Iron absorption by heterozygous carriers of the HFE C282Y mutation associated with hemochromatosis¹⁻⁴

Janet R Hunt and Huawei Zeng

ABSTRACT

**Background:** Research conducted before genotyping was possible suggested that subjects heterozygous for the genetic mutation associated with hemochromatosis absorbed nonheme iron more efficiently than did control subjects when tested with a fortified meal. Heme-iron absorption in these subjects has not been reported.

**Objective:** We compared the absorption of heme and nonheme iron from minimally or highly fortified test meals between HFE C282Y-heterozygous and wild-type control subjects.

**Design:** After prospective genotyping of 256 healthy volunteers, 11 C282Y-heterozygous and 12 wild-type control subjects were recruited, and their iron absorption was compared by using a hamburger test meal with or without added iron and ascorbic acid. After retrospective genotyping of 103 participants in previous iron-absorption studies, 5 C282Y-heterozygous subjects were compared with 72 wild-type control subjects.

**Results:** HFE C282Y-heterozygous subjects did not differ significantly from wild-type control subjects in their absorption of either heme or nonheme iron from minimally or highly fortified test meals. No differences were detected in blood indexes of iron status (including serum ferritin, transferrin saturation, and non-transferrin-bound iron) or in blood lipids or transaminases, but heterozygotes had significantly greater, although normal, fasting glucose concentrations than did wild-type control subjects. Compound heterozygotes (those who had both HFE C282Y and H63D mutations) absorbed more nonheme (but not heme) iron from meals with high (but not low) iron bioavailability.

**Conclusions:** HFE C282Y-heterozygous subjects did not absorb dietary iron more efficiently, even when foods were highly fortified with iron from ferrous sulfate and ascorbic acid, than did control subjects. Iron fortification of foods should not pose an additional health risk to HFE C282Y heterozygotes. Am J Clin Nutr 2004; 80:924–31.

**KEY WORDS** Nutrient-gene interactions, nonheme-iron absorption, heme-iron absorption, diet, iron bioavailability, iron fortification, hemochromatosis, serum ferritin, non-transferrin-bound iron, glucose

INTRODUCTION

Hemochromatosis is a disorder of excessive iron accumulation characterized by increased heme- and nonheme-iron absorption (1). In 1996, specific genetic mutations in the HFE gene were identified (2) in hemochromatosis patients of Northern European origin. Although it is not known how the HFE protein influences iron absorption, it may influence the affinity of plasma transferrin for its receptor, as part of an HFE, β₂-microglobulin, and transferrin receptor complex in the basolateral membrane of developing enterocytes (3). More than 80% of hemochromatosis patients are homozygous for a C282Y mutation in this gene, and a smaller proportion are compound heterozygous for both the C282Y mutation and an H63D mutation (2).

Identification of the C282Y mutation raised questions about the possible health effects of this common mutation for heterozygous carriers, who include 9.54% of non-Hispanic whites in the United States (4). Heterozygosity has been associated with shorter life expectancy in a Danish study (5), but not in other studies (6–8), and it has even been associated with longevity in some Italian women (9, 10). Increased risk of type 2 diabetes in C282Y heterozygotes found in one study (16) was unconfirmed in another (17). Genetic studies in healthy populations have identified many symptom-free C282Y homozygotes, which shows that the clinical phenotypic penetrance of the mutation is quite low (18–20). Heterozygosity has been inconsistently associated with slightly higher transferrin saturation and serum ferritin concentrations (18, 20–26). Non-transferrin-bound iron (NTBI) was reported in one small study to be greater in heterozygous subjects than in wild-type control subjects, but there was no difference in serum ferritin or transferrin saturation between these 2 groups (27).

Before C282Y genotyping was available, iron absorption by heterozygous carriers of hemochromatosis was tested by identifying as heterozygotes the children of patients with hemochromatosis or two siblings who shared a single HLA haplotype (1). No difference was found between heterozygous and control subjects in the absorption of nonheme iron from a hamburger meal (1). However, when the meal was fortified with 20 mg Fe (as...
ferrous sulfate) and 100 mg ascorbic acid, the heterozygous subjects absorbed 2.5 times as much nonheme iron as the control subjects absorbed. Because of the high frequency of the HFE C282Y mutation, such an increase in absorption may have public health implications for food fortification policies. The objective of the current study was to compare iron absorption in heterozygotes identified by the C282Y mutation with that in wild-type control subjects by feeding test meals similar to those used by Lynch et al (1). Secondary objectives were to examine iron absorption related to other HFE C282Y and H63D polymorphisms and to test for differences in clinical chemistry, including an increase in NTBI in subjects who were heterozygous for the C282Y mutation.

SUBJECTS AND METHODS

General protocol

The absorption of dietary iron in healthy subjects who are heterozygous for the HFE C282Y mutation and in wild-type control subjects was compared by using 2 sets of data. First, 256 healthy volunteers were prospectively genotyped (tested for both the HFE C282Y and H63D mutations), which led to the enrollment of 11 HFE C282Y-heterozygous and 12 wild-type control subjects for tests of heme- and nonheme-iron absorption from test meals prepared with and without extra fortification with iron (as ferrous sulfate) and ascorbic acid. Second, 103 participants in previous iron-absorption studies were retrospectively genotyped, which allowed the comparison of 5 subjects who were heterozygous for the C282Y mutation, 2 compound-heterozygous subjects, and subjects with other HFE genotypes with wild-type control subjects who had neither mutation.

Subjects

Healthy men and nonpregnant women were recruited through public advertising and selected after an interview and blood analysis helped ascertain that they had no apparent underlying disease and had blood hemoglobin concentrations of ≥120 g/L. Although race was not designated as a selection criterion, all volunteers were white and non-Hispanic. The participants gave written informed consent. The University of North Dakota’s Radioactive Drug Research Committee and Institutional Review Board and the US Department of Agriculture’s Human Studies Review and Radiological Safety committees reviewed and approved this human study.

Dietary treatments

Prospective genotyping study

Iron absorption was tested from weighed standard or fortified test meals similar to those used by Lynch et al (1). The standard meal consisted of ground beef (113 g), a refined-wheat bun (53 g), French-fried potatoes (68 g), tomato ketchup (20 g), and a vanilla milkshake (150 g). The refined-wheat bun was commercially enriched with iron according to common US practice for refined-wheat products (20 mg iron/460 g flour). The standard meal contained 906 kcal and 5.1 mg Fe, of which 1.2 mg was in the heme form. The fortified meal was similar, but it omitted the French-fried potatoes and milkshake and added 200 g orange juice supplemented with 100 mg ascorbic acid and 20 mg Fe as ferrous sulfate. The orange juice was prepared and mixed with supplements on the same morning that the meals were served. The fortified meal contained 590 kcal and 25.1 mg iron, of which 1.2 mg was in the heme form.

Retrospective genotyping study

Subjects with mutations of interest had previously participated in iron-absorption studies with 3 different sets of dietary treatments. The subjects’ iron absorption was tested 1 from a 2-d menu low in dietary iron bioavailability (no meat, small amounts of poultry and fish, and ≈20 mg ascorbic acid at each meal, with considerable black tea and phytic acid–containing foods) before and after 10 wk of consumption of the same diet (28, 29); 2 from a higher-bioavailability test meal containing beef, refined-wheat roll, French-fried potatoes, and milkshake before and after 12 wk of daily supplementation with a placebo (30); and 3 from 2 test meals of beef, refined-wheat roll, French-fried potatoes, and milkshake, with and without cheese, which were followed by a complete gastrointestinal lavage procedure 8 h after each meal (31). No C282Y mutations were identified in subjects formerly treated with a high-iron-bioavailability diet (28, 29) or with iron supplements (30). Subjects fasted for 8 h before and 4 h after single test meals and ate only the food provided when tested with complete menus for 2 d.

Iron-absorption measurements

Heme- and nonheme-iron absorption was measured by extrinsically labeling the foods (in the final stages of preparation) with 18.5 or 37 kBq 55Fe-labeled hemoglobin and 37 kBq 59FeCl3. Radiolabeled hemoglobin was obtained by intravenously injecting 74 MBq 55Fe into an iron-deficient, pathogen-free rabbit, bleeding the animal 2 wk later, isolating the red cells by centrifugation, and removing the stroma by lysing and further centrifugation (32). For the low-bioavailability diet involving 2 d of meals, the isotopes were added to the diet in proportion to the heme- and nonheme-iron contents of the meals, which yielded constant specific activities (ratios of 55Fe to dietary heme iron and of 59Fe to nonheme iron) for all 6 meals. The tracers were added to the foods that were the best sources of that form of iron in each meal. Meat, poultry, and fish dishes were cooked in advance, cooled, radiolabeled, and then minimally reheated in the microwave just before service. All labeled meals were consumed at the research center.

Nonheme-iron absorption was determined by whole-body scintillation counting that detected only the gamma-emitting 59Fe radioisotope. The custom-made whole-body counter used 32 crystal NaI(Tl) detectors, each 10 cm × 10 cm × 41 cm, arranged in 2 planes above and below the participant, who lay supine. The initial total-body activity was determined before any unabsorbed isotope was excreted but ≥1 h after the subjects finished the single meals (or 2 meals of the 2-d menu, with calculated adjustments for the fraction of the total activity administered). The percentage of nonheme-iron absorption was measured as the portion of initial whole-body activity that remained after 2 wk. Heme-iron absorption was ascertained by measuring the blood retention of 55Fe (together with 59Fe; 33) after 2 wk and by estimating total blood volume on the bases of body height, sex, and weight (34, 35) and of the assumption that 80% of the absorbed heme iron was incorporated into blood (36). All isotope determinations included corrections for physical decay and background activity measured 1–2 d before isotope administration.
Genotyping

Genomic DNA was extracted from buccal smears (for retrospective genotyping) or blood (prospective genotyping study) by using a DNA isolation kit (Qiagen, Valencia, CA). HFE C282Y and H63D mutations were ascertained by using a two-round polymerase chain reaction (PCR; semi-nested primers) approach (37, 38) and enzymatic digestion of the products similar to that in previous reports (2, 39). Briefly, genomic DNA was used as a first-round PCR DNA template, and then 0.5 μL of the first-round PCR product was used as the second-round PCR DNA template. As described by Feder et al (2), for the C282Y mutation, the first-round PCR primers were 5’-TGCGAAGGGTAACAGATCC-3’ and 5’-CCAATGAAACAGATGAGCACA-3’, and the second-round PCR primers were 5’-TGCGAAGGGTAACAGATCC-3’ and 5’-CTCAGGCACTCCTCACC-3’. For the H63D mutation, the first-round PCR primers were 5’-ACATGGTTAAGGCGTGTGTC-3’ and 5’-CTTGTGTTGTGGTTGATTCC-3’, and the second-round PCR primers were 5’-ACATGGTTAAGGCGTGTGTC-3’ and 5’-GGCACATCTGGCTGATT-3’ (2). DNA spanning C282Y (560 base pairs) and H63D (295 base pairs) mutations was amplified in the first round of PCR. The C282Y and H63D mutations were detected by amplification of 389 and 208 base pairs, respectively, in the second-round PCR.

Negative controls were included in each PCR. The PCR conditions were as follows: 94 °C for 2 min; then 25 cycles of 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 1 min; and, finally, 72 °C for 2 min. Aliquots of second-round PCR products were digested with Rsa I and Mbo I endonuclease restriction enzymes to identify the C282Y and H63D mutations, respectively, as described previously (39). The enzymatic digestion product was subsequently separated by electrophoresis in 3% agarose 2000 (Gibco-BRL, Carlsbad, CA) gels. Each subject’s genotype was determined independently by 2 staff members.

Clinical chemistry

Hemoglobin was measured by using a Celldyne 3500 System (Abbott Laboratories, Abbott Park, IL). Serum iron was determined colorimetrically by using a Cobas Fara Chemistry Analyzer (Hoffman-LaRoche Inc, Nutley, NJ) with a commercial chromogen (Ferene; Raichem Division of Hemagen Diagnostics, San Diego). Iron-binding capacity was similarly measured after the addition of a known amount of ferrous iron to the serum sample under alkaline conditions. The percentage of transferrin saturation was calculated from serum iron and total-iron-binding capacity. Serum ferritin concentrations were measured with the use of an enzyme-linked immunosorbent assay (Abbott Laboratories; or Diagnostic Products Corporation, Los Angeles). Serum NTBI was measured on subsamples of blood obtained at 3 different times from each subject and was detected colorimetrically after the addition of nitrosoic acid and treatment with cobalt (40).

Colorimetric methods were used to determine glucose, aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), triacylglycerol (41), total cholesterol (42), and HDL cholesterol (42) from fasting blood serum (all with the use of the Cobas Fara Chemistry Analyzer). VLDL cholesterol was estimated from triacylglycerol (43), and LDL cholesterol was calculated by difference (LDL = Total − HDL − VLDL).

Statistical analysis

Iron-absorption and serum ferritin data were logarithmically transformed, and geometric means are reported. Power curves were used to plot relations between iron absorption and serum ferritin concentrations (these relations are linear when the data are logarithmically transformed).

For statistical tests of the effect of genotype, the known inverse relation between iron absorption and serum ferritin was used to normalize iron-absorption values to an arbitrary serum ferritin concentration of 40 μg/L, as follows:

\[
\begin{align*}
\ln(\text{normalized percentage nonheme-iron absorption}) &= \ln(\text{percentage nonheme iron absorbed}) \\
&+ \ln(\text{ferritin in } \mu\text{g/L}) - \ln(40 \ \mu\text{g/L}) \quad (I) \\
\ln(\text{normalized percentage heme-iron absorption}) &= \ln(\text{percentage heme iron absorbed}) + 0.3 \\
&\times \ln(\text{ferritin in } \mu\text{g/L}) - 0.3 \times \ln(40 \ \mu\text{g/L}) \quad (2)
\end{align*}
\]

This normalization uses a slope of −1.0 for the regression of ln(nonheme-iron absorption) on ln(serum ferritin) (44) and a slope of −0.3 for a similar regression with heme-iron absorption (1, 30, 45). [The slope from Lynch et al (1) was estimated from calculations by Cook (46).] All statistical tests were conducted by using PC/SAS software (version 8.02; SAS Institute Inc, Cary, NC; 47). For the prospective genotype experiment, a possible interaction between genotype and meal fortification was evaluated by using repeated-measures analysis of variance (ANOVA; 47). For retrospective data, t tests were used to compare selected individual absorption measurements with those of wild-type control subjects in the same experiment (47). The chi-square test was used to compare national genotyping results with those of this project (47). P < 0.05 (with two-tailed testing) was considered significant.

RESULTS

Prospective genotyping data set

The genotyping results from this convenience sampling in Grand Forks, ND, did not differ significantly (by chi-square analysis) from a representative sampling of the non-Hispanic white US population (4; Table 1). Of 256 subjects prospectively screened, 20 (8%) were C282Y heterozygous, without the H63D mutation. Of these 20 subjects, 12 agreed to participate in the iron-absorption study. Reasons for nonparticipation included anemia (blood hemoglobin < 120 g/L; n = 2); pregnancy (n = 1); lack of willingness to follow experimental protocol (n = 2); and loss to follow-up contact (n = 3). One subject was subsequently eliminated because of elevated hematocrit and referral for possible polycythemia, which left 11 subjects (8 females and 3 males) who were C282Y heterozygous. Twelve control subjects (wild-type, with neither the HFE C282Y nor H63D mutation; 9 females and 3 males) who were C282Y heterozygous. Twelve control subjects (wild-type, with neither the HFE C282Y nor H63D mutation; 9 females and 3 males) were randomly selected. The control subjects were not paired with heterozygous subjects for analysis because the initial serum ferritin measurements were later judged to be from an unreliable commercial source and because several months had elapsed between
the initial serum ferritin measurements and the iron-absorption experiment. When it was concluded that the initial serum ferritin analyses were unreliable, this measurement was repeated with a different commercial kit, but only in those subjects who had participated in the iron-absorption measurements.

Iron absorption was not significantly affected by HFE C282Y heterozygosity. As expected, absorption of both forms of iron was inversely related to serum ferritin concentrations (Figure 1). After normalization of iron absorption to a serum ferritin concentration of 40 µg/L, there was no significant difference (by analysis of variance) in the absorption of either heme or nonheme iron by subjects with the 2 different genotypes, whether absorption was tested with the standard meal or with the meal fortified with additional iron (as ferrous sulfate) and ascorbic acid (Figure 2). Normalized absorption of nonheme iron in the heterozygous and wild-type subjects was, respectively, 10.9% and 7.5% from the standard meal and 7.7% and 7.5% from the fortified test meal. Normalized absorption of heme iron in the 2 groups was, respectively, 36% and 33% from the standard meal and 29% and 32% from the fortified test meal.

Several clinical chemistry measurements were compared between the C282Y-heterozygous (n = 11) and wild-type control (n = 12) subjects (Table 2). The control subjects had been selected for serum ferritin measurements, age, sex, and BMI that were similar to those of the C282Y-heterozygous subjects at the time of initial genotyping (several months before). At the time of the iron-absorption measurements, the 2 groups did not differ significantly, according to t tests, in any of the iron status measurements (Table 2). These included NTBI concentration, which was 0.65 ± 0.12 and 0.68 ± 0.08 µmol/L for the heterozygous and control subjects, respectively. No differences were found in serum lipid measurements or transaminases (AST or GGT). Of the clinical chemistry measurements conducted, only fasting serum glucose concentrations differed between the 2 groups (4.6 ± 0.1 and 4.1 ± 0.1 mmol/L for heterozygous and control subjects, respectively; P < 0.02 by t test; Table 2).

**Retrospective genotyping data set**

Only 5 subjects who were heterozygous for the C282Y mutation were identified from the retrospective genotyping (Table 1).

**TABLE 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NHANES III (n = 256)</th>
<th>Prospective (n = 256)</th>
<th>Retrospective (n = 103)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>n (%)</td>
<td>n</td>
</tr>
<tr>
<td>C282Y-homozygous</td>
<td>0.30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C282Y-heterozygous</td>
<td>9.54</td>
<td>20 (8)</td>
<td>5</td>
</tr>
<tr>
<td>H63D-homozygous</td>
<td>2.15</td>
<td>6 (2)</td>
<td>1</td>
</tr>
<tr>
<td>H63D-heterozygous</td>
<td>23.55</td>
<td>62 (24)</td>
<td>23</td>
</tr>
<tr>
<td>C282Y/H63D-compound-</td>
<td>2.35</td>
<td>3 (1)</td>
<td>2</td>
</tr>
<tr>
<td>heterozygous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type (control)</td>
<td>62</td>
<td>165 (64)</td>
<td>72</td>
</tr>
</tbody>
</table>

1 NHANES III, the third National Health and Nutrition Examination Survey. Neither the prospective nor the retrospective samples from Grand Forks differed significantly from the genotypic distribution in the nationally representative NHANES III (chi-square analysis).

These subjects did not absorb either heme or nonheme iron more efficiently than did the wild-type control subjects who had neither mutation. This conclusion was based on t tests (C282Y-heterozygous compared with wild-type control subjects) when the absorption data within a single experiment were normalized to a serum ferritin concentration of 40 µg/L. Iron absorption had been measured twice in each subject, and the data were highly reproducible between measurements (eg, the absorption results at week 10, shown in Figure 3, were similar to those at week 0). Two subjects were compound heterozygous for both the C282Y and H63D mutations, and their nonheme-iron absorption depended on the bioavailability of the test diet. The nonheme-iron absorption in one subject was substantially inhibited by the low-iron-bioavailability diet, which is similar to the results in the wild-type subjects (Figure 3). When the absorption data were normalized to a serum ferritin concentration of 40 µg/L (not shown), nonheme-iron absorption from the low-bioavailability diet in this compound-heterozygous subject did not differ significantly from that in the wild-type control subjects. The other
compound-heterozygous subject was tested with high-bioavailability meals and appeared to absorb nonheme iron from both a cheeseburger meal (Figure 4) and a similar hamburger meal (not shown) more efficiently than did the wild-type control subjects. When absorption measurements were normalized to a serum ferritin concentration of 40 μg/L, the compound-heterozygous subject absorbed significantly more of the nonheme iron from these high-bioavailability meals than did the 6 wild-type control subjects: 24% compared with 7.5 ± 4.7%, respectively, for the cheeseburger meal ($P < 0.01$; $t$ test) and 22% compared with 7.9 ± 4.8%, respectively, for the hamburger meal ($P < 0.02$; $t$ test). Heme-iron absorption was not affected by the compound-heterozygous condition (Figures 3 and 4). There was no indication of any difference in iron absorption between the wild-type control subjects and the single subject who was homozygous for the H63D mutation (Figure 4) or the subjects who were heterozygous for that mutation (Figures 3 and 4).

As indicated in Subjects and Methods, heme-iron absorption in this study was determined by measuring blood $^{55}$Fe 2 wk after isotope administration and assuming 80% erythrocyte incorporation of the absorbed isotope. Similar overall results were obtained if heme-iron absorption was determined without an assumption of 80% erythrocyte incorporation but with the assumption that the erythrocyte incorporation of $^{55}$Fe from administered heme iron was identical to that of the nonheme $^{59}$Fe, which was ascertained from blood and whole-body measurements. However, this latter calculation method resulted in an apparent outlier: one woman with a low serum ferritin concentration (9 μg/L) who was C282Y heterozygous had very low measurements of erythrocyte $^{59}$Fe incorporation (56% and 58% at 0 and 10 wk, respectively; calculated by dividing the fractional blood retention by the fractional whole-body retention of $^{59}$Fe). The use of this low erythrocyte incorporation of the nonheme radiotracer resulted in unusually high estimations of heme-iron absorption (78% and 58% at 0 and 10 wk, respectively). If erythrocyte incorporation of 80% was used in the absorption calculation, the estimated heme-iron absorption in this female subject

![FIGURE 2. Absorption of heme and nonheme iron as tested with the standard meal and the fortified meal in C282Y-heterozygous (●; n = 11) or wild-type control (○; n = 12) subjects. When absorption was normalized to a serum ferritin of 40 μg/L (see text), there was no significant difference ($P > 0.05$) in nonheme- and heme-iron absorption between the 2 groups of subjects, whether tested with the standard or the fortified meal.]

### TABLE 2
Comparison of age, BMI, and clinical chemistry results between prospectively genotyped C282Y-heterozygous and wild-type control subjects

<table>
<thead>
<tr>
<th></th>
<th>C282Y-heterozygous subjects</th>
<th>Wild-type control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($n = 11$)</td>
<td>($n = 12$)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>49 ± 4 (26–76)$^2$</td>
<td>46 ± 10 (25–57)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 1.3 (20.6–34.9)</td>
<td>26.5 ± 1.0 (21.6–32.2)</td>
</tr>
<tr>
<td>Serum ferritin (μg/L)</td>
<td>37 [25, 55] (3–254)$^d$</td>
<td>52 [43, 63] (14–199)</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>29 ± 4 (4–43)</td>
<td>30 ± 2 (19–45)</td>
</tr>
<tr>
<td>Serum iron (μmol/L)</td>
<td>15 ± 2 (2–23)</td>
<td>16 ± 1 (10–26)</td>
</tr>
<tr>
<td>Non-transferrin-bound iron (μmol/L)</td>
<td>0.65 ± 0.12 (0.14–1.4)</td>
<td>0.68 ± 0.08 (0.29–1.1)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.66 ± 0.42 (4.34–7.96)</td>
<td>5.66 ± 0.46 (3.50–7.94)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.16 ± 0.14 (0.75–2.30)</td>
<td>1.29 ± 0.11 (0.75–1.94)</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.11 ± 0.13 (0.58–1.93)</td>
<td>1.09 ± 0.18 (0.37–2.73)</td>
</tr>
<tr>
<td>Glucose (mmol/L)$^d$</td>
<td>4.6 ± 0.1 (3.9–5.3)</td>
<td>4.1 ± 0.1 (3.7–4.9)</td>
</tr>
<tr>
<td>AST (μkat/L)</td>
<td>0.35 ± 0.04 (0.22–0.60)</td>
<td>0.29 ± 0.02 (0.20–0.43)</td>
</tr>
<tr>
<td>GGT (μkat/L)</td>
<td>0.27 ± 0.02 (0.18–0.37)</td>
<td>0.30 ± 0.05 (0.13–0.82)</td>
</tr>
</tbody>
</table>

$^d$ AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase.

$^2$ $\bar{x}$ ± SE; range in parentheses (all such values). Results are untransformed.

$^d$ Geometric $\bar{x}$; 1 SE, + 1 SE in brackets; range in parentheses (all such values). Results are log transformed.

$^d$ Only fasting glucose differed significantly between genotype groups, $P < 0.02$ ($t$ test).
was not unusually high (55% and 42% at 0 and 10 wk, respectively; the latter value is shown in Figure 3). Although this subject’s erythrocyte 59Fe incorporation was unusually but reproducibly low for a woman with low serum ferritin, her blood retention of 59Fe was not unusually high. Accordingly, the heme-iron absorption results presented are based on an assumed 80% erythrocyte incorporation. In the prospective genotyping data set, the erythrocyte incorporation of 59Fe (percentage of ingested isotope) did not significantly differ between the 11 heterozygotes and the 12 control subjects (nonheme iron: $R^2 = 0.61$, $P < 0.01$; heme iron: $R^2 = 0.48$, $P < 0.01$). Similar measurements at week 0 gave similar results.

**DISCUSSION**

The results indicate that subjects who are heterozygous for the HFE C282Y mutation do not absorb dietary iron any more efficiently than do wild-type control subjects. These results confirm the finding of Lynch et al (1) that there is no difference between these groups in nonheme-iron absorption from a standard hamburger meal, but they are in contrast to the same group’s finding that heterozygous subjects absorb more nonheme iron from a meal fortified with additional iron (from ferrous sulfate) and ascorbic acid than do wild-type control subjects. Prospective power analyses had predicted that 10–15 subjects from each genotype would provide 90% power to detect an interaction (genotype × level of fortification) of the magnitude observed by Lynch et al (1)—ie, similar nonheme-iron absorption from the minimally fortified meal, but more than twice as much nonheme-iron absorption from the fortified meal. However, the present data gave no indication of this interaction (Figure 2). This difference in results is likely related to the method of genotyping. Because the heterozygous subjects of Lynch et al were identified by their relation to hemochromatosis patients and by HLA assessment, they were more likely to have additional unidentified genetic, ethnic, or lifestyle characteristics that resulted in a phenotypic expression of excessive iron accumulation, or 2 to be mistakenly typed as heterozygous when some may have been compound heterozygous or C282Y homozygous.
among either C282Y-homozygous, C282Y-heterozygous, or wild-type control subjects, which is consistent with the results of the present study (Table 2). The fasting glucose concentrations that we found should be interpreted with caution, by recognizing that this one minor difference in blood glucose arose from multiple t test comparisons (Table 2).

In conclusion, these results indicate no significant difference in heme- or nonheme-iron absorption by heterozygous carriers of the HFE C282Y genetic mutation associated with hemochromatosis, whether tested by using common meals or meals highly fortified with iron as ferrous sulfate and ascorbic acid. The results are consistent with greater absorption of nonheme iron from meals with high iron bioavailability in subjects who are compound heterozygous for the C282Y and H63D mutations. Although a substantial proportion of the US population is heterozygous for the C282Y mutation, the present results do not suggest any greater risk to these subjects due to consumption of foods that are highly fortified with this highly bioavailable form of iron or with ascorbic acid.

We gratefully acknowledge the contributions of members of our human studies research team, particularly Carol Ann Zito, who conducted the blood radioiron analyses and, together with Melissa Phelps and Cheryl Stjern, performed the genotyping assays. In addition, Emily J Nielsen managed the volunteer recruitment and scheduling; Bonita Hoverson supervised the planning and service of the controlled diets; Sandy K Gallagher supervised the clinical laboratory analyses; Glenn I Lykken designed and consulted in the use of the whole body counter; and LuAnn K Johnson performed the statistical analyses. We are especially grateful to the volunteers for their dedication and service.

JRH conceived and designed the study, supervised the iron-absorption measurements, and wrote the manuscript. HZ supervised genotyping measurements and contributed to the manuscript. Both were directly funded by the US Department of Agriculture Agricultural Research Service and had no conflict of interest.

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