Dietary factors associated with the risk of high iron stores in the elderly Framingham Heart Study cohort[1–4]

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ABSTRACT

Background: High body iron stores may increase the risk of several chronic diseases. Whether dietary factors contribute to the risk of high iron stores is unknown.

Objective: We assessed the relation between dietary factors and the risk of high iron stores in the elderly Framingham Heart Study cohort.

Design: We examined the relation between the usual intake of dietary factors (food-frequency questionnaire) and the risk of high iron stores (serum ferritin > 300 and 200 μg/L in men and women, respectively) in 614 subjects aged 68–93 y.

Results: The risk of high iron stores was significantly higher *1)* in subjects who took ≥ 30 mg supplemental Fe/d than in nonusers [odds ratio (OR): 4.32; 95% CI: 1.63, 11.47], *2)* in subjects who consumed > 21 servings of fruit/wk than in those who consumed ≤ 14 servings/wk (OR: 2.88; 95% CI: 1.26, 6.61), and *3)* in subjects who consumed > 4 but < 7 or ≥ 7 servings of red meat/wk than in those who consumed ≤ 4 servings/wk (ORs: 2.94 and 3.61, respectively; 95% CIs: 1.33, 6.47 and 1.57, 8.27, respectively). Whole-grain intake (> 7 servings/wk) was inversely associated (OR: 0.23; 95% CI: 0.07, 0.75).

Conclusions: Among elders, intakes of highly bioavailable forms of iron (supplemental iron and red meat) and of fruit, a dietary source of an enhancer of nonheme-iron absorption (vitamin C), promote high iron stores, whereas foods containing phytate (whole grains) decrease these stores. Individual dietary patterns may be important modulators of high iron stores. *Am J Clin Nutr* 2002;76:1375–84.

INTRODUCTION

It has long been known that the body has a considerable capacity to store iron (1). Iron overload denotes an excess of body iron stores. Aside from pathologic forms of primary and secondary iron overload (2, 3), moderately elevated iron stores may be an issue of concern because of a possible association with several chronic diseases, such as heart disease (4–6), cancer (7), and diabetes (8, 9). However, this association between iron stores and the risk of disease is controversial because there are limited data for diabetes and cancer and many studies did not find an association with heart disease (10).

Cross-sectional data from the second National Health and Nutrition Examination Survey showed that iron stores in noninstitutionalized US adults, which were estimated by serum ferritin (SF) concentrations (11, 12), increase with age until about the sixth decade of life, when they appear to plateau in both men and women (13). The cause of increased iron stores in otherwise healthy elderly people is unknown. The apparent stability of iron stores throughout the later years of life led some researchers to suggest a set point theory of iron-store regulation, such that once a person reaches his genetically determined maximum amount of storage iron, further increases in stores do not result from increases in dietary or supplemental iron intakes (14, 15). Other researchers suggested that the down-regulation of both heme- and nonheme-iron absorption in response to elevations in body iron stores is so effective that it is not possible in healthy subjects to induce iron overload from diet alone, even if the diet has a high iron content or the dietary iron has high bioavailability (16).

We recently reported that ≈13% of elderly American subjects from the Framingham Heart Study had high iron stores, defined as an SF concentration > 300 μg/L in men and > 200 μg/L in women (17). This prevalence of high iron stores was in marked contrast to the prevalence of iron deficiency (2.7%) and iron deficiency anemia (1.2%) and was still evident after the exclusion of subjects with possible pathologically elevated SF concentrations. Although we previously reported that heme iron, supplemental iron, dietary vitamin C, alcohol, meat, and fruit intakes were positively associated with SF concentrations and that coffee intake was negatively associated with SF concentrations in these elderly men and women (18), it is not known whether any of these dietary factors are specifically associated

with the risk of high iron stores in these elderly subjects. Therefore, we undertook the analyses in this article to address whether dietary factors are associated with a risk of high iron stores in this elderly American population.

SUBJECTS AND METHODS

Study population

All subjects in this analysis were members of The Framingham Heart Study cohort. Initiated in 1948–1950, The Framingham Heart Study is a longitudinal study of heart disease risk factors; the study is described in detail elsewhere (19). 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 5209 white men and women aged 30–62 y who were selected largely at random from the residents of Framingham, MA. Subjects have been invited for follow-up examinations every 2 y to ascertain the development of disease and changes in clinical, biochemical, and behavioral variables.

Surviving members (*n* = 1401) of the original cohort who were 67–96 y of age participated in the 20th cycle of data collection (cycle 20) between February 1988 and January 1990. All materials and data used for this analysis were collected at the cycle 20 examination. The rationale for and sequence of exclusions are described below and resulted in a final sample size of 614.

Biochemical variables

During cycle 20, nonfasting blood samples were collected by venipuncture into evacuated EDTA tubes. The samples for the clinical chemistries were received at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University in Boston 1 d after collection. Serum aliquots were stored in trace mineral-free Nunc vials (Fisher Scientific, Pittsburgh) at $-20\,°C$ for 3–5 y.

Body iron stores were estimated on the basis of SF concentrations (11, 12), which were measured with the Magic Ferritin ^{125}I radioimmunoassay (Ciba Corning, Norwood, MA). In our laboratory, assay of the World Health Organization International Ferritin Standard 80/578 with the Magic Ferritin radioimmunoassay yielded mean ferritin values that were within 5–10% of the stated concentrations. The rate of degradation of ferritin in specimens stored at $-20\,°C$ is $< 0.3\%$ per year (20). The Framingham cycle-20 sera were stored at $-20\,°C$ for 3–5 y before we assayed them for ferritin; this storage period translates into a small practical effect (0.9–1.5%) in terms of the original values.

The white blood cell count of whole blood specimens was measured on a System 9000 Diff Model Automated Cell Counter (Serono-Baker Diagnostics Inc, Allentown, PA). This measure was used to identify subjects with possible infection. C-reactive protein (CRP), which was used as an inflammatory index (21), was measured with an immunoturbidimetric method with the use of a CRP SPQ Test System Antibody Reagent Set II (INCSTAR, Stillwater, MN) on a Cobas Fara II Centrifugal Analyzer (Roche, Nutley, NJ). Because the detection limit of our CRP assay was 6 mg/L, we defined inflammation as a CRP concentration ≥ 6 mg/L. The liver enzymes alkaline phosphatase (EC 3.1.3.1), alanine aminotransferase (EC 2.6.1.2), and aspartate aminotransferase (EC 2.6.1.1) were measured with an in vitro diagnostic reagent system

(Roche) on a Cobas Fara II Centrifugal Analyzer to identify subjects with possible liver disease.

Dietary data

Dietary data were collected only for noninstitutionalized subjects. Dietary intake was estimated with the Willett 126-item, semiquantitative food-frequency questionnaire (FFQ), which has been validated for iron intake (22). The questionnaire was mailed to the subjects before their cycle 20 examination for completion at home. The completed forms were collected and checked at the examination and were then forwarded to the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, where they were reviewed, coded, and sent to the Harvard School of Public Health for nutrient analysis. Heme iron was estimated as 40% of the total iron in meat, fish, and poultry (23).

Sample exclusions

FFQs were completed by 1068 of the 1401 cycle 20 participants, leaving 333 subjects with no dietary intake data. Approximately 37% (*n* = 123) of these 333 subjects were examined either at home or in a nursing home and were therefore ineligible to complete a FFQ according to the FFQ protocol used at the cycle 20 examination. Four additional subjects had missing information on where their cycle 20 examination occurred, leaving ≈62% (*n* = 206) who were examined at Framingham and were therefore eligible to complete an FFQ but did not. These are the truly missing FFQs. The Framingham Heart Study did not provide us with any information on why these subjects did not complete their questionnaires. It may be that some subjects just forgot to bring them to their examination. Subjects with missing data for > 12 food items and those 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.

Ferritin is a positive acute phase protein whose synthesis and secretion by hepatic cells is increased by inflammatory cytokines (24). Thus, although SF typically reflects body iron stores (11, 12), in the presence of various acute or chronic disease conditions, such as inflammation, infection, liver disease, or malignancy, it becomes an unreliable indicator of iron status because its blood concentration becomes disproportionately elevated relative to actual iron stores (21, 25–28). We attempted to control for the possible confounding effects of these conditions by excluding subjects who met the following disease criteria, which were intended to be suggestive, not diagnostic. Inflammation was defined as a CRP concentration ≥ 6 mg/L (*n* = 45; 6.1%). 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) (*n* = 40; 5.5%). Possible liver disease was defined as an abnormal elevation of any 1 of 3 liver enzymes: alanine aminotransferase > 2 times the upper limit of the normal range (> 1.23 μkat/L, or > 74 U/L) (*n* = 10; 1.4%), aspartate aminotransferase > 2 times the upper limit of the normal range (> 1.13 μkat/L, or > 68 U/L) (*n* = 8; 1.1%), or alkaline phosphatase > 1.5 times the upper limit of the normal range (> 2.58 μkat/L, or > 154.5 U/L) (*n* = 16; 2.2%). Because we did not have a biochemical marker for cancer, subjects with active cancer at cycle 20 were excluded on the basis of an algorithm from

TABLE 1
Dietary and nondietary variables considered for inclusion in 2 multiple logistic regression models for predicting the risk of high iron stores in the elderly

Variable	Nutrient model	Food and beverage model
Dependent	High iron stores[1]	High iron stores[1]
Independent		
Nondietary factors	Sex	Sex
	Age (y)	Age
	BMI (kg/m^2)	BMI
	Smoking (yes or no)	Smoking
	Aspirin (yes or no)	Aspirin
	Medications[2]	Medications
		Supplemental iron[3]
Dietary factors	Total energy intake (kJ)[4]	Total energy intake[4]
	Heme iron (mg)	Fruit (servings/wk)[5]
	Nonheme iron (mg)	Vegetables
	Supplemental iron and vitamin C[6]	Beans and nuts
		Red meats
	Dietary vitamin C (mg)	Light meats
	Dietary calcium (mg)	Breads and pasta
	Supplemental calcium (yes or no)	Sweet baked goods
		Cold breakfast cereals
	Dietary fiber (g)	Whole grains
	Caffeine (mg)	Milk
	Alcohol (g)	Other dairy products
		Coffee and tea
		Alcohol[7]

[1] Defined as a serum ferritin concentration >300 μg/L in men and >200 μg/L in women.

[2] Antiplatelet medications; anticoagulants; nonaspirin, nonsteroidal antiinflammatory agents; and antiulcer medications.

[3] Adjusted in continuous form.

[4] Used as a control variable.

[5] All food groups were analyzed on the basis of servings/wk except for cold breakfast cereals, which were analyzed in 2 different ways: as mg/Fe wk from cold breakfast cereals and as either whole-grain or refined-grain cereals.

[6] Categorized as *1*) subjects who took neither supplemental iron nor supplemental vitamin C, *2*) subjects who took only vitamin C supplements, *3*) subjects who took ≤12 mg supplemental Fe with or without supplemental vitamin C, *4*) subjects who took >12 to <30 mg supplemental Fe with or without supplemental vitamin C, and *5*) subjects who took ≥30 mg supplemental Fe with or without supplemental vitamin C.

[7] Used as a control variable.

a Framingham master cancer file and a cycle 20 physician observation (*n* = 35; 4.7%).

Blood ferritin concentrations may be elevated in conditions of pathologic iron overload. The most common inherited form is hereditary hemochromatosis, an autosomal recessive disorder characterized by elevated iron absorption that affects ≈1 in 300 persons in populations of northern European descent (2). Although the elevations in SF concentration in hemochromatosis reflect actual increases in body iron stores, the etiology of these elevations is pathologic. We identified the subjects with the highest probability of being homozygous for hemochromatosis as those having the following 3 abnormal iron indexes: an SF concentration >300 μg/L, a serum iron concentration >32 μmol/L (>180 μg/dL), and transferrin saturation >0.50 (>50%) (29, 30). Three subjects met these criteria and were also excluded. An additional female subject with an SF concentration of 934 μg/L

was excluded because she had a hematologic profile that suggested polycythemia vera, a neoplastic stem cell disorder of unknown cause (31).

Because some subjects met more than one disease criterion, the final number of individuals excluded from subsequent analyses because of their possible confounding effect on the relation between dietary factors and the risk of elevated iron stores was 126. Consequently, the subpopulation used for the analyses of dietary factors and the risk of high iron stores was 614.

Definition of high iron stores

The normal range of SF concentrations is commonly considered to be ≈15–300 μg/L (32), suggesting that >300 μg/L is indicative of abnormally high iron stores in both sexes. However, sex has a marked effect on body iron stores (13). Our elderly men had a significantly higher (*P* = 0.0001; *t* test) geometric mean SF concentration (108 μg/L) than did the women (73 μg/L), and the 95th percentile value for SF concentrations among the men (441 μg/L) was 120 μg/L higher than that among the women (261 μg/L). Based on data from the second National Health and Nutrition Examination Survey, the 95th percentile values for SF concentration in women aged 45–64 y and in men aged 18–64 y were 193 and 299 μg/L, respectively (33). Therefore, we chose sex-specific cutoff criteria for defining high iron stores: an SF concentration >300 μg/L in men and >200 μg/L in women.

Statistical analyses

All statistical procedures were performed by using SAS for WINDOWS, version 8 (34). Multiple logistic regression analyses were carried out by using PROC LOGISTIC to test whether the probability of our elderly subjects having high iron stores was influenced by various dietary factors that were previously shown in the literature to affect iron bioavailability. Two separate models were developed to examine associations between the risk of high iron stores and nutrients and food and beverage groups. There were 2 types of independent variables in both models: nondietary factors associated with SF and dietary factors, as shown in **Table 1**. The dependent variable was high iron stores as defined above.

Dietary factors

The food and beverage groups were composed of items from the food-frequency questionnaire (22). The fruit group consisted of all fruit and fruit juice. All vegetables, as well as tomatoes and potatoes in various forms, composed the vegetable group. The bean group consisted of various legumes, tofu, nuts, and peanut butter. The red-meat group was composed of liver, beef, pork, or lamb, which were served either by themselves or in prepared dishes, as well as hot dogs, bacon, and processed meats. The light-meat group consisted of chicken, turkey, and seafood. Skim, low-fat, or whole milk composed the milk group, whereas the other dairy group consisted of all other forms of dairy products. Coffee, tea (not herbal), and decaffeinated coffee composed the coffee and tea group.

Fortified, refined grain products were classified as 2 different groups on the basis of the form of iron used for fortification: the bread and pasta group, which consisted of items fortified primarily with iron sulfate, and the sweet baked goods group, which consisted of items fortified exclusively with elemental iron powder produced by hydrogen reduction (ie, reduced iron) [P Ranum (Ceres Nutrition), personal communication,). White and dark

bread, English muffins, bagels, and rolls were included in the bread and pasta group. The sweet baked goods group included cake, pie, sweet rolls, cookies, brownies, doughnuts, pancakes, and waffles. We included crackers and cooked breakfast cereals, other than oatmeal, in this group because they are also fortified with reduced iron.

Although cold breakfast cereals are also fortified with reduced iron [P Ranum (Ceres Nutrition), personal communication, 2001], we examined them separately in 2 different ways: *1*) as mg Fe/wk from cold breakfast cereals and *2*) as either whole-grain or refined-grain cold breakfast cereals. A variable representing iron intake (in mg Fe/wk) from cold breakfast cereals was created by first combining the different brands of cold cereal with the same amount of iron per serving into groups and then multiplying by the variable for servings/wk of cold cereal. The resulting distribution of iron intake from cold breakfast cereal was then divided into 4 categories, with subjects who never or seldom consumed cold breakfast cereals in the lowest category. Cold breakfast cereals were divided into refined or whole-grain cereals on the basis of the content of whole grain or bran in the cereal, as reported by Jacobs et al (35) and Liu et al (36). A breakfast cereal was classified as a whole-grain cereal if it contained ≥25% (by wt) whole grain or bran and was classified as a refined cereal if it contained <25% whole grain or bran. A 3-category variable was then created to compare the risk of high iron stores between subjects who consumed refined cold cereal, subjects who consumed whole-grain cold cereal, and nonusers of cold breakfast cereals. The whole-grain food group consisted of whole-grain cold breakfast cereals as defined above, brown rice, oatmeal, popcorn, bran, and wheat germ.

Nondietary factors

Nondietary factors previously shown to be associated with SF include male sex (13), age (13), and body mass index (BMI; in kg/m^2) (18), smoking status over the past year (yes or no) (37), and aspirin use (yes or no) (38). We created a variable (yes or no) for use within the past year of ≥1 of the following 3 categories of drugs believed to influence iron stores by blood loss: antiplatelet medications, anticoagulants, and nonaspirin, nonsteroidal antiinflammatory agents. Although they do not directly increase the risk of bleeding, antiulcer medications were also included because patients who use them are at increased risk of blood loss due to ulcers.

Total energy intake was included in both models to adjust for possible systematic overreporting or underreporting of dietary intake (22). Because alcohol intake is known to be positively associated with SF concentrations (39–41), we examined its association with the risk of high iron stores by category in the nutrient model and included it as a continuous variable with the other covariates in the food and beverage model. We also adjusted for supplemental iron intake expressed as a continuous variable in the food and beverage model.

Nutrient model

To evaluate whether there was a graded association between the intake of various nutrients and the risk of elevated iron stores, nutrients were divided into quartile categories, and the risks for the subjects in the second, third, and fourth quartiles were compared with the risk for subjects in the lowest or referent quartile. Nonheme iron was calculated as the difference between dietary iron intake and heme-iron intake. Caffeine intake (mg) was

expressed in quartile categories. Alcohol intake (g) was expressed in 3 categories: nondrinkers, who served as the referent group; moderate drinkers (≤2 drinks/d); and heavy drinkers (>2 drinks/d). We defined one drink as 13.2 g alcohol, which is the mean value in one shot of liquor (29.56 mL; 1 oz), one beer (354.72 mL; 12 oz), and one glass of wine (162.58 mL; 5.5 oz) (42).

We also created a variable (yes or no) for supplemental calcium intake. We created 3 categories of combined supplemental iron and supplemental vitamin C use for the following reasons. Of the 16.4% (*n* = 101) of our elderly sample who took iron in supplemental form, 91% (*n* = 92) also took vitamin C in supplemental form, mostly (*n* = 87) in the form of multivitamins. Only 9 subjects took iron supplements but not vitamin C supplements; therefore, we left those subjects in the iron and vitamin C supplement group. In contrast, of the 31% (*n* = 188) who took vitamin C supplements, 51% (*n* = 96) did not concomitantly take iron supplements. We further subdivided the iron supplement users according to dose. The resulting variable for supplemental iron and vitamin C included 5 categories: *1*) subjects who took neither supplemental iron nor supplemental vitamin C, *2*) subjects who took only vitamin C supplements, *3*) subjects who took ≤12 mg supplemental Fe with or without supplemental vitamin C, *4*) subjects who took >12 to <30 mg supplemental Fe with or without supplemental vitamin C, and *5*) subjects who took ≥30 mg supplemental Fe with or without supplemental vitamin C.

Food and beverage model

To evaluate whether there was a graded association between the intake of various food and beverage groups and the risk of elevated iron stores, each food or beverage group was divided into 3 or 4 groups on the basis of the number of servings per week. If <10% of the population were nonconsumers of a food or beverage group, the nonconsumers were included in the lowest intake group; if >10% were nonconsumers, the nonconsumers were the lowest intake, or referent, group. Nonconsumers were the subjects who selected "never, or less than once per month" for average use over the past year. The risks for the subjects in the higher intake groups were compared with the risk for the subjects in the lowest intake group.

Model-building procedure

We began with 2 initial models each composed of nondietary covariates of SF and dietary factors. The dietary factors in one model were nutrients, and those in the other model were food and beverage groups. Our model-building goal was to describe the most parsimonious models of dietary factors significantly associated with the risk of high iron stores, after adjustment for nondietary factors associated with SF. We performed backward elimination on the initial models to determine the final reduced models, ie, that final subset of dietary factors associated with the risk of high iron stores. The initial food and beverage model included both cold cereal variables. In our backward elimination procedure, all levels of each dietary factor were treated as one variable (ie, either all were removed or all were retained), and the 0.05 level of significance was used for the exit level of the variables. Once the final models were determined, overall goodness of fit was assessed by the Hosmer-Lemeshow statistic. In each final model, interactions between sex and each independent variable were tested one at a time. Interactions between dietary factors retained in the final food and beverage model were also tested one at a time. The significance of the trend for each dietary factor in the final model was

determined by the *P* value for the regression coefficient for each dietary factor when all variables were entered into the final model in continuous form and no simplification was performed.

Because reduction procedures can result in unstable models, bootstrap analysis was performed according to Efron and Tibshirani (43) to check the stability of our reduced models. A bootstrap sample is a random sample of the original data set that has the same number of observations as the original data set and is drawn with replacement. One hundred bootstrap samples were generated for both the nutrient model and the food and beverage model, and backward elimination was performed on each sample with the same methods used to derive the original reduced models.

Information on the use of aspirin and other drugs and on BMI was missing for 36, 9, and 5 subjects, respectively. Information on the intake of vegetables, light meats, breads and pasta, sweet baked goods, whole grains, cold breakfast cereals, and milk was missing for 3, 2, 14, 17, 12, 3, and 18 subjects, respectively. Missing food intake data represent nonresponders to FFQ items. Because of these missing data, the actual sample size for the final multiple logistic regression models was 571 and 542 for the nutrient model and the food and beverage model, respectively. Reported prevalences and medians for the descriptive statistics were based on different sample sizes because of missing data. Student's *t* tests and chi-square tests (or Fisher's exact test when one or more expected cell counts was < 5) were used to test between-group differences for means and proportions, respectively.

Limitations

We did not account for some factors in our analysis, such as blood donation, estrogen use, undetected inflammation, and hereditary hemochromatosis genotypes that may alter SF concentrations. Blood donation is unlikely to have affected our values in this elderly sample (68–93 y) because US blood banks, until very recently, generally mandated a maximum donor age of 65 y (14, 44). It is well known that estrogen use may cause breakthrough bleeding in postmenopausal women and thus may have affected SF concentrations in our elderly female subjects. We consider this to be of small practical significance because only 21 women (6%) reported current estrogen use at cycle 20. We defined inflammation as a CRP concentration ≥ 6 mg/L because that was the detection limit of our laboratory method. Ultrasensitive CRP assays have been developed that have detection limits as low as 0.05 mg/L and are thus able to measure low-grade inflammation (25, 45). Because our assay's detection limit was 6 mg CRP/L, we could not identify subjects with mild inflammation. Various heterozygous genotypes for the *HFE* gene influence iron stores (46–48). Although we did not genotype our subjects, we expect that the effect of these heterozygous genotypes on the risk of high iron stores in our cohort was small. Although the prevalence of C282Y *HFE*–related hemochromatosis heterozygotes is probably 10–15% in this population (49, 50), it has been shown that only ≈8–20% of *HFE* heterozygotes have high SF concentrations, defined either as exceeding the 95th percentile value for age-matched controls (49) or as being above the upper threshold of the normal reference range for the population (51). On that basis, we would expect that only ≈3% of our population would be predicted to have high iron stores attributable to the C282Y *HFE* mutation. Because most of our elderly subjects took supplemental iron as part of a multivitamin that included vitamin C, we could not determine whether the risk of high iron stores associated with the use of supplemental iron was due to the iron salts alone or to the enhancing effect of

vitamin C on their absorption. Finally, because our cross-sectional study design precludes any causal inferences, further observational and experimental studies are needed before any firm conclusions can be drawn in regard to the influence of specific dietary factors on the risk of high iron stores.

RESULTS

Descriptive characteristics of the sample

Nondietary factors

The sample was composed of 246 (40%) men and 368 women with a mean (± SD) age of 75.3 ± 5.0 y (range: 68–93 y). The mean BMI was 26.4 ± 4.4. Thirteen percent of these elderly subjects were smokers, and there was no significant difference between the proportions of male and female smokers. Thirty-four percent of the subjects took aspirin, and the proportion of the men who did so was significantly higher than that of the women (*P* = 0.003). The use of other medications associated with an increased risk of bleeding was as follows: antiplatelet medications, 3.9% (*n* = 24); anticoagulants, 2.4% (*n* = 15); nonaspirin, nonsteroidal antiinflammatory drugs, 16.1% (*n* = 99); and antiulcer medications, 7% (*n* = 43). Of the 319 (52% of the sample) subjects who used these medications, 265 subjects took 1 kind of drug and 54 subjects took ≥ 2 different kinds of medications.

Serum ferritin and prevalence of high iron stores

The median SF concentration of the sample was 94 μg/L, and the 10th and 90th percentile values were 25 and 255 μg/L, respectively. The men had a significantly (*P* = 0.0001) higher geometric mean SF concentration (108 μg/L) than did the women (73 μg/L). There was no significant difference between the men and the women in the proportion who had sex-defined elevated iron stores; 11.4% of the total sample had sex-defined elevated iron stores. There was no significant difference in geometric mean total iron intake between the subjects with elevated iron stores and those without them (15.1 mg compared with 14.2 mg, respectively; *P* = 0.43).

Dietary factors associated with the risk of high iron stores

Nutrient model

The median intakes of nutrients and nonnutrient dietary factors and the proportions of the total sample who consumed them are shown in **Table 2**. The final nutrient model is shown in **Table 3**. The backward elimination procedure on the initial model resulted in a final model that included one independent predictor of high iron stores: supplemental iron and vitamin C. There was no evidence of lack of fit (*P* = 0.43, Hosmer-Lemeshow statistic). The sex interaction was not significant.

In the final model, we observed an increasing risk of high iron stores with increasing intakes of supplemental iron and vitamin C. The risk of high iron stores in the subjects who took > 12 but < 30 mg supplemental Fe/d with or without supplemental vitamin C was 2.4-fold that in the nonusers [adjusted odds ratio (OR): 2.44; 95% CI: 0.97, 6.12; *P* = 0.06], and the risk of high iron stores in the subjects who took ≥ 30 mg supplemental Fe/d with or without supplemental vitamin C was 4.3-fold that in the nonusers (adjusted OR: 4.32; 95% CI: 1.63, 11.47; *P* = 0.003).

The use of supplemental iron and vitamin C was an independent predictor of high iron stores in 88 of 100 bootstrap samples,

TABLE 2
Nutrient model: descriptive characteristics of nutrient and nonnutrient dietary factors considered for inclusion in the final multiple logistic regression model for predicting the risk of high iron stores in the elderly

Variable	Intake[1]
Nutrients	
Iron (mg)	
Total	13.2 [100]
Dietary	12.2 [100]
Heme	0.77 [99.7]
Nonheme	11.19 [100]
Supplemental	20 [16.4]
Vitamin C (mg)	
Total	166.7 [100]
Dietary	146.1 [100]
Supplemental	134 [30.6]
Calcium (mg)	
Total	712.8 [100]
Dietary	663 [100]
Supplemental	300 [18.4]
Nonnutrient dietary factors	
Dietary fiber (g)	18.4 [100]
Caffeine (mg)	147.8 [98.2]
Alcohol (g)	11.1 [57.5]

[1] Median; the percentage of the total sample who consumed each nutrient or nonnutrient dietary factor is in brackets.

indicating that the final model produced from the original data set was quite stable and would be found regularly in different comparable samples. Heme iron was positively associated with the risk of high iron stores in 67 bootstrap samples, nonheme iron was inversely related to the risk of high iron stores in 58 samples, and alcohol was positively related in 50 samples. All other nutrient and nonnutrient factors that were tried in the original backward logistic regression were retained as significant independent variables in <50% of the samples (between 6 and 35 samples).

Food and beverage model

The median intakes of food and beverage groups and the proportion of the total sample who consumed items from each group

TABLE 3
Final nutrient multiple logistic regression model for predicting the risk of high iron stores in the elderly[1]

Variable	OR[2]	P[3]
Supplemental intakes of iron and vitamin C		
Nonusers of both (n = 417)		
Supplemental iron and vitamin C		
>0 to ≤12 mg Fe/d (n = 38)	1.36 (0.49, 3.80)	0.56
>12 to <30 mg Fe/d (n = 38)	2.44 (0.97, 6.12)	0.06
≥30 mg Fe/d (n = 25)	4.32 (1.63, 11.47)	0.003
Supplemental vitamin C only (n = 96)	0.54 (0.20, 1.42)	0.21

[1] OR, odds ratio. The final model was derived from a backward elimination procedure on the dietary variables (nutrient model) shown in Table 1. Nonusers of both supplemental iron and supplemental vitamin C are the referent group.

[2] 95% CI in parentheses. Adjusted for sex, age, BMI, total energy intake, smoking status, aspirin use, and use of various medications known to be associated with an increased risk of bleeding.

[3] P for trend (0.001) was derived from the P value for the regression coefficient for supplemental iron as a continuous variable in the final model.

TABLE 4
Food and beverage model: descriptive characteristics of food and beverage groups considered for inclusion in the final multiple logistic regression model for predicting the risk of high iron stores in the elderly[1]

Food or beverage group[2]	Intake[3]
	Servings/wk
Fruit (n = 614)	17 [99.3]
Vegetables (n = 611)	18.8 [100]
Beans and nuts (n = 614)	2.4 [92.8]
Red meat (n = 614)	2.5 [96.9]
Light meat (n = 612)	4 [99.2]
Breads and pasta (n = 600)	10 [99.7]
Sweet baked goods (n = 597)	10 [97.2]
Cold breakfast cereals (n = 611)	3 [72.3]
Refined cereals (n = 165)	3 [37][4]
Whole-grain cereals (n = 277)	3 [63][4]
Whole grains (n = 602)	4.5 [79.6]
Milk (n = 596)	5.5 [79.9]
Other dairy products (n = 614)	5.4 [94.6]
Coffee and tea (n = 614)	14 [96.7]

[1] n Values vary because of missing data. Missing data represent nonresponders to items on the food-frequency questionnaire.

[2] See Subjects and Methods for description of food and beverage groups.

[3] Median; the percentage of the total sample who consumed items from each food or beverage group is in brackets. Nonconsumers were the subjects who selected "never, or less than once per month" for average consumption over the past year of items on the food-frequency questionnaire.

[4] Percentage of subjects who consumed cold breakfast cereals (n = 442)

are shown in **Table 4**. The final food and beverage model is shown in **Table 5**. The backward elimination procedure on the initial model resulted in a final model that included 4 independent predictors of high iron stores: fruit, red meat, sweet baked goods, and whole grains. There was no evidence of lack of fit (P = 0.89, Hosmer-Lemeshow statistic). There were no significant interactions.

In the final model, the risk of high iron stores in the subjects who consumed >21 servings of fruit or fruit juice/wk was ≈3-fold that in the subjects who consumed ≤14 servings/wk (adjusted OR: 2.88; 95% CI: 1.26, 6.61; P = 0.01); the P for trend was 0.04. The risk of high iron stores in the subjects who consumed >4 but <7 servings of red meat/wk was ≈3-fold that in the subjects who consumed ≤4 servings/wk (adjusted OR: 2.94; 95% CI: 1.33, 6.47; P = 0.008). The risk of high iron stores in the subjects who consumed ≥7 servings of red meat/wk was 3.6-fold that in the subjects who consumed ≤4 servings/wk (adjusted OR: 3.61; 95% CI: 1.57, 8.27; P = 0.004). The P for trend in the red meat group was 0.004. The risk of high iron stores in the subjects who consumed >14 servings of sweet baked goods/wk was 60% of that in the subjects who consumed <7 servings/wk (adjusted OR: 0.40; 95% CI: 0.17, 0.91; P = 0.03); the P for trend was 0.08. The risk of high iron stores in the subjects who consumed >7 servings of whole grains/wk (>1 serving/d) was 77% of that in the subjects who did not consume whole grains (adjusted OR: 0.23; 95% CI: 0.07, 0.75; P = 0.01); the P for trend was 0.13. This difference remained significant after a Bonferroni adjustment for multiple comparisons (P = 0.03). Furthermore, we compared the final food model with and without whole grains by using a likelihood ratio test to see whether whole grains added significant predictive capability. The difference was highly significant, suggesting that the whole-grain category makes a significant contribution to the final food model. Of the covariates, age was only marginally associated

TABLE 5
Final food and beverage multiple logistic regression model for predicting the risk of high iron stores in the elderly[1]

Variable (servings/wk)	OR[2]	P
Fruit[3]		
≤14 (n = 230)		
>14 to ≤21 (n = 181)	2.03 (0.91, 4.53)	0.08
>21 (n = 203)	2.88 (1.26, 6.61)	0.01
Red meat[4]		
≤4 (n = 318)		
>4 to <7 (n = 145)	2.94 (1.33, 6.47)	0.008
≥7 (n = 151)	3.61 (1.57, 8.27)	0.004
Sweet baked goods[5]		
<7 (n = 205)		
≥7 to ≤14 (n = 175)	0.55 (0.26, 1.14)	0.11
>14 (n = 217)	0.40 (0.17, 0.91)	0.03
Whole grains[6]		
≤0.47 (n = 184)		
>0.47 to ≤4 (n = 176)	1.12 (0.55, 2.29)	0.75
>4 to ≤7 (n = 98)	1.44 (0.62, 3.36)	0.40
>7 (n = 141)	0.23 (0.07, 0.75)	0.01

[1] OR, odds ratio. The lowest intake group for each food or beverage group was the referent group. The final model was derived from a backward elimination procedure of dietary variables (food and beverage model) shown in Table 1. P for trend values were derived from the P value for the regression coefficient for each dietary factor in the final model when all variables in the model were continuous.
[2] 95% CI in parentheses. Adjusted for sex, age, BMI, total energy intake, smoking status, aspirin use, use of various medications known to be associated with an increased risk of bleeding, alcohol intake, and use of iron-containing supplements.
[3] P for trend = 0.04.
[4] P for trend = 0.004.
[5] P for trend = 0.08.
[6] P for trend = 0.13.

($P = 0.06$) with the risk of high iron stores, whereas supplemental iron and vitamin C ($P < 0.001$), alcohol ($P = 0.01$), and BMI ($P = 0.05$) were significantly associated with risk.

Fruit and red meat were positive, independent predictors of high iron stores in 80 and 75 of 100 bootstrap samples, respectively, whereas whole grains and sweet baked goods were inversely related to the risk of high iron stores in 85 and 48 samples, respectively. This indicates that the model produced from the original data set, with the exception of sweet baked goods, was reasonably stable and would be found regularly in different comparable samples. All other food groups that were tried in the original backward logistic regression were retained as significant independent variables in < 50% of the samples (between 14 and 41 samples).

DISCUSSION

To our knowledge, our results are the first to show that dietary factors are significantly associated with the risk of high iron stores. These findings are noteworthy because they seem to contradict experimental evidence from Hallberg and coworkers (16, 52, 53) suggesting that dietary iron cannot result in iron overload. These researchers have shown down-regulation of both heme- and nonheme-iron absorption at SF concentrations of ≈60 μg/L to a concentration just sufficient to cover basal iron losses; they conclude that there is no risk in otherwise healthy, iron-replete individuals of developing iron overload by iron absorption from a diet containing highly bioavailable forms of dietary iron. In contrast, we observed that 70% of the subjects in our iron-replete elderly population had SF concentrations > 60 μg/L and that consumption of iron-containing supplements and of certain dietary factors were significant risk factors for high iron stores after rigorous control for potential confounders.

We suggest 3 possible reasons for this discrepancy. First, their results are derived from metabolic studies involving short-term dietary interventions, whereas our findings are based on observational data involving estimations of long-term dietary intake and SF concentrations reflecting accumulated iron stores from all sources. Second, we may have inadequately controlled for potential confounders of the association between dietary factors and the risk of high iron stores. Third, because their subjects were young and our study population was elderly, our divergent findings may be due to age-related changes in the regulation of iron absorption. No firm conclusions can be drawn from the existing literature on this subject, which is both scarce and often contradictory (54).

Dietary factors associated with a higher risk of high iron stores

Fruit and fruit juice

Elderly subjects who consumed > 3 servings of fruit or fruit juice/d (30% of the population) had a significantly higher risk of high iron stores than did those who consumed ≤2 servings/d. Fruit and fruit juices are rich dietary sources of organic acids such as ascorbic (vitamin C), citric, malic, and tartaric acids. Thus, we suggest that this positive association may largely have been due to the combined effects of vitamin C and other organic acids in enhancing nonheme iron bioavailability (55–57).

Red meat and light meat

We found that the risk of high iron stores in the subjects who consumed > 4 servings of red meat products/wk (approximately one-half of our elderly population) was ≈3-fold that in the subjects who consumed ≤4 servings/wk. This was probably due to the highly bioavailable heme iron that is found only in meat, of which red meats are the richest dietary sources.

Our findings suggest that not all types of meat consumption are associated with a high risk of high iron stores, because light meat (poultry and seafood) intake was unrelated to risk. We speculate that this may have been due to a difference between red meat and light meat in the amount and bioavailability of iron. Red meats have a higher total iron content and a greater proportion of heme iron than do light meats (58–61). Mean heme iron as a percentage of total iron varies from 50% to 80% in cooked red meats and from 25% to 40% in cooked light meats (chicken and turkey) (60).

Supplemental iron and vitamin C

The dose-dependent increase in the risk of high iron stores that was associated with iron-containing supplements is not surprising. The increase in risk was probably due to chronic consumption of high amounts of supplemental iron (≥ 30 mg supplemental Fe/d) normally meant for the short-term clinical treatment of iron-deficiency anemia. However, it also should be noted that we found an increased risk of high iron stores in persons who reported consuming > 12 but < 30 mg supplemental Fe/d, an amount of iron that is included in multivitamin supplements commonly consumed by iron-replete persons.

Dietary factors associated with a lower risk of high iron stores

Whole grains

We observed that the subjects who consumed >7 servings of whole grains/wk (>1 serving/d), representing a median dietary fiber intake of 23 g, had a 77% lower risk of high iron stores than did the subjects who did not consume whole grains. We suggest that this inverse association was probably due to the well-known inhibitory effect of the phytate found in fiber on the absorption of nonheme iron (62–69). Although we did not have information on when the foods were consumed, we speculate that the subjects who consumed ≤7 servings/wk probably ate whole grains at breakfast in the form of iron-fortified, whole-grain breakfast cereals. We may not have seen a deleterious effect on iron status because of the fortification iron. However, the subjects who consumed >7 servings/wk probably consumed whole-grain products that were not iron-fortified at meals other than breakfast, which allowed the whole-grain effect on the risk of high iron stores to be seen in that group. To investigate this speculation, we deleted whole-grain breakfast cereal users from the final food model and ran the model again. We observed an inverse trend across the whole-grain groups; the adjusted odd ratios for the 3 groups (ie, >0.47 to ≤4, >4 to ≤7, and >7) were 1.05, 0.50, and 0.14, respectively. The P for trend was not significant (0.17), which was probably due to lack of power because we deleted >200 whole-grain breakfast cereal consumers. This suggests that there was an inverse trend with increasing whole-grain intake that was masked by the consumption of iron-fortified, whole-grain breakfast cereals by our study population.

Sweet baked goods

The finding that >2 servings of sweet baked goods/d was associated with a 60% lower risk of high iron stores must be interpreted with caution because this food group was a significant predictor of high iron stores in only 48 of 100 bootstrap samples. This suggests instability in this estimate of risk and the need for further confirmation in additional studies. Nevertheless, it is possible that this inverse association may have been due to the combined effect of 2 inhibitors of iron absorption that are present in refined (white) flour: phytate and calcium (70, 71).

Epidemiologic implications

Findings have been inconclusive among studies investigating the association between total dietary iron intake and the risk of various heart disease outcomes (4–6, 72–81). Because heme iron is much more bioavailable than is nonheme iron, it may be beneficial to make a distinction between the 2. Of the studies cited above, only 2 examined the association between heme-iron intake and the risk of heart disease, and both found heme to be significantly associated with an increased risk of myocardial infarction (6, 73, 75). Furthermore, meat (red meat and poultry combined) has been shown to be associated with an increased risk of both fatal ischemic heart disease (82) and myocardial infarction (4, 73). However, the latter association was not significant. Although a causal relation between excess body iron and the risk of heart disease is controversial (4, 6, 83–87), our finding of a significant association between the intake of red meat, which is rich in heme iron, and the risk of high iron stores suggests that the reported associations of dietary heme iron (6, 73, 75) and meat intake (82) with an increased risk of myocardial infarction are compatible with the possibility that high iron stores may increase the risk of coronary disease.

Conclusions

Our findings are noteworthy because we showed that several dietary factors are associated with the risk of high iron stores in a noninstitutionalized, elderly American cohort. Our observation that red meat intake was associated with a significantly higher risk of high iron stores suggests that many US elderly persons who consume a Western diet may be at an increased risk of high iron stores because of their typical intake of red meat. Because the effect of heme iron on the bioavailability of dietary iron is substantial (88), our finding that red meat and light meat have different effects on the risk of high iron stores suggests that the effect of heme iron may differ markedly depending on dietary patterns of meat consumption. Furthermore, this analysis provides clear support for our previous suggestion (17) that the unprescribed use of supplemental iron by elderly Americans on a Western-style diet is unnecessary. The fiber intakes associated with a lower risk of high iron stores in our elderly, iron-replete subjects were within the range of recommended intakes for Americans (≈20–35 g/d; 89–91). The possible adverse consequences of the reduced dietary iron bioavailability produced by high fiber intakes on the iron status of subgroups of the American population who are vulnerable to iron deficiency, ie, young children and women of reproductive age, need additional investigation. If the association between high iron stores and the risk of chronic diseases proves to be causal, our data are important because they suggest that modifying dietary patterns and avoiding iron-containing dietary supplements could be helpful in lowering the risk of developing high iron stores and, thus, the risk of developing disease.

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