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# Dietary determinants of iron stores in a free-living elderly population: The Framingham Heart Study<sup>1-4</sup>

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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  $-20^{\circ}\text{C}$ . 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 semi-quantitative 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  $^{125}\text{I}$  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  $^{125}\text{I}$ . 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  $-20^{\circ}\text{C}$  is  $<0.3\%/y$  (75). Cycle 20 sera from The Framingham Study were stored at  $-20^{\circ}\text{C}$  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 ( $\geq 6$  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 [ $> 1.23$   $\mu\text{kat/L}$  ( $> 74$  U/L)], aspartate aminotransferase more than two times the upper limit for normal [ $> 1.13$   $\mu\text{kat/L}$  ( $> 68$  U/L)], alkaline phosphatase  $> 1.5$  times the upper limit for normal [ $> 2.58$   $\mu\text{kat/L}$  ( $> 154.5$  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  $\approx 1$  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  $> 300$   $\mu\text{g/L}$ , serum iron  $> 32$   $\mu\text{mol/L}$  ( $> 180$   $\mu\text{g/dL}$ ), 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  $> 16736$  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  $\mu\text{g/L}$  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  $6.7 \times 10^6/\text{mm}^3$ ), 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  $\mu\text{g/L}$ , which was significantly greater ( $P < 0.05$ ) than the mean for the remaining 634 individuals (85  $\mu\text{g/L}$ ), 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 yes/no 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<sup>1</sup>

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

<sup>1</sup> Age in y, BMI in kg/m<sup>2</sup>, 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  $\geq 33\%$  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  $\pm 1.96 \times SE$ .

## RESULTS

### Characteristics of the study population for serum ferritin and covariates

The sample was composed of 254 men (40%) and 380 women, whose mean ( $\pm SD$ ) age was  $75.3 \pm 5.0$  y. The geometric mean

serum ferritin of the population and its 95% CI was  $85 \mu\text{g/L}$  (79, 92  $\mu\text{g/L}$ ). There was a significant sex difference in geometric mean ferritin concentrations between men and women ( $P = 0.0001$ ): men,  $108 \mu\text{g/L}$  (95% CI: 95, 122); women,  $72 \mu\text{g/L}$  (95% CI: 66, 79). The mean ( $\pm SD$ ) BMI was  $26.4 \pm 4.4$  with a range of 16 to 51 (5th percentile = 20.5, 95th percentile = 33.8). The mean ( $\pm SD$ ) energy intake for the population was  $7368 \pm 2573$  kJ/d ( $1761 \pm 614.9$  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 ( $\pm SD$ ) intake of total iron was  $17.8 \pm 15.1$  mg/d. The mean ( $\pm SD$ ) daily intakes of dietary iron without supplements, heme iron, and nonheme iron were  $13.5 \pm 7.6$ ,  $0.90 \pm 0.53$ , and  $12.6 \pm 7.4$  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 ( $\pm SD$ ) daily intake of total vitamin C was  $251 \pm 282$  mg. Mean ( $\pm SD$ ) 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 <sup>1</sup>
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
	Lentils, peas, limas, other beans	470 mL
Beans and legumes	Tofu, soybeans	85.2–113.6 g
	Chicken and turkey, with or without skin	113.6–170.4 g
Poultry	Liver	85.2–113.6 g
	Hamburger patty	1
Meat	Beef, pork, lamb in sandwich or mixed dish	< 113.6 g
	Beef, pork, lamb as main dish	113.6–170.4 g
	Bacon	2 slices
Processed meats	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

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

plements was  $153 \pm 79$  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 ( $\pm$ SD) total daily calcium intake was  $807 \pm 437$  mg. The mean ( $\pm$ SD) intake of dietary calcium without supplements was  $720 \pm 351$  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 ( $\pm$ SD) daily intake of dietary fiber was  $19.3 \pm 8$  g. The mean ( $\pm$ SD) daily caffeine intake was  $192 \pm 162$  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  $\approx 19$  servings/wk or almost 3 servings/d. In terms of fresh fruit eaten, that is  $\approx 2.5$  pieces of fruit/d. Ninety-seven percent of these elderly subjects consumed dark

green and orange vegetables, the users averaging  $\approx 6$  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  $\approx 1.5$  muffins per day, for example. The 73% who ate cold breakfast cereals had a mean intake of 940 mL/wk (4 cups/wk) or  $\approx 160$  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  $\approx 354$  mL/d (1.5 cups/d). The 58% who were tea drinkers averaged  $\approx 1.77$  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<sup>1</sup>

	Percentage users		Intake <i>servings/wk</i> <sup>2</sup>
	%		
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

<sup>1</sup> For a detailed delineation of food variables and serving sizes see Table 2.

<sup>2</sup>  $\bar{x} \pm SD$ .

( $P = 0.007$ ), 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%, 1%) 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 nega-

tively 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  $\approx 10$  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)<sup>1</sup>

Dietary factors	Model 1 <sup>2</sup>		Model 2 <sup>3</sup>	
	Regression coefficients ± SE <sup>4</sup>	P	Regression coefficients ± SE <sup>4</sup>	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 <sup>5</sup>			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 <sup>5</sup>			-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 <sup>5</sup>			-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

<sup>1</sup> 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.

<sup>2</sup>  $R^2 = 0.12$ .

<sup>3</sup>  $R^2 = 0.17$ .

<sup>4</sup> Difference in log-transformed serum ferritin for each indicated amount of nutrient intake per day.

<sup>5</sup> 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  $\approx 20$ –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  $\leq 45$   $\mu\text{g/L}$ ) 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)<sup>1</sup>

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

<sup>1</sup> 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.  $R^2 = 0.18$ .

<sup>2</sup> For a detailed delineation of the food variables see Table 2.

<sup>3</sup> 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 nutrition (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  $\geq 500$  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 ( $\leq 0.45$ ) 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. ☞

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