ferritin, suggesting that greater intakes of nonheme iron were not associated with greater iron stores in our elderly population. This striking absence of an influence of nonheme iron on serum ferritin may be related to its relatively low bioavailability in this iron-sufficient population.

Bioavailability of nonheme iron is markedly influenced by both iron status (117) and meal composition (42). Absorption can differ as widely as 2% from a meal containing inhibitors of nonheme-iron absorption to 45% from a meal with enhancers (42).We observed a significant interaction of sex with dietary nonheme iron intake, in which higher nonheme iron intakes were associated with lower serum ferritin in men. Because this relation does not make biological sense and was not observed in women, it is likely an artifact of our data. For clarification, it would be helpful to test this interaction in another group of elderly subjects.

The fact that supplemental iron was a significant positive predictor of serum ferritin when only 16% of elderly subjects in our study used some form of supplemental iron is not surprising. Iron supplements, in doses as low as 20 mg, can significantly increase serum ferritin concentrations even in young women with regular blood loss (121, 122). Furthermore, Holyoake et al (83) showed a rise in plasma ferritin concentrations in elderly patients (serum ferritin ≤45 μg/L)with iron supplementation.

The enhancing effect of ascorbic acid on nonheme-iron absorption, both as a supplement (46, 123) and in food (29, 46, 124, 125), is widely known. We found that total vitamin C intake was not related to serum ferritin in model 1; however, when separated into dietary and supplemental vitamin C in model 2, dietary vitamin C was a significant positive predictor ($P = 0.04$),

TABLE 5
Results of regression of serum ferritin on food variables in cycle 20 of The Framingham Heart Study cohort (model 3)[1]

Food variable[2]	Regression coefficient ± SE [3]	*P*
Milk	−0.029 ± 0.02	0.15
Fruit	0.0087 ± 0.0041	0.04
Dark green and orange vegetables	0.0088 ± 0.011	0.42
Other vegetables	0.0023 ± 0.007	0.74
Beans and legumes	0.011 ± 0.032	0.73
Poultry	−0.043 ± 0.026	0.09
Meat	0.062 ± 0.020	0.002
Processed meats	0.038 ± 0.016	0.02
Fish	0.018 ± 0.021	0.39
Sweet baked goods	0.0018 ± 0.0048	0.71
Breads	−0.0039 ± 0.005	0.45
Cold cereals	0.019 ± 0.015	0.21
Tea	−0.0059 ± 0.0059	0.31
Coffee	−0.014 ± 0.0049	0.006

[1] Serum ferritin was natural log transformed to normalize the distribution and equalize the variance. The covariates considered for model 3 include age, sex, BMI, smoking status, use of aspirin and medications, total energy intake, supplemental iron, supplemental vitamin C, and supplemental calcium. $R^2 = 0.18$.

[2] For a detailed delineation of the food variables *see* Table 2.

[3] Difference in log-transformed serum ferritin for each serving per week.

whereas supplemental vitamin C was not. These observations contrast with previous epidemiologic findings in 38 nonpregnant, healthy, premenopausal women (71) in whom intake of vitamin C supplements was positively associated with serum ferritin concentration. However, others have also noted a significant positive correlation between mealtime vitamin C intake and serum ferritin but not total vitamin C intake (109). This observation suggests that total daily vitamin C may not be the best indicator of the influence of ascorbic acid on iron stores. Our results also seem to suggest the importance of mealtime vitamin C, which may be due to the following observations. First, vitamin C has an enhancing effect only if it is ingested with meals (126). Consequently, vitamin C taken at other times would play no role in iron balance. Furthermore, it has been shown that the first 50–100 mg ascorbic acid provide the most marked enhancement of iron absorption (40), additional quantities result in small effects. In other words, if vitamin C is added to a diet normally high in ascorbic acid, it would have a limited effect on iron nutriture (40). The average dietary vitamin C intake in Framingham elderly subjects who took vitamin C supplements was quite high, 164 mg, therefore, it is possible that supplemental vitamin C taken at meals provides little additional enhancement of iron absorption, and that taken between meals is irrelevant to iron balance. In addition, several studies have shown that supplementation with ≥500 mg ascorbic acid at two to three meals every day for 5-10 wk does not result in significant increases in iron stores (75, 127–129).

It has been shown in the literature that other organic acids present in fruit (eg, citric, malic, and tartaric acids) have similar, although independent, and less marked enhancing effects on nonheme-iron absorption (57, 125). Thus, the positive association between fruit consumption and serum ferritin shown in model 3 may not be due to the action of ascorbic acid alone.

It was not surprising to find both sex and alcohol intake to be strong predictors of iron stores in the Framingham elderly. There is a well-known sex difference in average serum ferritin concentrations; men have significantly higher concentrations than women (7). Although some have suggested that this sex difference in serum ferritin disappears in the elderly (15, 130), our data agree with others who maintain that concentrations in elderly women begin to approach the mean concentrations of men, but that a difference persists (1, 16, 17, 81, 83, 90, 92, 111, 131). Furthermore, the sex difference in ferritin concentrations in our elderly population was not explained by the other dietary and nondietary variables included in our analyses.

We have confirmed a positive association between serum ferritin and alcohol intake in healthy elderly subjects. Jacques et al (132) showed a dose-dependent increase in serum ferritin with increasing alcohol intake, suggesting that blood concentrations of ferritin increase with increasing alcohol intake in elderly individuals. The mechanisms for this are unclear because there are so many unanswered questions about alcohol's role in dietary iron absorption and liver iron homeostasis (133). Understanding is further complicated by the distinction between the acute and chronic effects of alcohol. Acute effects of alcohol on iron absorption are controversial. Some studies have reported increased nonheme-iron absorption (58, 94, 95, 134–136), and others have shown little or no effect (95, 96, 137). Alcohol may increase dietary intake of iron primarily through the iron content of wine, but wines have complex and diverse effects on iron bioavailability because of their variable iron and polyphenol contents, and the latter may inhibit iron absorption (96, 137–139). It has been shown that alcohol induces ferritin secretion in vivo in alcoholics (100), although the mechanism is unknown. Furthermore, evidence from animal studies suggests that the acute effect of alcohol may be mediated through acetaldehyde production, which inhibits ferritin uptake by the liver (140). To date, it is not clearly understood how chronic alcohol ingestion influences serum ferritin concentrations. Further work is needed to explain the association between increased serum ferritin concentrations and alcohol intake.

It is noteworthy that BMI in our elderly population was positively associated with serum ferritin. Increased BMI in the elderly likely reflects increased body fat, not increased muscle mass. The reason for the association between BMI and iron stores is unknown. Interestingly, Moirand et al (24) recently described a new syndrome of iron overload not genetically linked to the HLA locus, which is characterized by normal transferrin saturation (≤ 0.45) and serum iron combined with an abnormal serum ferritin concentration. The patients described were obese, hyperlipidemic, or hypertensive, or had abnormal glucose metabolism. Moirand et al suggested a possible link between iron excess and metabolic disorders associated with the insulin resistance syndrome, the exact nature of which is currently unknown.

Negative predictors of serum ferritin

Caffeine in models 1 and 2 was a significant negative predictor of serum ferritin. Caffeine intake represents the consumption of coffee, tea, and caffeinated soft drinks. Because few subjects drank caffeinated soft drinks (colas: 19%; diet colas: 12%) compared with coffee (67%) and tea (55%), caffeine is likely acting as a surrogate for coffee and tea consumption. The potent inhibitory effects of both coffee and tea on nonheme-iron absorption are well known (41, 46, 47, 57–59, 114, 134, 141, 142). Although both beverages contain caffeine, there is evidence suggesting that polyphenols, not caffeine

(58, 143), are responsible for these effects (58, 59, 141). This would be consistent with our observation that coffee intake was a significant negative predictor of iron stores in model 3, confirming previous epidemiologic findings (69). Although tea has been shown to be a negative predictor of iron stores (71), it was not a significant predictor in our elderly population. In the experimental literature, tea is a more potent inhibitor of iron absorption than coffee (134, 144), and the quantitatively greater tannin content in tea is considered a key factor (46, 47, 58, 134, 144). Because the inhibitory effects of these beverages in humans occur when ingested with a meal or 1 h later (144), our results may reflect differences in consumption patterns. Although information in this regard is lacking, it is possible that coffee was ingested with or right after meals, whereas tea was consumed between meals or later in the evening.

Although an age-associated increase in iron stores is commonly proposed (1, 9, 13–15, 91–93), it is noteworthy that age was not related to serum ferritin in our elderly population. Our results concur with those who suggest that iron stores do not increase with age (16, 22, 90, 91, 131). However, longitudinal data are needed to answer this question more adequately.

Our epidemiologic findings in this population-based sample of elderly Americans corroborate experimental data from single-meal iron-absorption studies that showed the potentially important role of dietary enhancers and inhibitors of iron absorption on iron stores. Because both low and high body iron stores can be deleterious, there appears to be an optimal range of body iron stores that is consistent with good health. Variations in dietary pattern can influence the relative amounts of some key dietary factors that modulate iron bioavailability and the accumulation of body iron stores. Our analyses of this elderly population suggest that with a Western diet containing meat, the positive dietary determinants of serum ferritin in the elderly are heme iron, supplemental iron, dietary vitamin C, and alcohol intake, whereas coffee intake has a negative effect on serum ferritin. Variations in dietary pattern that change the consumption of these key dietary components may have an important influence on body iron stores and health.

REFERENCES

1. Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. Blood 1976;48:449–55.
2. World Health Organization. Nutritional anemias. World Health Organ Tech Rep Ser 1968;405.
3. Cook JD, Finch CA. Assessing iron status of a population. Am J Clin Nutr 1979;32:2115–9.
4. Hallberg L. Iron in clinical medicine—an update. J Intern Med 1989;226:281–3 (editorial).
5. Expert Scientific Working Group. Summary of a report on assessment of the iron nutritional status of the United States population. Am J Clin Nutr 1985;42:1318–30.
6. Dallman PR, Siimes MA, Stekel A. Iron deficiency in infancy and childhood. Am J Clin Nutr 1980;33:86–118.
7. Cook JD, Lipschitz DA, Miles LE, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. Am J Clin Nutr 1974;27:681–7.
8. Prasad AN, Prasad C. Iron deficiency: non-hematological manifestations. Prog Food Nutr Sci 1991;15:255–83.
9. Johnson MA, Fischer JG, Bowman BA, Gunter EW. Iron nutriture in elderly individuals. FASEB J 1994;8:609–21.
10. Dallman PR. Iron deficiency: does it matter? J Intern Med 1989;226:367–72.
11. Beard J. One person's view of iron deficiency, development, and cognitive function. Am J Clin Nutr 1995;62:709–10.
12. Baynes RD. Iron deficiency. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron metabolism in health and disease. London: WB Saunders Co Ltd, 1994.
13. Lynch SR, Finch CA, Monsen ER, Cook JD. Iron status of elderly Americans. Am J Clin Nutr 1982;36:1032–45.
14. Garry PJ, Goodwin JS, Hunt WC. Iron status and anemia in the elderly: new findings and a review of previous studies. J Am Geriatr Soc 1983;31:389–99.
15. Qvist I, Norden A, Olofsson T. Serum ferritin in the elderly. Scand J Clin Lab Invest 1980;40:609–13.
16. Milman N, Andersen HC, Strandberg Pedersen N. Serum ferritin and iron status in 'healthy' elderly individuals. Scand J Clin Lab Invest 1986;46:19–26.
17. Marie B, Cals M, De Jaeger C, Lowenstein W, Durand H, Ekindjian OG. Indicators of iron status in nonanemic elderly subjects: influence of sex and age. Clin Chem 1994;40:1779–81.
18. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992;86:803–11.
19. Lauffer RB. Iron stores and the international variation in mortality from coronary artery disease. Med Hypoth 1990;35:96–102.
20. Sullivan JL. The iron paradigm of ischaemic heart disease. Am Heart J 1989;117:1177–88.
21. Stevens RG, Jones DY, Micozzi MS, Taylor PR. Body iron stores and the risk of cancer. N Engl J Med 1988;319:1047–52.
22. Woo J, Mak YT, Law LK, Swaminathan R. Plasma ferritin in an elderly population living in the community. J Med 1989;20:123–34.
23. Dinneen SF, O'Mahony MS, O'Brien T, Cronin CC, Murray DM. Serum ferritin in newly diagnosed and poorly controlled diabetes mellitus. Ir J Med Sci 1992;161:636–8.
24. Moirand R, Mortaji AM, Loreal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. Lancet 1997;349:95–7.
25. McCance RA, Widdowson EM. The absorption and excretion of iron following oral and intravenous administration. J Physiol 1938;94:148–54.
26. McCance RA, Widdowson EM. Absorption and excretion of iron. Lancet 1937;2:680–4.
27. Hallberg L, Bjorn-Rasmussen E. Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as haem and non-haem iron. Scand J Haematol 1972;9:193–7.
28. Cook JD, Layrisse M, Martinez-Torres C, Walker R, Monsen E, Finch CA. Food iron absorption measured by an extrinsic tag. J Clin Invest 1972;51:805–15.
29. Layrisse M, Martinez-Torres C, Gonzales M. Measurement of the total daily dietary iron absorption by the extrinsic tag model. Am J Clin Nutr 1974;27:152–62.
30. Bjorn-Rasmussen E. Food iron absorption in man. IV. Validity of the extrinsic tag two-pool method for measurement of dietary non-heme iron absorption in patients with various clinical disorders. Scand J Gastroenterol 1973;8:645–50.
31. Bjorn-Rasmussen E, Hallberg L, Walker RB. Food iron absorption in man. I. Isotopic exchange between food iron and inorganic iron salt added to food: studies on maize, wheat, and eggs. Am J Clin Nutr 1972;25:317–23.
32. Layrisse M, Martinez-Torres C. Model for measuring dietary absorption of heme iron: test with a complete meal. Am J Clin Nutr 1972;25:401–11.
33. Hallberg L. The pool concept in food iron absorption and some of its implications. Proc Nutr Soc 1974;33:285–91.
34. Bjorn-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B. Food iron absorption in man. Applications of the two-pool extrinsic tag method to measure heme and nonheme-iron absorption from the whole diet. J Clin Invest 1974;53:247–55.

35. Martinez-Torres C, Layrisse M. Iron absorption from veal muscle. Am J Clin Nutr 1971;24:531–40.
36. Turnbull A, Cleton F, Finch CA. Iron absorption. IV. The absorption of hemoglobin iron. J Clin Invest 1962;41:1897–907.
37. Conrad ME, Weintraub LR, Sears DA, Crosby WH. Absorption of hemoglobin iron. Am J Physiol 1966;211:1123–30.
38. Callender ST, Mallett BJ, Smith MD. Absorption of hemoglobin iron. Br J Haematol 1957;3:186–92.
39. Layrisse M, Martinez-Torres C, Roche M. Effect of interaction of various foods on iron absorption. Am J Clin Nutr 1968;21:1175–83.
40. Hallberg L, Brune M, Rossander L. The role of vitamin C in iron absorption. Int J Vitam Nutr Res Suppl 1989;30:103–8.
41. Disler PB, Lynch SR, Charlton RW, et al. The effect of tea on iron absorption. Gut 1975;16:193–200.
42. Hallberg L. Bioavailability of dietary iron in man. Annu Rev Nutr 1981;1:123–47.
43. Hallberg L, Rossander-Hulthen L, Brune M, Gleerup A. Inhibition of haem-iron absorption in man by calcium. Br J Nutr 1992;69:533–40.
44. Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am J Clin Nutr 1991;53:112–9.
45. Brise H, Hallberg L. Effect of ascorbic acid on iron absorption. Acta Med Scand Suppl 376 1962;171:51–8.
46. Hallberg L, Brune M, Rossander L. Effect of ascorbic acid on iron absorption from different types of meals. Hum Nutr Appl Nutr 1986;40A:97–113.
47. Rossander L, Hallberg L, Bjorn-Rasmussen E. Absorption of iron from breakfast meals. Am J Clin Nutr 1979;32:2484–9.
48. Hazell T, Ledward D, Neale R. Iron availability from meat. Br J Nutr 1978;39:631–8.
49. Layrisse M, Martinez-Torres C, Leets I, Taylor P, Ramirez J. Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. J Nutr 1984;114:217–23.
50. Sandberg AS. The effect of food processing on phytate hydrolysis and availability of iron and zinc. In: Friedman M, ed. Nutritional and toxicological consequences of food processing. New York: Plenum Press, 1991:499–508.
51. Brune M, Rossander-Hulten L, Hallberg L, Gleerup A, Sandberg AS. Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inosital phosphates with different numbers of phosphate groups. J Nutr 1992;122:442–9.
52. Morris ER, Ellis R. Bioavailability to rats of iron and zinc in wheat bran: response to low-phytate bran and effect of the phytate/zinc molar ratio. J Nutr 1980;110:2000–10.
53. Reddy MB, Hurrell RF, Juillerat MA, Cook JD. The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans. Am J Clin Nutr 1996;63:203–7.
54. Simpson KM, Morris ER, Cook JD. The inhibitory effect of bran on iron absorption in man. Am J Clin Nutr 1981;34:1469–78.
55. Widdowson EM, McCance RA. Iron exchanges of adults on white and brown bread diets. Lancet 1942;1:588–90.
56. Bjorn-Rasmussen E. Iron absorption from wheat bread. Influence of various amounts of bran. Nutr Metab 1974;16:101–10.
57. Gillooly M, Bothwell TH, Torrance JD, et al. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br J Nutr 1983;49:331–42.
58. Disler PB, Lynch SR, Torrance JD, Sayers MH, Bothwell TH, Charlton RW. The mechanism of the inhibition of iron absorption by tea. S Afr J Med Sci 1975;40:109–16.
59. Brune M, Rossander L, Hallberg L. Iron absorption and phenolic compounds: importance of different phenolic structures. Eur J Clin Nutr 1989;43:547–58.
60. Macfarlane BJ, Bezwoda WR, Bothwell TH, et al. Inhibitory effect of nuts on iron absorption. Am J Clin Nutr 1988;47:270–4.
61. Tuntawiroon M, Sritongkul N, Brune M, et al. Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in men. Am J Clin Nutr 1991;53:554–7.
62. Hallberg L, Rossander-Hulten L, Brune M, Gleerup A. Calcium and iron absorption: mechanism of action and nutritional importance. Eur J Clin Nutr 1992;46:317–27.
63. Galan P, Cherouvrier F, Preziosi P, Hercberg S. Effects of the increasing consumption of dairy products upon iron absorption. Eur J Clin Nutr 1991;45:553–9.
64. Reddy MB, Cook JD. Effect of calcium intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 1997;65:1820–5.
65. Bothwell TH, Baynes RD, MacFarlane BJ, MacPhail AP. Nutritional iron requirements and food iron absorption. J Intern Med 1989;226:357–65.
66. Monsen ER, Hallberg L, Layrisse M, et al. Estimation of available dietary iron. Am J Clin Nutr 1978;31:134–41.
67. Taylor PG, Mendez-Castellanos H, Martinez-Torres C, et al. Iron bioavailability from diets consumed by different socioeconomic strata of the Venezuelan population. J Nutr 1995;125:1860–8.
68. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 1991;54:717–22.
69. Soustre Y, Dop MC, Galan P, Hercberg S. Dietary determinants of the iron status in menstruating women. Int J Vitam Nutr Res 1986;56:281–6.
70. Preziosi P, Hercberg S, Galan P, Devanlay M, Cherouvrier F, Dupin H. Iron status of a healthy French population: factors determining biochemical markers. Ann Nutr Metab 1994;38:192–202.
71. Yokoi K, Alcock NW, Sandstead HH. Iron and zinc nutriture of premenopausal women: associations of diet with serum ferritin and plasma zinc disappearance and of serum ferritin with plasma zinc and plasma zinc disappearance. J Lab Clin Med 1994;124:852–61.
72. Payette H, Gray-Donald K. Dietary intake and biochemical indices of nutritional status in an elderly population, with estimates of the precision of the 7-d food record. Am J Clin Nutr 1991;54:478–88.
73. Dawber TR, Meadors GF, Moore FE. Epidemiological approaches to heart disease: the Framingham study. Am J Public Health 1951;41:279–86.
74. Willett W. Nutritional epidemiology. New York: Oxford University Press, 1990.
75. Cook JD, Watson SS, Simpson KM, Lipschitz DA, Skikne BS. The effect of high ascorbic acid supplementation on body iron stores. Blood 1984;64:721–6.
76. Johnson MA. Iron: nutrition monitoring and nutrition status assessment. J Nutr 1990;120:1486–91.
77. Cartwright GE. The anemia of chronic disorders. Semin Hematol 1966;3:351–75.
78. Means RT Jr, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease. Blood 1992;80:1639–47.
79. Yip R, Dallman PR. The roles of inflammation and iron deficiency as causes of anemia. Am J Clin Nutr 1988;48:1295–300.
80. Means RT. Pathogenesis of the anemia of chronic disease: a cytokine-mediated anemia. Stem Cells 1995;13:32–7.
81. Cook JD, Skikne BS, Lynch SR, Reusser ME. Estimates of iron sufficiency in the US population. Blood 1986;68:726–31.
82. Lipschitz DA, Mitchell CO, Thompson C. The anemia of senescence. Am J Hematol 1981;11:47–54.
83. Holyoake TL, Stott DJ, McKay PJ, Hendry A, MacDonald JB, Lucie NP. Use of plasma ferritin concentration to diagnose iron deficiency in elderly patients. J Clin Pathol 1993;46:857–60.
84. Fairbanks VF. Iron in medicine and nutrition. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. Philadelphia: Lea and Febiger, 1994:185–213.
85. Fairbanks VF. Laboratory testing for iron status. Hosp Pract (Off Ed) 1991;3(suppl 26):17–24.
86. Halliday JW, Powell LW. Iron overload. Semin Hematol 1982;19:42–53.
87. Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL, eds. Harrison's principles of internal medicine. 13th ed. New York: McGraw-Hill Inc, 1994.

88. SAS Institute. SAS/STAT user's guide, version 6. 4th ed. Cary, NC: SAS Institute, Inc, 1989.
89. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia. J Gen Intern Med 1992;7:145–53.
90. Touitou Y, Proust J, Carayon A, et al. Plasma ferritin in old age. Influence of biological and pathological factors in a large elderly population. Clin Chim Acta 1985;149:37–45.
91. Cals MJ, Bories PN, Devanlay M, et al. Extensive laboratory assessment of nutritional status in fit, health-conscious, elderly people living in the Paris area. J Am Coll Nutr 1994;13:646–57.
92. Loria A, Hershko C, Konijn AM. Serum ferritin in an elderly population. J Gerontol 1979;34:521–4.
93. Freedman ML. Iron deficiency in the elderly. Hosp Pract (Off Ed) 1986;21:115–22, 127, 130 passim.
94. Charlton RW, Jacobs P, Seftel H, Bothwell TH. Effect of alcohol on iron absorption. Br Med J 1964;2:1427–9.
95. Chapman RW, Morgan MY, Boss AM, Sherlock S. Acute and chronic effects of alcohol on iron absorption. Dig Dis Sci 1983;28:321–7.
96. Celada A, Rudolf H, Donath A. Effect of a single ingestion of alcohol on iron absorption. Am J Hematol 1978;5:225–37.
97. Meyer TE, Kassianides C, Bothwell TH, Green A. Effects of heavy alcohol consumption on serum ferritin concentrations. S Afr Med J 1984;66:573–5.
98. Lundin L, Hallgren R, Birgegard G, Wide L. Serum ferritin in alcoholics and the relation to liver damage, iron state and erythropoietic activity. Acta Med Scand 1981;209:327–31.
99. Moirand R, Lescoat G, Hubert N, Dezier JF, Pasdeloup N, Brissot P. Alcohol induction of ferritin expression in a human hepatoblastoma cell line (HEP G2). Alcohol Clin Exp Res 1990;14:847–52.
100. Moirand R, Lescoat G, Delamaire D, et al. Increase in glycosylated and nonglycosylated serum ferritin in chronic alcoholism and their evolution during alcohol withdrawal. Alcohol Clin Exp Res 1991;15:963–9.
101. Kristenson H, Fex G, Trell E. Serum ferritin, gammaglutamyl-transferase and alcohol consumption in healthy middle-aged men. Drug Alcohol Depend 1981;8:43–50.
102. Bezwoda WR, Bothwell TH, Torrance JD, et al. The relationship between marrow iron stores, plasma ferritin concentrations and iron absorption. Scand J Haematol 1979;22:113–20.
103. Walters GO, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. J Clin Pathol 1973;26:770–2.
104. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. Br Med J 1972;4:206–8.
105. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
106. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. N Engl J Med 1974;290:1213–6.
107. Galan P, Hercberg S, Soustre Y, Dop MC, Dupin H. Factors affecting iron stores in French female students. Hum Nutr Clin Nutr 1985;39C:279–87.
108. Hallberg L, Bengtsson C, Lapidus L, Lindstedt G, Lundberg P, Hulten L. Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. Br J Haematol 1993;85:787–98.
109. Razagui IB, Barlow PJ, Izmeth MGA, Taylor KDA. Iron status in a group of long-stay mentally handicapped menstruating women: some dietary considerations. Eur J Clin Nutr 1991;45:331–40.
110. Milman N, Ingerslev J, Graudal N. Serum ferritin and iron status in a population of 'healthy' 85-year-old individuals. Scand J Clin Lab Invest 1990;50:77–83.
111. Milman N, Schultz-Larsen K. Iron stores in 70-year-old Danish men and women. Evaluation in 469 individuals by serum ferritin and hemoglobin. Aging Clin Exp Res 1994;6:97–103.
112. Thomas AJ, Bunker VW, Stansfield MF, Sodha NK, Clayton BE. Iron status of hospitalized and housebound elderly people: dietary intake, metabolic balances, haematological and biochemical indices. Q J Med 1989;70:175–84.
113. Garry PJ, Wayne SJ, Koehler KM, Pathak DR, Baumgartner RN, Simon TL. Prediction of iron absorption based on iron status of female blood donors. Am J Clin Nutr 1992;56:691–8.
114. Lynch SR, Dassenko SA, Morck TA, Beard JL, Cook JD. Soy protein products and heme iron absorption in humans. Am J Clin Nutr 1985;41:13–20.
115. Hallberg L, Solvell L. Absorption of hemoglobin iron in man. Acta Med Scand 1967;181:335–54.
116. Hallberg L, Bjorn-Rasmussen E, Howard L, Rossander L. Dietary heme iron absorption. A discussion of possible mechanisms for the absorption-promoting effect of meat and for the regulation of iron absorption. Scand J Gastroenterol 1979;14:769–79.
117. Lynch SR, Skikne BS, Cook JD. Food iron absorption in idiopathic hemochromatosis. Blood 1989;74:2187–93.
118. Bezwoda WR, Bothwell TH, Charlton RW, et al. The relative dietary importance of haem and non-haem iron. S Afr Med J 1983;64:552–6.
119. Cook JD. Adaptation in iron metabolism. Am J Clin Nutr 1990;51:301–8.
120. Hallberg L, Hulten L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? Am J Clin Nutr 1997;66:347–56.
121. Borch-Iohnsen B, Meltzer HM, Stenberg V, Reinskou T, Trygg K. Bioavailability of daily low dose iron supplements in menstruating women with low iron stores. Eur J Clin Nutr 1990;44:29–34.
122. Hercberg S, Galan P, Soustre Y, Dop MC, Devanlay M, Dupin H. Effects of iron supplementation on serum ferritin and other hematological indices of iron status in menstruating women. Ann Nutr Metab 1985;29:232–8.
123. Moore CV, Dubach R. Observations on the absorption of iron from foods tagged with radioiron. Trans Assoc Am Phys 1951;64:245–56.
124. Callender ST, Marney SR, Warner GT. Eggs and iron absorption. Br J Haematol 1970;19:657–65.
125. Ballot D, Baynes RD, Bothwell TH. The effects of fruit juices and fruits on the absorption of iron from a rice meal. Br J Nutr 1987;57:331–43.
126. Cook JD, Monsen ER. Vitamin C, the common cold, and iron absorption. Am J Clin Nutr 1977;30:235–41.
127. Malone HE, Kevany JP, Scott JM, O'Broin SD, O'Connor G. Ascorbic acid supplementation: its effects on body iron stores. Ir J Med Sci 1986;155:74–9.
128. Hunt JR, Gallagher SK, Johnson LK. Effect of ascorbic acid on apparent iron absorption by women with low iron stores. Am J Clin Nutr 1994;59:1381–5.
129. Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH. Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. Am J Clin Nutr 1990;51:649–55.
130. Jacobs A, Worwood M. The biochemistry of ferritin and its clinical implications. In: Brown EB, ed. Progress in hematology IX. New York: Grune & Stratton, 1975.
131. Inelmen EM, D'Alessio M, Gatto MRA, et al. Descriptive analysis of the prevalence of anemia in a randomly selected sample of elderly people living at home: some results of an Italian multicentric study. Aging Clin Exp Res 1994;6:81–9.
132. Jacques PF, Sulsky S, Hartz SC, Russell RM. Moderate alcohol intake and nutritional status in nonalcoholic elderly subjects. Am J Clin Nutr 1989;50:875–83.
133. Zhang H, Loney LA, Potter BJ. Effect of chronic alcohol feeding on hepatic iron status and ferritin uptake by rat hepatocytes. Alcohol Clin Exp Res 1993;17:394–400.
134. Hallberg L, Rossander L. Effect of different drinks on the absorption of non-heme iron from composite meals. Hum Nutr Appl Nutr 1982;36A:116–23.
135. Sorensen EW. Studies on iron absorption. V. The effect of ascorbic acid and ethyl alcohol on the absorption of iron in iron-deficient subjects. Acta Med Scand 1966;180:241–4.

136. Duane P, Raja KB, Simpson RJ, Peters TJ. Intestinal iron absorption in chronic alcoholics. Alcohol Alcohol 1992;27:539–44.
137. Cook JD, Reddy MB, Hurrell RF. The effect of red and white wines on nonheme-iron absorption in humans. Am J Clin Nutr 1995;61:800–4.
138. Bezwoda WR, Torrance JD, Bothwell TH, Macphail AP, Graham B, Mills W. Iron absorption from red and while wines. Scand J Haematol 1985;34:121–7.
139. MacDonald RA, Baumslag N. Iron in alcoholic beverages. Possible significance for hemochromatosis. Am J Med Sci 1964;247:649–54.
140. Zhang H, Potter BJ. The effect of ethanol metabolism on ferritin uptake by freshly isolated rat hepatocytes: is acetaldehyde responsible for this alteration? Alcohol Clin Exp Res 1992;16:301–7.
141. Siegenberg D, Baynes RD, Bothwell TH, et al. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am J Clin Nutr 1991;53:537–41.
142. DeAlarcon PA, Donovan ME, Forbes GB, Landaw SA, Stockman JA. Iron absorption in the thalassemia syndromes and its inhibition by tea. N Engl J Med 1979;300:5–8.
143. Munoz LM, Lonnerdal B, Keen CL, Dewey KG. Coffee consumption as a factor in iron deficiency anemia among pregnant women and their infants in Costa Rica. Am J Clin Nutr 1988;48:645–51.
144. Morck TA, Lynch SR, Cook JD. Inhibition of food iron absorption by coffee. Am J Clin Nutr 1983;37:416–20.