Relevance of dietary iron intake and bioavailability in the management of *HFE* hemochromatosis: a systematic review[1–3]

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ABSTRACT

Background: Hereditary hemochromatosis (HH) leads to iron loading because of a disturbance in the negative-feedback mechanism between dietary iron absorption and iron status. The management of HH is achieved by repeated phlebotomies.

Objective: We investigated whether HH patients would benefit from a diet with low iron intake and bioavailability.

Design: We performed a systematic review of studies that linked iron bioavailability and status with dietary factors in subjects with diagnosed HH. Studies on heterozygotes for the *HFE* mutation were excluded.

Results: No prospective, randomized study was reported. Nine studies that directly measured iron bioavailability from test meals in HH patients have been described as well as 3 small, prospective, longitudinal studies in HH patients. Eight cross-sectional studies were identified that investigated the effect of dietary composition on iron status. Calculations of iron bioavailability in HH were made by extrapolating data on hepcidin concentrations and their association with iron bioavailability. The potential reduction in the yearly amount of blood to be phlebotomized when restricting dietary iron absorbed was estimated in the 3 longitudinal studies and ranged between 0.5 and 1.5 L. This amount would be dependent on individual disease penetrance as well as the dietary intervention.

Conclusions: Despite the limited quantitative evidence and the lack of randomized, prospective trials, dietary interventions that modify iron intake and bioavailability may affect iron accumulation in HH patients. Although this measure may be welcome in patients willing to contribute to their disease management, limited data exist on the clinical and quality of life benefit.   *Am J Clin Nutr* doi: 10.3945/ajcn.112.048264.

INTRODUCTION

Hereditary hemochromatosis (HH) is a heterogeneous group of disorders that is characterized by excessive iron bioavailability and deposition in the body. It is caused by a limited ability to downregulate iron absorption in the presence of sufficient iron stores (1–4). The most prevalent form by far is *HFE*-related HH and can be ascribed to homozygosity for the p.Cys282Tyr mutation in the *HFE* gene (5). The mutation is estimated to affect 1:200–1:300 subjects of Northern European descent (6). The clinical penetrance is lower and ranges between 2% and 38% in men and 1% and 10% in women (5, 7, 8). The low penetrance indicates that other genetic, epigenetic, and environmental factors play a role in the development of the disease (5). Although cross-sectional studies have indicated that male sex, age, and alcohol consumption are predictors of phenotypic expression, other factors, such as diet, may also be involved (9).

Dietary heme and nonheme iron are absorbed by distinct pathways (10–12); nonheme iron has to be reduced by dietary components or by duodenal cytochrome b before it can be taken up by dimetal transporter 1. In contrast, heme iron is absorbed intact (13) and is more independent from effects of the food matrix (10). Enterocyte iron is released to the blood via the cellular iron exporter ferroportin on the basolateral membrane. The regulation of this transport is reduced by the hepatocyte-derived peptide hormone hepcidin, which binds to ferroportin, leads to its internalization and degradation (11, 14, 15). In patients with *HFE* hemochromatosis, the duodenal expression of dimetal transporter 1, duodenal cytochrome b (16), and ferroportin (17, 18) is increased and consistent with the gene-expression profile encountered in iron-deficient duodenal enterocytes (19). Furthermore, lower serum hepcidin concentrations relative to ferritin concentrations have been reported in HH patients compared with those of control subjects (20) .

Dietary iron intake and bioavailability are determinants of iron status in the general population (21, 22). However, little is known about potential diet-related effects on iron accumulation in HH. Dietary recommendations for subjects with *HFE* HH are typically limited to general recommendations to follow a healthy diversified diet (*see* Supplemental Table 1 under "Supplemental data" in the online issue). An expert consensus is that patients should avoid iron-containing food supplements and alcohol. Patients diagnosed with HH are treated with a schedule of phlebotomies, which is an approach that has been shown to be safe and effective (23, 24). It is a commonly encountered attitude that patients wish active involvement in their own treatment, and a significant number of HH patients request more-detailed dietary

advice (Dutch Hemochromatosis Society, personal communication, May 2011).

We aimed to review the literature on iron bioavailability in subjects with *HFE*-related p.Cys282Tyr HH as well as idiopathic HH and to estimate whether and to which extent dietary iron restriction and modulation of dietary iron bioavailability could support treatment in the management and prevention of HH.

METHODS

Literature search

Online literature databases the Web of Science (Thomson Reuters; http://thomsonreuters.com/web-of-science/) and PubMed (National Centre for Biotechnology Information, US National Library of Medicine; www.pubmed.gov) were searched for articles that investigated iron bioavailability and iron status in subjects with HH. Studies were reviewed that included *HFE* homozygous p.Cys282Tyr subjects. Earlier studies on subjects with idiopathic HH conducted before the discovery of the *HFE* gene (25) were included in the review because homozygosity for the p.Cys282Tyr mutation in the *HFE* gene explains the great majority of these cases (25). Original research, including both observational and prospective studies, was included. Relevant-outcome measures were direct measurements of iron bio-availability, hepcidin concentrations, iron status markers, and the quantity of iron removed by phlebotomy under varying dietary regimens. The primary search was conducted between January and July 2011. An update search was conducted from May to July 2012. Relevant articles published thereafter but before the end of 2012 were also included. The literature search was conducted by 2 persons separately. The following search terms were used: iron status, hereditary hemochromatosis, iron overload, idiopathic, iron bioavailability, iron absorption, iron status, ferritin, hepcidin, diet, inhibitors, enhancers, homozygotes, and *HFE* gene. All original studies that reported the effect of dietary and lifestyle factors on iron status in human hemochromatosis patients were included. Studies and outcomes that focused exclusively on heterozygotes for the *HFE* gene were excluded. Articles that investigated the fecal excretion of radioisotopic tracers were not included in the review. Studies and data were not pooled into a meta-analysis but analyzed qualitatively and summarized in tables. No formal assessment of publication or reporting bias was performed. The study and protocol are also available under PROSPERO (International prospective registry of systematic reviews; http://www.crd.york.ac.uk/prospero/; registration no. CRD42012003501).

Calculation of iron absorption and iron balance in HH

Zimmermann et al (26) previously established the regression curve between hepcidin concentrations and iron absorption from a standard test meal in healthy individuals as follows:

$$\text{Iron absorption (\%)} = -3.9656 \ln[\text{hepcidin (nmol/L)}] + 13.238 \quad (1)$$

This regression curve was obtained by concomitantly assessing hepcidin concentration and iron bioavailability from an isotopically labeled test meal in 89 subjects with either a serum ferritin (SF) concentration <25 μg/L or who were iron sufficient (SF concentration >40 μg/L). These inclusion criteria were chosen to cover a wide range of iron statuses. With the assumptions that hepcidin is the primary determinant of iron absorption both in subjects with and without HH, we used this equation to estimate iron absorption in p.Cys282Tyr homozygotes by imputing average serum and plasma hepcidin concentrations at different stages of phlebotomy (20). In both of these studies (20, 26), the hepcidin concentration was measured at the Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands (Hepcidinanalysis.com) by using weak cation exchange time-of-flight mass spectrometry. The synthetic analog hepcidin-24 of hepcidin-25 was used as an internal standard for quantification (27, 28).

The effect of diet-related factors on iron balance was calculated for some of the studies under the following assumptions: *1*) the hemoglobin concentration was 150 g/L, *2*) the iron content in hemoglobin was 3.47 mg Fe/g hemoglobin, and *3*) the phlebotomy session (one unit of blood) was equal to 450 mL blood.

RESULTS

A total of 64 full-text articles were assessed for eligibility (*see* Supplemental Figure 1 under "Supplemental data" in the online issue). Of these articles, 20 studies were excluded because they were dedicated to heterozygotes for the p.Cys282Tyr mutation, 13 studies were clinical observations without a dietary component, and 7 studies investigated other conditions nonrelated to diet in HH. Furthermore, 3 studies investigated the fecal excretion of isotopic labels. A total of 21 studies were included in the final qualitative assessment. Nine studies that directly measured iron bioavailability from test meals (**Table 1**), 3 small, nonrandomized longitudinal, prospective studies (**Table 2**), and 8 cross-sectional studies, which were cited in 9 publications (**Table 3**), were found. No randomized prospective study has been reported to date.

Iron absorption studies in HH patients

Of the 9 radio or stable isotope studies shown in the literature, only the most-recent studies included an explicit characterization of the *HFE* gene in participating subjects (34, 35). Iron absorption from isotopically labeled iron dosages and test meals was repeatedly reported to be higher in subjects with idiopathic HH than in healthy control individuals (2, 3, 29, 31, 32, 35).

Since body iron stores are the most important determinant of iron absorption in the general population (10), it may confound data from absorption studies if not taken into account. Walters et al (2) showed that iron absorption from a chicken soup meal was 21.9% in patients with HH compared with 12.6% in the control group, regardless of iron status. In addition, the authors compared regression lines that linked iron absorption to serum ferritin concentrations and showed a smaller decrease in iron absorption with increasing iron stores in the HH group. In a secondary regression analysis, a nearly similar iron absorption of 27% and 26% in HH patients at SF values of 20 and 200 μg/L was estimated, respectively, whereas in healthy control subjects, absorption was decreased from 26% to 2.5%, which corresponded

TABLE 1
Iron-absorption studies in idiopathic HH patients or carriers of one or more *HFE* gene mutations[1]

First author, year of publication (reference)	Study population	Methods and outcome measures	Results[2]	Conclusions and comments
Smith, 1969 (29)	Subjects with idiopathic HH 1) Before start of venisection: n = 13 2) After venisection: n = 25 3) Healthy control subjects: n = 15	Radio-isotope absorption study; subjects with liver cirrhosis. Test meal composed of potato salad, corned beef, and fruit with ferric citrate tracer	1) Iron absorption: 13.4% 2) Iron absorption 1–3 y later: 63%; 3–5 y later: 52%; 5–10 y later: 44% 3) Iron absorption: 14.1%	Increase in iron absorption after initiation of phlebotomy therapy
Williams, 1965 (30)	Patients with idiopathic HH 1) Before phlebotomy 2) During phlebotomy 3) After phlebotomy	Radio-isotope absorption study (%) with ferric chloride given with a corned beef and potato salad meal with fruit; iron status assessed as the percentage of TS	1) Iron absorption: 6.7% (1–15%); percentage of TS: 85% 2) 31% (15–66%); percentage of TS: 78% 3) 65% (41–100%); percentage of TS: 52%	Increase in iron absorption with decreasing percentage of TS
Walters, 1975 (2)	1) Idiopathic HH: n = 52 a) SF <250 µg/L b) SF >250 µg/L 2) Control subjects: n = 21	Radio-isotope absorption study (%) with ferric citrate in a chicken soup test meal	1) Iron absorption: 21.9%; 2) 12.6% 1a) 30.0% (2 compared with 1a, $P < 0.05\%$) 1b) 16.9% (2 compared with 1b, NS) Secondary regression analysis SF: 20 µg/L: 1) compared with 2): 27.1% compared with 17.2% SF: 200 µg/L: 1) compared with 2): 26.0% compared with 2.5%	Higher iron absorption in idiopathic HH patients, especially at low SF
Bezwoda, 1976 (31)	1) Iron-deficient patients; SF < 25 µg/L; n = 18 2) Idiopathic HH; SF <25 µg/L; n = 8 3) Anemic women: SF < 25 µg/L; n = 12	Radio-isotope absorption study (%) with A) nonheme iron added to whole-wheat flour B) Labeled heme iron added to lamb with hemolyzed blood cells C) Ascorbic acid (60 mg)	1) compared with 2) compared with 3) A) Nonheme iron absorption: 1) 18.9% compared with 2) 36.4% compared with 3) 5.8% ($P < 0.05$) B) Heme iron absorption 1) 29.8% compared with 2) 37.1% compared with 3) 31.6% (NS) C) 1) 46.8% compared with 2) 74.2% compared with 3) 53.7% ($P < 0.05$)	High nonheme-iron absorption in idiopathic HH patients at low SF; at low SF concentrations heme iron is highly absorbed in all subjects; iron given with ascorbate without food is more bioavailable in HH patients than in control subjects
Valberg, 1979 (32)	1) Subjects with idiopathic HH; SF: 441.5 µg/L; n = 4 2) Healthy control subjects; SF: 64.5 µg/L; n = 33	Radio-isotope absorption study (%) Reference dose of ferric ascorbate given without food matrix	1) Iron absorption 74% compared with 2) 46%	Absorption of ferric ascorbate without food is higher in HH than in control subjects

TABLE 1 (*Continued*)

First author, year of publication (reference)	Study population	Methods and outcome measures	Results[2]	Conclusions and comments
Bezwoda, 1981 (33)	Idiopathic HH; mean SF: 25 μg/L; $n = 7$	Radio-isotope absorption study (%) Maizemeal porridge A) 5 mg Fe-chloride + 60 mg AA B) 10 mg Fe chloride + 60 mg AA C) 3 mg Fe sulfate + 30 mg AA in water (no food matrix)	A) Iron absorption: 25% B) 20% C) 72%	Absorption of iron is decreased in a maizemeal porridge with AA compared with ferric ascorbate without a food matrix in subjects with HH
Lynch, 1989 (3)	1) Control subjects: $n = 75$ 2) Idiopathic HH: $n = 15$ 3) Heterozygotes	Radio-isotope absorption study (%) A) Labeled heme and nonheme iron added to a standard meal B) Labeled nonheme iron added to standard meal (A) + 20 mg Fe sulfate + 100 mg vitamin C from orange juice Test meal (B) was tested only in groups 1) and 3)	A) Heme-iron absorption lower in 1) than in 2) but comparable at SF < 50 μg/L Nonheme-iron absorption in 1) lower than in 2) but similar to 3) Substudy in ID subjects A) Heme-iron absorption in ID control subjects: 21%, in ID HH subjects: 41% B) 1) 3.4% compared with 3) 9.2%	Higher heme- and nonheme-iron absorption in idiopathic HH and heterozygous HH compared with control subjects; differences in slopes relating SF and bioavailability in HH and control patients for both heme and nonheme iron
Kaltwasser, 1998 (34)	p.Cys282Tyr HH: $n = 18$	Radio-isotope absorption study (%) of meal with A) black tea and B) no tea Subjects acted as their own control subjects	Iron absorption: A) 6.9%; B) 22.1%; $P < 0.05$ Mean ($\pm$SEM) SF in the study group: 191 $\pm$ 18 μg/L	Tea consumption with the meal reduced iron absorption significantly
Hutchinson, 2008 (35)	1) Control subjects: $n = 14$ 2) p.Cys282Tyr HH: $n = 12$ 3) Heterozygotes for p.Cys282Tyr: $n = 7$ 4) Iron-deficient anemic: $n = 10$	Serum iron-appearance study, administration of 13.1 mg nonheme iron in the form of $FeCl_3$ (10 mg) vegetables in tomato sauce, potato mash, fruit, and orange juice (3.1 mg)	Test meal contained 260 mg vitamin C 1) SF: 115 μg/L; 2) SF: 94.3 μg/L; 3) SF: 64 μg/L; 4) SF: 9.9 μg/L Serum iron increase in 1) highest for 1) and 2) significantly different to 3) ($P < 0.0001$); no difference between 1) compared with 2) and 3) compared with 4)	Higher serum iron appearance in IDA and p.Cys282Tyr HH compared with control subjects

[1] AA, ascorbic acid; HH, hereditary hemochromatosis; ID, iron deficient; IDA, iron deficiency anemia; SF, serum ferritin; TS, transferrin saturation.
[2] All values are means (ranges in parentheses) unless otherwise indicated,

TABLE 2
Longitudinal iron intervention studies in subjects with hereditary hemochromatosis[1]

First author, year of publication (reference)	Study population	Methods	Outcome	Results[2]	Conclusions and comments
Olsson, 1997 (36)	Idiopathic HH; M; SF: 14–16 μg/L; $n = 16$	Longitudinal cohort study comparing 2 periods of 1 y A) Study period 1: iron-fortified wheat flour (65 mg/kg) available B) Study period 2: no iron-fortified flour available	SF; absorbed iron (calculated); interval (d) between phlebotomy	A) compared with B Absorbed iron: 4.27 compared with 3.36 mg Interval between phlebotomies: 59 compared with 69 d Difference in SF between 2 study periods despite increased interval of phlebotomy: 21.2 compared with 14.2 μg/L.	Iron-fortified wheat flour led to higher SF and a shorter period to the next phlebotomy and higher iron absorbed
Kaltwasser, 1998 (34)	p.Cys282Tyr HH: $n = 18$	Nonrandomized intervention study of 1 y Treatments: with main meals A) Black tea: $n = 9$ B) No tea: $n = 9$	SF; quantity of iron removed with phlebotomy	A) compared with B ΔSF: 2.78 μg/L ($r = 0.95$, $P < 0.05$) compared with 4.26 μg/L ($r = 0.98$, $P < 0.05$) Iron-loading: 1436 compared with 1085 mg (NS) Phlebotomy interval: 14 compared with 13 wk	Significant increase in SF in both groups but a smaller nonsignificant increase when tea was consumed with meals
Hutchinson, 2007 (37)	Patients with p.Cys282Tyr mutation in venesection therapy with the use of PPI drugs (lanzoprazole, omeprazole) to decrease gastric acid secretion ($n = 7$)	Longitudinal cohort study comparing annual phlebotomy requirements to maintain SF at 50 μg/L. 1) Before PPIs (6.1 y) 2) After initiation of PPIs (3.8 y) Outcomes A) Quantification of amount of blood removed with phlebotomy/y B) Serum iron response to an iron test meal containing 14.5 mg Fe		A) 2.5 compared with 0.5 L B) 50% reduction ($P < 0.05$) in serum iron AUC when a test meal was administered before or after PPIs	PPIs reduce iron bioavailability and decreases phlebotomy requirements in patients with HH

[1] HH, hereditary hemochromatosis; PPI, proton pump inhibitor; SF, serum ferritin.
[2] All values are means.

TABLE 3
Cross-sectional studies investigating associations between dietary intake and iron status indicators in hereditary hemochromatosis[1]

First author, year of publication (reference)	Study population	Study designs and outcome measures	Results[2]	Conclusions
Scotet, 2003 (38)	p.Cys282Tyr/p.Cys282Tyr: n = 378 1) Moderate alcohol intake (<60 g/d): n = 345 2) High alcohol intake (≥60 g/d): n = 33	Cross-sectional comparison of iron status (SF, SFe, and percentage of TS) between 1) and 2)	1) compared with 2) SF[2]: 969 μg/L compared with 1745 μg/L (P < 0.05) SFe[3]: 36 μmol/liter compared with 40 μmol/liter (P < 0.05) Percentage of TS: 80% compared with 87% (P < 0.05)	High alcohol intake is associated with higher SF in p.Cys282Tyr/p.Cys282Tyr
Greenwood, 2005 (39); Cade, 2005 (40)	1) wt/wt (p.Cys282Tyr): n = 5815 2) wt/wt (p.His63Asp): n = 4850 3) p.Cys282Tyr/wt: n ~ 901 4) p.His63Asp/wt: n ~ 1750 5) p.Cys282Tyr/p.Cys282Tyr: n = 31 a) Premenopausal + perimenopausal: n = 8 b) Postmenopausal: n = 21 6) p.Cys282Tyr/p.His63Asp: n ~ 170 7) p.His63Asp/p.His63Asp: n ~ 170	Multivariate linear regression analysis for associations between heme iron intake and Fe status (SF); assessment of long-term diet by using a food-frequency questionnaire	Significant diet-gene interaction (P < 0.05) for heme iron intake and p.Cys282Tyr homozygosity; extra 1- mg heme Fe/d increases SF by 41% (95% CI: 32–51%) in p.Cys282Tyr homozygotes; no higher iron status or diet-gene interaction for p.His63Asp homozygotes	Strong association between heme iron intake and iron status in p.Cys282Tyr/p.Cys282Tyr; no additional associations with dietary factors; treatment of diagnosed patients unknown; low heme iron intake in population; SF may be confounded by infection or inflammation
van der A, 2006 (41)	F: n = 1611 1) wt/wt, SF: 72 μg/L; n = 1035 2) p.Cys282Tyr/wt, p.His63Asp/wt, p.His63Asp/p.His63Asp: SF: 83; n = 550 3) p.Cys282Tyr/p.Cys282Tyr, p.Cys282Tyr/ p.His63Asp, SF: 288: n = 26	Cross-sectional association between heme iron intake and iron status (SF)	Positive association between SF and heme iron intake in all study groups No significant interaction of genotype × heme iron intake Higher SF in group 3)	Heme iron intake is associated with increased SF in women with p.Cys282Tyr/p.Cys282Tyr and p.Cys282Tyr/p.His63Asp Note: no quantitative interaction effect measures reported; higher SF with increased heme iron intake in all groups
McCune, 2006 (42)	First-degree relatives of p.Cys282Tyr/ p.Cys282Tyr with different genotypes or phenotypes identified clinically or via screening: n = 165	Cross-sectional risk association study. Estimation of relative contribution of HFE gene to risk of iron overload phenotype defined as the percentage of TS >50% + SF: M >300 μg/L, premenoposal F >200 μg/L Fruit consumption: ≤7 compared with >7 portions/wk; alcohol intake: >5 compared with ≤5 U/wk	ORs (95% CIs) for low compared with high fruit consumption of 3.28 (1.05, 11.42; P < 0.05) and for high compared with low alcohol intake of 2.30 (1.01, 5.31; P < 0.05)	Higher risk of iron overload with low fruit consumption and with high alcohol consumption.
Allen, 2007 (8)	p.Cys282Tyr/p.Cys282Tyr;n = 46 1) Normal SF (M <300 μg/L, F <200 μg/L): n = 23 2) High SF (n = 23)	Cross sectional investigation on the association between alcohol (<20 compared with >20 g/d) and meat consumption with iron overload	Alcohol intake <20g/d Alcohol intake >20g/d	Observed higher alcohol intake in high-compared with low-SF group; higher meat consumption in women with high SF; relation was nonsignificant

(Continued)

TABLE 3 (*Continued*)

First author, year of publication (reference)	Study population	Study designs and outcome measures	Results[2]	Conclusions
Milward, 2008 (43)	1) wt/wt: *n* = 1303 2) p.Cys282Tyr/p.Cys282Tyr + p.Cys282Tyr/p.His63Asp: *n* = 57 3) p.Cys282Tyr/wt/ p.His63Asp/ wt: *n* = 873	Multivariate linear regression for association between dietary intake on iron status in presence of *HFE* gene mutations; dietary intake assessed qualitatively (frequency of consumption from food groups)	Frequency of red meat and alcohol intake associated with higher SF in men and women. Frequency of fresh fruit intake associated with lower SF in men. Cooked vegetable intake associated with higher SF in women. Significant interaction between *HFE* genotype and alcohol consumption in women.	Alcohol consumption interacts with genotype on SF in women; no diet × genotype interaction reported on other dietary factors
Jacobs, 2009 (44)	First-degree relatives of *HFE* p.Cys282Tyr/p.Cys282Tyr subjects with genotypes: of p.Cys282Tyr/p.Cys282Tyr, p.Cys282Tyr/p.His63Asp, p.Cys282Tyr/wt and wt/wt: *n* = 735	Multivariate logistic regression of genotype, and lifestyle factors on percentage of TS and SF in first-degree relatives	Familial iron severity OR (95% CI): 1.04 (1.10, 1.08) and age-of-testing OR (95% CI): 1.02 (1.003, 1.05) related to elevated SF concentrations; high meat consumption (>200 g/d) related to elevated SF OR (95% CI) of 1.61 (1.01, 2.56)	Heme iron intake affects SF concentrations in first-degree relatives of subjects with the *HFE* p.Cys282Tyr/p.Cys282Tyr genotype
Gordeuk, 2012 (45)	p.Cys282Tyr/p.Cys282Tyr: *n* = 213 M: *n* = 133 (mean ± SD age: 50 ± 13 y) F: *n* = 80 (mean ± SD age: 52 ± 14 y)	Estimation of dietary iron and alcohol consumption via a quantitative food-frequency questionnaire; multivariate linear regression of ln SF concentration, with nonheme iron, heme iron, supplemental iron, age, race, C-reactive protein, and ALT concentrations as predictors	No association of nonheme- or heme-iron intake with serum ferritin concentrations. Age, sex, ALT, and alcohol consumption significantly correlated with SF	No detected effect of supplemental iron, heme iron, and nonheme iron intakes on serum ferritin concentrations in this population

[1] ALT, alanine aminotransferase; SF, serum ferritin; SFe: serum iron; TS, transferrin saturation.
[2] All values are means unless otherwise indicated.

to a 10-fold higher iron bioavailability in HH patients at high SF concentrations (2). Other studies in subjects with low SF (<25 μg/L) have reported iron absorption from nonheme iron to be 36% in subjects with HH compared with a range between 5.8% and 19% in control subjects (31). While further investigating the relation between SF and nonheme iron absorption from a test meal, Lynch et al (3) reported a significant slope of −0,936 (log Fe absorption, %)/log (SF, μg/liter) on a logarithmic scale in normal subjects for nonheme iron absorption compared with a significant slope of −0.405 (log Fe absorption, %)/log (SF, μgliter/L) in patients with HH. At a standardized SF concentration of 100 μg/L, these values corresponded with a nonheme iron bioavailability of ∼2% and ∼18% in control subjects and HH patients, respectively (data not shown). In contrast to this and unlike in healthy volunteers, heme iron bioavailability was unrelated to iron stores (no significant correlation) in patients with HH (3). These studies indicated abnormally high dietary iron absorption, for both heme and nonheme iron, in subjects with HH and a weaker regulatory feedback mechanism on iron absorption at high SF concentrations, particularly for heme iron in HH subjects.

It is well known that iron bioavailability from supplemental iron consumed without any food, such as reference doses administered in isotopic studies (46), is higher than when consumed together with a meal. Part of the variation in iron absorption observed in bioavailability studies can be explained by the presence or the nature of the food matrix because enhancement or the inhibition of iron absorption in healthy subjects can occur despite low iron status or physiologic upregulation (21, 47). Similar effects are plausible in subjects with HH, but the magnitude of the effect may differ from that in subjects without HH. Various studies have investigated this relation. One study investigated iron absorption that compared iron-deficient anemic subjects and patients with HH. All had SF concentrations <25 μg/L. Subjects received 3 types of meals as follows: 1) a whole-maize porridge rich in absorption inhibitors, 2) a gravy that contained heme iron, and 3) a dose of iron ascorbate solution without any food matrix. HH patients absorbed significantly more iron from all nonheme iron–containing meals, whereas the absorption of heme iron was similar in HH subjects (37.1%) and iron-deficient anemic subjects (31.6%) (31). The solution that contained ferrous ascorbate given without any additional food was ∼50% more bioavailable in HH than control subjects (31). In another study conducted in idiopathic HH patients with low iron status (mean SF concentration: 25 μg/L), iron absorption was 20% from a maize porridge with ascorbic acid and 72% from an iron ascorbate solution, respectively, which indicated a strong inhibiting effect of the food matrix on iron bioavailability in iron-depleted HH subjects (33). Subjects with diagnosed genetic HH who regularly underwent a phlebotomy (mean SF concentration: 122 μg/L) and drank black tea with a mixed meal that consisted of rice, potatoes, beef, and spinach were reported to absorb only 6.9% of the iron content in the meal compared with an absorption of 22.1% when the same meal was given without tea to the same subjects. This study confirmed that polyphenols from black tea decrease iron bioavailability in patients with HH (34), similar to findings in normal subjects (48, 49). Overall, these studies indicated that certain diet-related components can have an inhibiting effect on iron absorption in HH patients.

Longitudinal studies

Although isotopic studies of iron absorption allow the quantification of food-matrix and physiologic effects on bioavailability, a longitudinal observation allows the long-term estimation of iron balance (Table 2). The iron-accumulation rate was reported to be highly variable in idiopathic HH patients (1.2–241 μg SF/L), with a mean yearly rise of 99 μg SF/L (50). However, not all p. Cys282Tyr homozygotes appear to show increased iron accumulation compared with wild-type counterparts (51, 52), which explains, in part, the high variation in iron accumulation over time and the low clinical penetrance of homozygosity of the p.Cys282Tyr mutation (52). Kaltwasser et al (29) showed that patients with clinically proven HH who regularly underwent a maintenance phlebotomy before the observation period, had a mean increase of 276.9 μg SF/L in 1 y (29). It can be calculated that a mean increase of 100 μg SF/L would correspond to ∼790 mg body Fe/y or 1.5 L blood to be phlebotomized (assuming that 1 μg SF/L is ∼8 mg body Fe) (53, 54).

In a cohort study conducted in Sweden in which HH subjects who underwent a phlebotomy acted as their own control subjects, the amount of iron absorbed was estimated to be 5.4 mg Fe/d, which corresponded to an iron bioavailability of 35% (estimated intake: 15.4 mg/d) (36). Subjects were maintained depleted with a mean SF concentration of 16 μg/L and a percentage of transferrin saturation of 34% during the course of phlebotomy. During the 1-y study, iron fortification of common flour in the country was discontinued, and the authors of the study estimated that this decreased iron intake to 11.3 mg/d, which resulted in 4.8 mg absorbed Fe/d without fortification (bioavailability estimated at 42%). This difference in iron intake resulted in a net difference of 0.65 mg absorbed Fe/d compared with in the reference period without iron fortification of flour. The described difference in absorbed iron would correspond to 240 mg Fe/y or ∼0.5 L blood to be phlebotomized (36) (data not shown in Table 2). Although the Fe bioavailability assessed in this study seemed to be consistent with data obtained from stable-isotope studies in subjects with similarly low iron status (SF concentration: 16 μg/L), a possible limitation in this study was the accuracy of the assessment of iron intake in the cohort subjects involved in this trial as well as the lack of a true control group (subjects without HH) during the study period.

Another nonrandomized study was conducted in HH patients that investigated the effect of consuming 250 mL black tea with each meal 3 times/d to inhibit iron absorption during a period of 52 wk (34). The control group consumed other drinks ad libitum and were free to choose their preferred beverage or not to drink; subjects were nonrandomly allocated to the 2 different arms of the study for compliance-related reasons. Both groups had significantly higher SF values after 1 y. At the end of the study, the amount of iron accumulated was assessed by phlebotomizing all subjects until an SF concentration of 50 μg/L was reached. The amount of iron removed was ∼50% higher in the control group than in the tea-drinking group. However, this difference was not significantly different between groups with means ± SEMs of 256 ± 173 and 827 ± 105 mg Fe absorbed, respectively (34).

Antacids are known to decrease iron bioavailability by lowering the amount of iron in solution in gastric and intestinal contents without interacting with iron-uptake mechanisms at the cellular level. With antacids, less iron would be solubilized from

food (48), and a smaller amount of dietary iron would be available for uptake. In a study in patients homozygous for the p. Cys282Tyr mutation, the use of proton pump inhibitors reduced gastric acid secretion and decreased the need for maintenance phlebotomy from 2.5 to 0.5 L/y (37). This effect would correspond to a decrease in iron removal of 1000 mg/y. However, the magnitude of the effect is difficult to generalize because of the small sample size included in this study ($n = 7$) and requires confirmation.

Stochastic modeling (Monte Carlo) was used in a longitudinal study to estimate iron accumulation in patients with HH (55). To construct the model, demographic and dietary intake data were taken from NHANES III, whereas estimates of iron bioavailability were taken from the studies by Lynch et al (3) and Bezwoda et al (31). For this analysis, 3 dietary modifications were tested by the model by setting iron intake to 200% and 100% of the Recommended Dietary Allowance, respectively, and defortification of iron-fortified flour. Estimated reductions in iron accumulation were more evident in men and were more pronounced with a stricter dietary change (such as capping iron intake to 100% of the Recommended Dietary Allowance). However, the constructed model was strongly sensitive to estimates from the regression line that related iron bioavailability and iron stores. The authors concluded that lifelong dietary habits may affect the rate of iron accumulation in HH and that the model assumed that all HH patients would have similar degrees of impairment in absorption control (55).

Cross-sectional studies that investigated associations

In a cross-sectional study in the United Kingdom, heme iron intake and p.Cys282Tyr homozygosity interacted significantly in increasing SF concentrations (39). The study indicated that heme iron intake had a 2 times greater effect on SF in p.Cys282Tyr homozygotes than in other groups studied (heterozygotes and wild-type individuals), whereas for nonheme iron, no difference was reported (Table 3). Similar results were reported in a study done in the Netherlands, where a significant association between heme iron intake and SF was shown in all study groups, as well as a higher SF in the combined group of p.Cys282Tyr homozygotes and compound heterozygotes (p.Cys282Tyr/His63Asp). However, despite higher SF with increasing heme iron intake, no significant intake-genotype interaction on SF was reported in the study (41). Both of these studies used a validated food-frequency questionnaire to assess dietary intakes in the study population. Other cross-sectional studies that assessed the intake of animal-source foods did not identify an association between iron status and heme iron intake in p.Cys282Tyr homozygotes (42, 43, 45). In a study on first-degree relatives of p.Cys282Tyr homozygotes, the relative contribution of lifestyle and genetic factors to the presence of iron overload (defined as the percentage of transferrin saturation $>50\%$ and SF concentrations >300 μg/L in men and >200 μg/L in women) was investigated with logistic regression (42). Genotype explained 42% of the variation in the model, whereas sex explained 6% of the variation in the model. Lifestyle factors were used to compute a propensity score and explained an additional 6% of the variation. These factors were being a carrier of p.His63Asp, a history of liver disease, current or past blood donorship, fresh-fruit consumption, alcohol consumption, and regular aspirin intake. Low fruit consumption (<7 portions/wk) was identified as a significant factor that contributed to an iron overload, together with a high intake of alcohol (>5 units/wk) (42). Similarly, high noncitrus fruit intake, low meat intake, and low alcohol intake were associated with lower concentrations of SF in a population not restricted to p.Cys282Tyr homozygotes only (43). In the same study, a significant interaction between alcohol intake and genotype in women was reported (43).

This finding was also indicated in a study by Scotet et al (38) in p.Cys282Tyr homozygotes, in which significant associations between higher iron indexes with increased alcohol intake were reported (38). In an additional study, first-degree relatives of p. Cys282Tyr homozygotes had a significant higher ferritin OR (95% CI) of 1.61 (1.01, 2.56) if they were identified as high meat consumers (>200 g/d) (44). In contrast to this finding, Gordeuk et al (45) did not find an association between nonheme and heme iron intakes and serum ferritin concentrations in a population of newly identified middle-aged p.Cys282Tyr homozygotes in whom iron intake was estimated by using a food-frequency questionnaire (45).

Calculation of iron absorption in HH

In the past, the relation between SF and iron bioavailability has been considered a potential diagnostic tool for early disease diagnosis (32). Because serum hepcidin concentrations relative to ferritin concentrations are significantly lower in HH subjects (20), the serum hepcidin:ferritin ratio has also been suggested as a useful diagnostic tool for the early detection of p.Cys282Tyr homozygotes at risk of developing iron overload as well as for monitoring phlebotomy treatment (14, 56). The relation between iron bioavailability and hepcidin concentrations has been assessed in normal subjects (26, 57). With the assumption that hepcidin regulates iron bioavailability similarly in *HFE* p. Cys282Tyr HH subjects and healthy control subjects, it can be estimated that iron absorption from a standardized meal (rice with vegetable sauce) ranges between 12.2% and 15.3% and from 6.6% to 12.4% in HH subjects and healthy control subjects, respectively, at normal SF concentrations (32–162 μg/L). In contrast, in HH subjects with elevated ferritin concentrations (330–1045 μg/L), food iron absorption would range between 8.6% and 11.3% (**Figure 1**).

DISCUSSION

Studies that have measured iron absorption in HH subjects indicated that the iron bioavailability in clinically penetrant HH patients *1*) is generally 2–10-folds higher than in wild-type individuals depending on the standardized iron status at which the groups were compared; *2*) is high for iron stores, particularly for heme iron, *3*) is influenced by the food matrix, and *4*) may stabilize at a range of 15–35% dietary iron bioavailability at high iron stores (>300 μg SF/L) and, therefore, is similar to iron absorption in non-HH iron-deficient subjects.

It has been shown that duodenal enterocytes in HH patients have an expression of iron transport proteins elevated for their iron-store concentration (18), and repeated phlebotomies induce an increased expression, which is likely responsible for increased mucosal transfer (18). The absorption studies reviewed (3, 31) suggested that the choice of dietary iron source (heme or nonheme
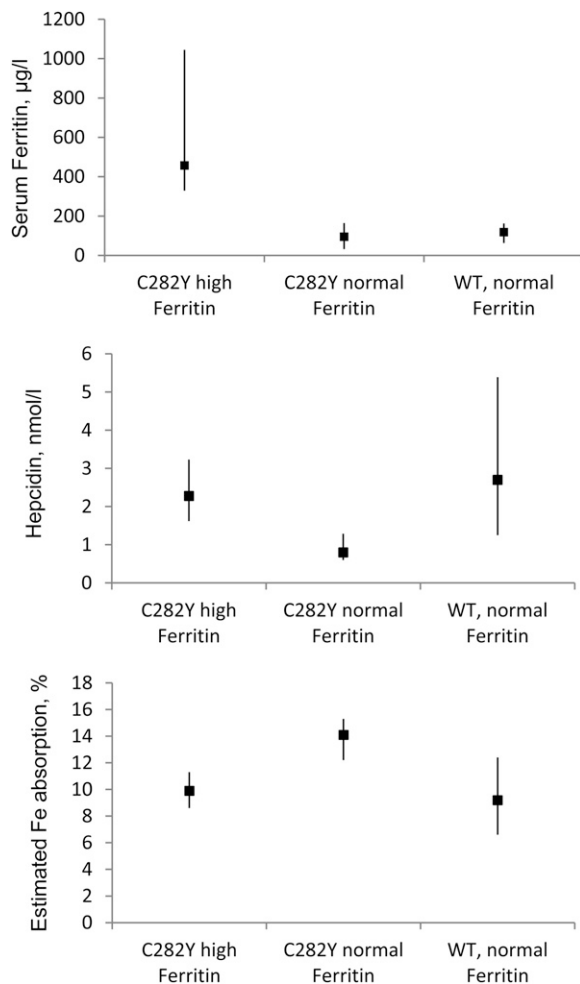
FIGURE 1. IQRs (lines) and medians (dots) of serum ferritin, hepcidin, and estimated iron bioavailability in HH patients with high or normal ferritin values compared with those of their WT counterparts. The estimated iron bioavailability was based on the extrapolation of hepcidin concentrations in HH patients as published by van Dijk et al (20) with the following regression formula: iron absorption (%) = -3.9656 ln[hepcidin (nmol/L)] + 13.238 (26), relating hepcidin concentrations with iron bioavailability in healthy subjects. HH, hereditary hemochromatosis; WT, wild-type.

iron) can affect the iron absorption and balance in HH patients. In addition, the modulation of the amount of dietary iron intake (36) or iron bioavailability (34) may decrease the rate of iron accumulation in patients with HH. It has also been shown that the presence of a food matrix per se (31) influences iron bioavailability in HH patients. The factors that would elicit an effect on iron bioavailability are similar to enhancers and inhibitors of iron absorption for non-HH subjects (58). The prospective, longitudinal studies discussed in this review also suggested an effect by dietary modulation on iron balance in HH patients (34, 36). However, the overall (nonsignificant) effect reported in the study that investigated the effect of tea on long-term body iron status may have been a result of other dietary and lifestyle factors associated with frequent tea drinking because subjects were not randomly assigned to treatment. Furthermore, the latter study had a small sample size ($n = 19$) (34). Although the results seen may not have been solely because of tea drinking, the effect size suggested a decrease in absorbed iron over the course of 1 y of $\sim$400 mg, one-third in iron accumulation (34),

and would correspond to a yearly decrease in phlebotomy need of 0.7 L blood.

Despite this suggestive evidence, limited direct evidence was shown to support the hypothesis that dietary modulation can influence iron accumulation in HH patients in a clinically relevant manner. This was a result of several important limitations.

First, the evidence from cross-sectional studies was difficult to interpret because of the potential confounding effect of chronic subclinical inflammation (ie, diabetes, the metabolic syndrome, and cardiovascular disease), which influences iron-status markers, which was likely to confound the relation between dietary iron intake and iron status. In one of the cross-sectional studies (42), low fruit intake was associated with higher risk of having an iron-overload phenotype in first-degree relatives of HH patients. This result was somewhat surprising because an opposite effect may be expected because of the vitamin C content of fruit. However, it is possible that fruit intake was a proxy for the dietary quality and a healthier lifestyle. This limitation may also apply to studies that investigate the bioavailability of iron in relation with SF, in which SF may not always reflect iron stores in the presence of subclinical inflammation.

A second limitation was that fully penetrant HH is a rare disease, which makes it difficult to conduct large prospective studies with iron status as the primary outcome. Therefore, studies have thus far been mostly conducted in small groups of subjects ($n = 16$–18), which has limited the statistical power of inference (59, 60). New approaches to study design such as the use of population pharmacokinetics to describe changes in iron status in longitudinal studies may allow studies to be conducted with smaller populations of HH patients and more closely describe the development of iron status over time following specific dietary or lifestyle patterns (61). With the use of the quantity of iron removed by a phlebotomy as an outcome measure may be promising because it would be less affected by short-term changes in iron-status markers because of subclinical inflammation.

Third, as noted by Tao et al (55), the calculation of the SF to iron bioavailability regression equation assumes that all subjects with clinically penetrant HH have a similar impairment of iron absorption regulation, which may not be the case (51) because a small proportion of homozygotes develop iron overload, and iron stores may plateau before reaching the critical level (52). A range of genetic and environmental factors, including dietary factors (15), may influence iron intake and bodily iron distribution and, thus, may influence disease penetrance. The prediction of the bioavailability in HH patients by using the serum hepcidin concentration may provide indications about the rate of iron loading in individual subjects at any given time but would require reference data that link the hepcidin concentration to iron bioavailability in clinically confirmed HH subjects. Studies that explicitly link genetic mutations and environmental and epigenetic factors to iron bioavailability in clinically confirmed HH subjects may provide additional leads.

Fourth, in only one of the studies that directly assessed iron bioavailability, the *HFE* genotype was assessed. The remaining studies in idiopathic HH patients, although conducted in clinically confirmed HH patients, may not have been entirely representative of the population of *HFE*-related HH patients and because isotopic studies have typically been conducted in a limited number of subjects. However, the likelihood of including

a subject with a rare genotype other than homozygosity for the p. Cys282Tyr mutation in the *HFE* gene or in other HH-related genes is low because of the high prevalence of p.Cys282Tyr homozygosity in clinically affected HH patients (25).

In HH patients with low to normal iron status who consume a typical Western diet that contains 16–18 mg/d Fe (62), a dietary iron absorption of 20–40% for heme and nonheme iron combined as shown in the studied literature would imply a long-term positive iron balance of ∼3–7 mg/d. It is very unlikely that such a positive balance could be reduced to zero with an exclusive dietary intervention. However, a dietary modulation may be a useful accessory measure to reduce the rapid reaccumulation of iron in clinically diagnosed HH patients who are undergoing a phlebotomy, especially in the maintenance phase. Depletion through a phlebotomy of HH patients until a very low SF concentration (50 µg/L) is reached (63) will upregulate the iron absorption in HH patients. Therefore, the inhibition or reduction of absorbed iron by dietary modulation could help to avoid exacerbating the excess release of iron into the circulation, which results in a vicious circle of more-frequent maintenance phlebotomies in HH patients (20, 64)

In conclusion, dietary modification may provide an auxiliary measure to inhibit iron accumulation and reduce the number of required phlebotomies in clinically confirmed HH patients. This result could increase the patient's active involvement in treatment and, as such, may be beneficial for prospective disease outcomes (65). However, additional longitudinal research would be required to formulate and test an effective dietary strategy for this patient group and quantify the clinical benefit in the number of phlebotomies avoided as well as patient wellbeing. Such a dietary strategy would comprise lowering dietary iron intake and reducing iron bioavailability while maintaining adequate intakes of other essential nutrients that are typically consumed as part of an iron-rich diet (ie, zinc, vitamin C, and vitamin B-12).

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