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Dietary iron intake and serum ferritin concentration in 213 patients homozygous for the *HFE*^{C282Y} hemochromatosis mutation

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BACKGROUND: *HFE*^{C282Y} homozygotes have an increased risk for developing increased iron stores and related disorders. It is controversial whether dietary iron restrictions should be recommended to such individuals.

OBJECTIVE: To determine whether dietary iron content influences iron stores in *HFE*^{C282Y} homozygotes as assessed by serum ferritin concentration.

DESIGN: Serum ferritin concentration was measured and a dietary iron questionnaire was completed as part of the evaluation of 213 *HFE*^{C282Y} homozygotes who were identified through screening of >100,000 primary care patients at five HEmochromatosis and IRon Overload Screening (HEIRS) Study Field Centers in the United States and Canada.

RESULTS: No significant relationships between serum ferritin concentration and dietary heme iron content, dietary nonheme iron content or reports of supplemental iron use were found.

CONCLUSION: These results do not support recommending dietary heme or nonheme iron restrictions for *HFEC*^{282Y} homozygotes diagnosed through screening in North America.

Key Words: *Haemochromatosis; Hemochromatosis; Iron overload; Iron supplementation*

La consommation de fer d'origine alimentaire et la concentration de ferritine sérique chez 213 patients homozygotes à la mutation d'hémochromatose *HFE*^{C282Y}

HISTORIQUE : Les homozygotes *HFE*^{C282Y} présentent un risque accru d'emmagasiner des réserves de fer et de présenter des troubles connexes. La décision de recommander des restrictions de fer d'origine alimentaire à ces individus est controversée.

OBJECTIF : Déterminer si le contenu en fer d'origine alimentaire influe sur les réserves en fer chez les homozygotes *HFE*^{C282Y}, telles qu'elles sont évaluées par la concentration de ferritine sérique.

MÉTHODOLOGIE : Les chercheurs ont mesuré la concentration de ferritine sérique et ont fait remplir un questionnaire sur le fer d'origine alimentaire dans le cadre de l'évaluation de 213 homozygotes *HFE*^{C282Y} recrutés par dépistage de plus de 100 000 patients en soins primaires de cinq centres d'études HEIR sur le dépistage sur le terrain de l'hémochromatose et de la surcharge en fer aux États-Unis et au Canada.

RÉSULTATS : Les chercheurs n'ont décelé aucune relation significative entre la concentration de ferritine sérique et le contenu en fer hémique d'origine alimentaire, le contenu en fer non hémique d'origine alimentaire ou les déclarations d'utilisation de suppléments de fer.

CONCLUSION : Ces résultats n'appuient pas la recommandation de restreindre le fer hémique ou non hémique chez les homozygotes *HFE*^{C282Y} diagnostiqués par dépistage en Amérique du Nord.

Iron is absorbed from the diet as a part of the heme molecule or as ferrous iron not bound to heme (1). Heme is found primarily in such proteins as hemoglobin and myoglobin. Iron not bound to heme is present in vegetables, cereals and other foodstuffs. Iron absorption occurs most efficiently in the duodenum, and heme iron is absorbed more readily than iron not bound to heme (2). Heme crosses from the lumen into the enterocyte (3) by an unknown mechanism, after which iron is removed from the protoporphyrin ring through the action of heme oxygenase (4). Nonheme iron uptake into the enterocyte is mediated by the iron transporter, divalent metal transporter 1 (5). Divalent iron is exported across the basolateral membrane of enterocytes to the portal blood stream by ferroportin 1 (6), and the membrane-associated ferroxidase, hephaestin (7), assists in converting the iron to the ferric form that is bound by plasma transferrin. Several studies indicate that dietary heme iron content is a significant predictor of iron stores among individuals homozygous for the C282Y mutation of the *HFE* gene on chromosome 6p (8,9). Population studies conducted predominantly among Caucasians and Hispanics have shown that amounts of dietary heme iron, but not those of dietary nonheme iron, are positively associated with serum ferritin (SF) concentration (10,11).

The HEmochromatosis and IRon Overload Screening (HEIRS) Study is a multicentre, National Institutes of Health-sponsored study

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designed to determine the prevalence of primary iron overload in adult primary care patients of various ethnicities who reside in the United States (US) and Canada (12). More than 100,000 participants were screened by testing for HFE^{C282Y} and HFE^{H63D} mutations and measuring SF concentration and transferrin saturation (TS) (13). Participants with HFE^{C282Y} homozygosity or combined elevations of SF and TS levels were invited to return for clinical follow-up, which included a dietary questionnaire. The purpose of the present research was to determine whether dietary nonheme iron, dietary heme iron and supplemental iron use influence SF concentration in HFE^{C282Y} homozygotes identified in screening programs.

METHODS

The present study was approved by the institutional review board of each participating institution and written informed consent was obtained from each participant.

Screening phase of the study

A description of the HEIRS Study design has been reported (12). The study was approved by the institutional review board of each participating institution and written informed consent was obtained from each participant. Participants identified through primary care clinics and medical blood drawing laboratories were screened over a two-year period (February 2001 to March 2003) at five HEIRS Field Centres (Washington DC; Birmingham, Alabama; Irvine, California; Portland, Oregon – Honolulu, Hawaii [USA]; and London, Ontario). Both patients and other persons accompanying the patient were potential participants. Eligibility criteria included age ≥25 years and an ability to understand the informed consent. Participants were asked how they heard about the study and whether they had been previously diagnosed with iron overload or hemochromatosis. Race/ethnicity was determined by self-reported answers to two questions: one asking about Hispanic background and one asking for nonexclusive choice of five racial groups: Caucasian, African American, Asian, Pacific Islander and American Indian. Participants affirming Hispanic background were classified as Hispanic. All participants were asked about a history of hemochromatosis or iron overload, liver disease, diabetes, arthritis, congestive heart failure, impotence and infertility before receiving any genetic test results. HFE^{C282Y} alleles were detected from blood spots using a modification of the Invader assay (Third Wave Technologies, USA) that increases the allele-specific fluorescent signal by including 12 cycles of locus-specific polymerase chain reaction before the cleavase reaction (14).

Clinical evaluation

Of 299 HFE^{C282Y} homozygotes identified in the HEIRS Study, 227 were not previously diagnosed with iron overload (13) and, were therefore, eligible for the present study. All were invited to participate in a postinitial screening clinical evaluation and 219 (96%) participated. Of these, 213 were included in the present analysis (216 completed questionnaires but three were excluded for extreme dietary values: see Statistical analysis section below). The clinical evaluations were performed over a two-and-one-half year period from July 2001 to February 2004. The mean ± SD number of days between initial screening and the clinical evaluation was 257±204. Participants in the clinical evaluation gave written informed consent in addition to the consent obtained in the initial screening part of the study. The purpose of the present clinical evaluation was to assess possible signs, symptoms and complications of iron overload, and to collect information regarding dietary and other factors possibly related to iron overload. Medical history, including the use of medications, vitamins, herbal remedies, and other supplements, were recorded on standardized forms and a food frequency questionnaire was completed (15). A limited physical examination was performed to detect signs of liver disease, heart disease, arthropathy or abnormal skin pigmentation. Venous blood was collected, usually in the fasting state.

Laboratory techniques

SF concentration was measured by turbidometric immunoassay (Roche Applied Science/Hitachi 911, USA). Serum iron concentration and unsaturated iron-binding capacity were measured spectrophotmetrically and the TS was calculated. Serum concentrations of glucose, alanine aminotransferase (ALT), aspartate aminotransferase, gamma-glutamyl transferase and C-reactive protein were determined using a Hitachi 911 analyzer (Roche Diagnostics/Boehringer Mannheim Corporation, USA). Serum insulin concentrations were measured using a DPC Immulite Analyzer (Diagnostic Products Corporation, USA). Complete blood counts were measured using the Beckman Coulter GenS (Beckman/Coulter, USA). Participants with hemoglobin level ≤132 g/L (men) or ≤116 g/L (women), or mean corpuscular volume ≤77 fL, underwent analysis of reticulocyte count by flow cytometry, serum concentrations of haptoglobin, lactate dehydrogenase, direct and indirect bilirubin (Hitachi 911 analyzer), and hemoglobin electrophoresis with hemoglobin A2 and F quantification (high-performance liquid chromatography methodology). Participants with elevated ALT levels (≥40 IU/L in men or ≥31 IU/L in women) were tested for hepatitis B surface antigen and antibody to hepatitis C virus (Vitros ECi, Ortho-Clinical Diagnostics, Inc, USA).

Estimation of dietary iron content and alcohol consumption

To estimate dietary iron content and alcohol consumption, participants completed the University of Hawaii Multi-Ethnic Dietary Questionnaire. The correlation of nutrient intake estimated from this questionnaire with information from 24 h recalls has been documented (15). The questionnaire asks about average eating habits over the past year. The questionnaire was analyzed at the University of Hawaii and the results of the analysis provided estimates for average daily total dietary iron and the amount of dietary iron derived from meat, fish and poultry. An estimate for supplemental iron intake was also provided. The iron derived from meat, fish, and poultry was classified as heme iron, and the difference between total dietary iron and dietary heme iron as nonheme iron.

Statistical analysis

Statistical analyses were performed using SAS version 9.1.3 and Splus 7.0 (SAS Institute, USA) for Windows (Microsoft Corporation, USA). SF concentration was natural log transformed in all analyses. Multivariate linear regression was used to investigate the relationship between natural log SF concentrations and dietary iron content in HFE^{C282Y} homozygotes, after considering potentially confounding factors such as age, sex, ethnicity, phlebotomy history, alcohol consumption, tea consumption, liver function tests and markers of inflammation. The antilog of regression coefficients shows the per cent change in SF concentration for a one unit difference in the independent variable. CIs for the coefficients were computed as the antilog of the geometric mean ± 1.96 × SE.

The primary analysis was multivariate linear regression of natural log SF concentration as a function of dietary nonheme iron, dietary heme iron and supplemental iron in the past two years (expressed as mg/kg/day), calories (kcal/day), age, race (white or nonwhite), male sex, alcohol consumption, C-reactive protein and ALT levels in participants with no history of blood transfusions or phlebotomy therapy. Two participants were excluded from the analysis because they reported extreme values of heme iron and one participant was excluded due to reporting extreme values for supplemental iron.

RESULTS

Characteristics of study participants

The characteristics of the study participants (HFE^{C282Y} homozygotes who presented for clinical evaluation who completed a dietary questionnaire, and did not undergo previous quantitative phlebotomy or have >10 units of blood transfused) are summarized in Table 1. HFE^{C282Y} homozygotes in this cohort were predominantly Caucasian, predominantly female and had a low prevalence of hepatitis, HIV infection or inflammatory

TABLE 1
Characteristics of *HFE*^{C282Y} homogyzotes identified by screening of a primary care population* (n=213)

Clinical	Women (n=133)	Men (n=80)
Age, years, mean ± SD	50±13	52±14
Ethnicity, n		
Caucasian	127	72
African American	1	3
Hispanic	1	2
Native American	1	0
Other	3	3
History of hepatitis, n (%)	9 (6)	3 (4)
History of infections or HIV, n (%)	0 (0)	0 (0)
History of other inflammatory conditions, n (%)	5 (4)	1 (1)
History of malignancy, n (%)	18 (14)	12 (15)
Serum ferritin, µg/L	253 (98–542)	666 (428–1310)
Hemoglobin, g/L, mean ± SD	138.8±11.0	154.4±11.0
MCV, fL, mean ± SD	94.3±5.1	95.0±5.1
ALT, U/L	17 (12–22)	28 (21–41)
CRP, mg/L	4.5 (2.0–8.1)	2.0 (2.0–4.5)
Dietary questionaire		
Calories, kcal/kg/day	25 (18–33)	24 (18–28)
Nonheme iron, mg/kg/day	0.16 (0.11–0.025)	0.15 (0.11–0.21)
Heme iron, mg/kg/day	0.02 (0.02–0.04)	0.03 (0.02–0.04)
Supplemental iron, mg/kg/day	0.00 (0.00–0.19)	0.00 (0.00–0.15)
Supplemental iron taken at least 2 years, mg/kg/day	0.00 (0.00–0.05)	0.00 (0.00–0.14)
Alcohol, g/kg/day	0.01 (0.00–0.09)	0.00 (0.00–0.13)
Tea consumed during past year, n (%)†		
Never to once a month	38 (28.6)	40 (50.0)
2–3 times a month – once/week	26 (19.5)	9 (11.3)
2–3 times a week – once/day	37 (27.8)	14 (17.5)
More than once a day	19 (14.3)	7 (8.8)
Not answered	13 (9.8)	10 (12.5)

*Data presented as median (interquartile range) unless otherwise indicated. *Persons who attended the comprehensive clinical evaluation, completed the dietary questionnaire, and did not undergo quantitative phlebotomy or have >10 units blood transfused in their lifetime. Forms with <500 cal/day or >8000 cal/day were excluded; †Maximum of black tea, or herbal/green/other tea. ALT Alanine aminotransferase; CRP C-reactive protein; MCV Mean corpuscular volume*

conditions according to the medical history. Fourteen per cent had a history of malignancy.

Relationship between SF concentration and dietary iron content

The relationship between dietary iron and natural log SF is shown in Figure 1. After adjustment for the factors listed in the Methods section, neither heme nor nonheme iron was significantly associated with natural log SF (Table 2). Known adjustment factors of age, alcohol consumption, ALT and male sex were significantly associated with higher natural log SF.

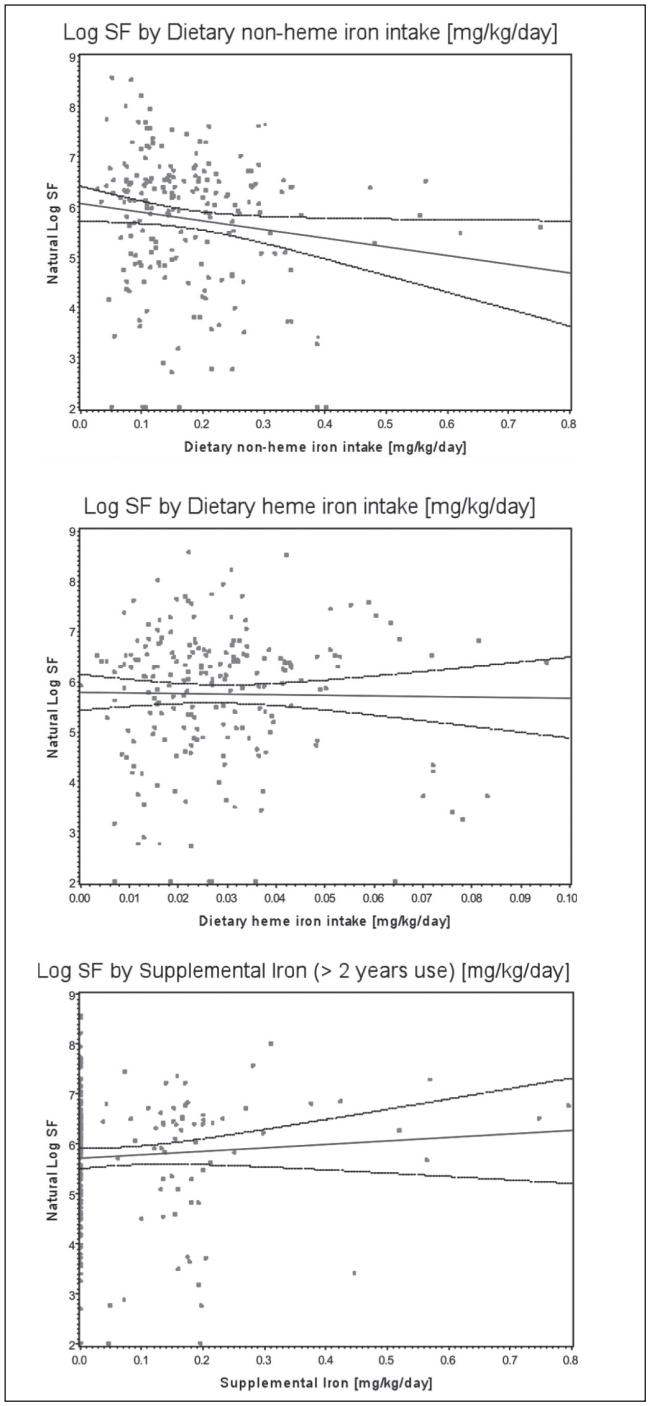

Figure 1) *Scatterplots of log serum ferritin (SF) concentration and dietary nonheme iron, heme iron and supplemental iron (expressed as mg/kg/day) in* HFE^{C282Y} *homozygotes with no history of blood transfusions or phlebotomy*

DISCUSSION

The present study did not find evidence that dietary iron content, either heme iron or nonheme iron, influences SF concentration in *HFE*^{C282Y} homozygotes identified by screening primary care patients in North America.

In previous studies by other investigators, the absorption of heme and nonheme iron from an unfortified meal (16) or nonheme iron from an oral iron test dose (17) by hemochromatosis patients diagnosed in medical care was greater than would be predicted from the relationship between iron absorption and SF levels observed in normal volunteers. There was a significant inverse relationship of absorption of nonheme iron with increasing SF. In contrast, heme iron absorption was not significantly related to SF concentration (16). In women in the United Kingdom, *HFE*^{C282Y} homozygotes had SF concentrations 2.4 times higher than women with wild-type *HFE* genotype. The association between heme iron intake estimated using food frequency questionnaires and SF concentration was stronger in *HFE*^{C282Y} homozygotes than in subjects with other *HFE* genotypes; nonheme iron intake had little effect on SF concentration (9,18,19). In the Netherlands, women >50 years of age, *HFE*^{C282Y} homozygotes and *HFE*$^{C282Y/H63D}$ compound heterozygotes had significantly higher SF concentrations than women with other *HFE* genotypes. *HFE*^{C282Y}

TABLE 2
Multiple linear regression model for associations of dietary iron intake and other covariates with log serum ferritin concentrations in *HFE*^{C282} homozygotes

Variable	% increase in serum ferritin level with a one unit change in variable (95% CI)	P
Heme iron, mg/kg/day	–28 (–100 to 10000)	0.96
Nonheme iron, mg/kg/day	–25 (–91 to 515)	0.79
Supplemental iron taken >2 years (yes/no)	–18 (–42 to 14)	0.24
Caucasian race	58 (–15 to 197)	0.15
Age 45–64 years compared with age <45 years	30 (–10 to 86)	0.16
Age ≥65 years compared with age <45 years	154 (59 to 307)	0.0001
Alcohol, g/kg/day	153 (26 to 408)	0.0098
ALT, U/L	2 (1 to 2)	<0.0001
Male sex	116 (53 to 205)	<0.0001
C-reactive protein, mg/L	–4 (–28 to 27)	0.78
Calories, kcal/kg/day	–0.4 (–3 to 2)	0.73

ALT Alanine aminotransferase

homozygotes and *HFE*$^{C282Y/H63D}$ compound heterozygotes who consumed relatively high amounts of heme iron had the highest SF concentration (20). It has been proposed that the high rate of expression of iron overload and associated manifestations in hemochromatosis homozygotes diagnosed in medical care in Australia is attributable to the high national rate of meat consumption (21). Red meat consumption was positively associated with higher SF concentrations among adults in Busselton, Australia, regardless of *HFE* genotype (22,23). Some persons with *HFE*^{C282Y} homozygosity may be especially susceptible to iron loading from diets in which a high proportion of available iron is present as heme, although they may also absorb increased fractions of nonheme iron.

Other studies have suggested that fortification of food with iron may increase the severity of iron overload in persons with hemochromatosis. In seven treated hemochromatosis patients, the absorption of nonheme iron from a test meal was measured using the extrinsic tag technique to simulate the effects of fortification (24). Doubling of the iron dose produced a proportional increase similar to that in normal subjects (25). In Sweden, fortification before 1995 accounted for 42% of the mean daily dietary iron content, although Swedish hemochromatosis homozygotes had lower iron burdens, on average, than those in Australia (26). Decreased fortification in Sweden since 1995 is expected to decrease the rate of iron accumulation in persons with hemochromatosis (27). It is similarly predicted that iron fortification of wheat flour at the current US levels would accelerate the initial rate of iron loading in persons with hemochromatosis, and that the accelerated evolution of clinical disease in susceptible individuals is directly proportional to the amount of fortification iron added (24,28).

The iron phenotypes of *HFE*^{C282Y} homozygotes diagnosed in screening are typically less severe than those of hemochromatosis patients diagnosed in medical care. This could account for some differences observed in the present and in previous studies. There are several other limitations to the present study. For example, SF concentration was measured at one point in time and did not address the effects of dietary therapy in a randomized trial. We did not use direct measures of body or liver iron. Dietary iron content in the present study was determined by recall questionnaire; the amounts are, therefore, approximations rather than precise amounts. The questionnaire may not have had sufficient sensitivity to fully reflect the variability in dietary iron content. Nevertheless, these results do not support recommending dietary iron restrictions to patients identified through screening programs.

Formulating an iron-restricted diet is difficult because iron is ubiquitous in fruits, vegetables and meat products and, in the US, all products that contain flour are fortified with iron. Regardless, the concept of dietary iron restriction for management of hemochromatosis and iron overload continues to hold appeal for patients and is often advocated by patient support groups as a way to limit iron accumulation. Dietary iron deficiency has been reported in developing countries; however, in these studies it is difficult to exclude the effects of enteric infections such as *Helicobacter pylori* and parasitic infestations. Vegan diets have been associated with iron deficiency (29). In persons with hemochromatosis diagnosed in medical care, iron absorption decreases as body iron stores increase (17,30,31). Iron absorption can also be affected by other dietary factors such as tea consumption, alcohol consumption and the use of proton pump inhibitors that suppress gastric acid secretion (32). Iron depletion by periodic phlebotomy removes iron efficiently, whereas dietary manipulation does not remove absorbed iron and has little effect on the rate of iron accumulation. It is likely that less emphasis on an iron-restricted diet could improve the quality of life of patients and simplify long-term management of hemochromatosis.

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