Plasma hepcidin concentrations significantly predict interindividual variation in iron absorption in healthy men

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ABSTRACT

Background: Iron absorption is proposed to be regulated by circulating hepcidin, but, to date, little data are available to evaluate this relation in humans.

Objective: Stored samples from a human iron absorption study were used to test the hypothesis that differences in plasma hepcidin explain interindividual variation in iron absorption.

Design: Hepcidin-25 concentrations were measured in fasting samples from men aged ≥40 y (n = 33) recruited to a study investigating the relation between the HFE genotype, iron absorption, and iron status.

Results: Log iron absorption was negatively correlated with serum ferritin (r = −0.59, P < 0.001) and with plasma hepcidin (r = −0.55, P < 0.001) but was unaffected by genotype. There was a positive correlation (r = 0.82, P < 0.001) between hepcidin (mean: 2.3; range: 0.1–7.8 nmol/L) and ferritin (mean: 70; range: 9–208 μg/L). Multiple linear regression models showed that plasma hepcidin in isolation significantly predicted 36% of the interindividual variation in iron absorption.

Conclusions: Plasma hepcidin and serum ferritin concentrations are highly correlated, and, in the normal range of plasma hepcidin values, 36% of interindividual differences in iron absorption are explained by differences in circulating plasma hepcidin. Am J Clin Nutr 2009;89:1088–91.

INTRODUCTION

Circulating hepcidin is proposed to regulate iron absorption (1, 2) by modulating iron export by ferroportin at the basolateral membrane of the duodenal mucosal cells (3) and/or uptake into the cells at the apical membrane by DMT1 (4). Hepcidin expression is regulated by body iron requirements (5). Urine and plasma concentrations of hepcidin appear to reflect body iron status (6–8), although, as yet, the relation has not been well characterized. Importantly, because the measurement of hepcidin in plasma has met with many technical challenges (7–9), until very recently effort has been focused on mRNA expression in cell culture and animal model systems and urinary rather than plasma concentrations in humans. To date, only Young et al (10) have studied the relation between plasma hepcidin concentrations and iron absorption in humans. In the present study, we made use of stored samples from a human iron absorption study to further test the hypothesis that plasma hepcidin explains interindividual variation in iron absorption.

SUBJECTS AND METHODS

Study population

Hepcidin-25 concentrations were measured in plasma samples collected between May 2002 and January 2003 in a human iron absorption study. Samples were stored at −80°C before hepcidin analysis in July 2008. Men aged ≥40 y were recruited to investigate the relation between genotype, iron absorption, and iron status. The study was approved by the Norwich District Ethics Committee and is reported in full elsewhere (11). A total of 35 men took part in the study: 15 HFE–wild type/wild-type controls, 15 C282Y/wild-type heterozygotes, and 5 C282Y/H63D compound heterozygotes. The subjects consumed 3 meals of high iron bioavailability for 2 d (extrinsically labeled with a total dose of 18 mg Fe-57) and 2 meals with fortified cereal products for the next 3 d (extrinsically labeled with a total dose of 45 mg Fe-54).

Measurement of hepcidin

Hepcidin was measured in fasting plasma samples collected on the first day that subjects consumed test meals; there was insufficient sample volume to measure hepcidin in 2 volunteers (one heterozygote and one compound heterozygote). The concentration of hepcidin-25 in the plasma samples was determined by using a combination of weak cation exchange chromatography and surface-enhanced laser-desorption/ionization time-of-flight mass spectrometry (7). The synthetic analog hepcidin-24 of hepcidin-25 was used as an internal standard for quantification. The intrarun variation in plasma hepcidin was 3.5% (range: 3.5–7.5 nmol/L), and the interrun variation was 7.5% (4 nmol/L). The lower limit of detection was 0.5 nmol/L.

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The hepcidin concentration of samples stored at −80°C was stable when assessed over 8 mo (DW Swinkels and C Laarakkers, unpublished observations, 2008), and the samples were stable when subjected to freeze-thaw cycles (12); however, it should be noted that the samples analyzed for this study were stored frozen at −80°C for 66 to 74 mo before analysis.

**Measurement of iron absorption and iron status**

Iron absorption from test meals was calculated from the isotopic enrichment of red blood cells 14 d after the last test meal and was corrected for utilization of absorbed iron by means of an intravenous infusion of Fe-58, as previously described by Roe et al (11).

Hemoglobin, serum iron, total-iron-binding capacity, transferrin saturation, serum ferritin, and C-reactive protein were measured by the Chemical Pathology Department of the BUPA Hospital (Norwich, United Kingdom). Serum ferritin was measured with a commercial chemiluminescent immunoassay analyzer (Immulite 2000; Diagnostic Products, Los Angeles, CA).

**Statistical analysis**

Statistical analyses were performed by using R data analysis software (13). Standard linear regression models were used where the response variable was the logarithm of the percentage of stable-isotope-labeled iron absorbed from the test meal. The explanatory variables for the linear regression were, initially, as follows: plasma hepcidin, serum ferritin, serum iron, total-iron-binding capacity, and plasma transferrin saturation. After the data were fitted to the regression model, nonsignificant variables were removed in a stepwise manner by backward elimination, and the model was refitted. This process was repeated until the final model only contained variables that had a significant explanatory effect on the response variable. For all models, regression diagnostics were checked to determine whether data transformations, outlier omissions, or alternative nonparametric models were required. Below the limit-of-detection measurements of plasma hepcidin-25 concentration (<0.5 nmol/L) were dealt with by using an accepted technique of modeling by a randomly generated number from the uniform distribution in the range 0–0.5 nmol/L.

**RESULTS**

Subject characteristics, indexes of iron status at the time of the absorption test, and percentage absorption from the test meals are shown in Table 1. C-reactive protein was measured as a marker of inflammation, and all values were within the normal range (0–10 mg/L). Iron absorption from fortified cereal products was highly correlated with absorption from the high-bioavailability iron test meals ($r = 0.82, P < 0.001$); therefore, only absorption from fortified cereal products was included in further analysis. Log iron absorption was negatively correlated with serum ferritin ($r = -0.59, P < 0.001$) and with plasma hepcidin ($r = -0.55, P < 0.001$; Figure 1). Serum ferritin was strongly correlated with plasma hepcidin ($r = 0.82, P < 0.001$; Figure 2). A one-factor analysis of variance indicated that the HFE genotype (C282Y/wild type, C282Y/H63D, wild type/wild type) had no significant influence on log iron absorption, and genotype was not significantly associated with either serum ferritin or plasma hepcidin. Utilization of absorbed iron in red blood cells was not significantly associated with plasma hepcidin or serum ferritin. In a multiple linear regression analysis, the continuous variables plasma hepcidin, serum ferritin, serum iron, total-iron-binding capacity, transferrin saturation, and plasma transferrin receptor significantly predicted log iron absorption (adjusted $R^2 = 0.39, P = 0.003$). In a stepwise regression procedure the reduced model that best predicted log iron absorption contained only serum ferritin (coefficient $= -0.004, P < 0.001$) and total-iron-binding capacity (coefficient $= 0.015, P = 0.034$) and explained 40% of the variance of log iron absorption (adjusted $R^2 = 0.40, P < 0.001$). Because serum ferritin was highly correlated with plasma hepcidin, multiple linear regression analysis was repeated using the same continuous variables but with serum ferritin excluded (adjusted $R^2 = 0.38, P = 0.0026$). In a stepwise regression procedure, the reduced model that best predicted log iron absorption contained only plasma hepcidin (coefficient $= -0.100, P < 0.001$) and total-iron-binding capacity (coefficient $= 0.015, P = 0.038$) and explained 36% of the variance of log iron absorption (adjusted $R^2 = 0.36, P < 0.001$).

**DISCUSSION**

Hepcidin undoubtedly plays a key role in iron homeostasis (14), although the precise mechanism by which it regulates

**TABLE 1**

Subject characteristics, indexes of iron status, plasma hepcidin concentrations, and percentage iron absorption measured on study day 1 and categorized by HFE genotype

<table>
<thead>
<tr>
<th></th>
<th>Wild type/wild type (n = 15)</th>
<th>C282Y/wild type (n = 14)</th>
<th>C282Y/H63D (n = 4)</th>
<th>Combined (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>60 ± 6 (48–69)</td>
<td>60 ± 7 (47–68)</td>
<td>60 ± 8 (48–66)</td>
<td>60 ± 6 (47–69)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 3.4 (20.9–34.5)</td>
<td>25.8 ± 3.1 (19.9–30.5)</td>
<td>23.9 ± 0.9 (23.1–27.4)</td>
<td>25.6 ± 3.1 (19.9–34.5)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.5 ± 0.8 (13.4–16.2)</td>
<td>14.7 ± 0.6 (13.4–15.7)</td>
<td>14.7 ± 0.6 (14.0–15.5)</td>
<td>14.6 ± 0.7 (13.4–16.2)</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>62 ± 50 (9–208)</td>
<td>72 ± 44 (32–149)</td>
<td>91 ± 56 (17–139)</td>
<td>70 ± 48 (9–208)</td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td>20.7 ± 5.3 (11.7–32.7)</td>
<td>22.6 ± 5.2 (15.4–30.9)</td>
<td>23.2 ± 5.0 (17.9–29.6)</td>
<td>21.8 ± 5.2 (11.7–32.7)</td>
</tr>
<tr>
<td>Total-iron-binding capacity (µmol/L)</td>
<td>64.6 ± 6.5 (52.7–77.4)</td>
<td>62.9 ± 6.5 (53.5–74.2)</td>
<td>60.5 ± 7.8 (50.9–68.4)</td>
<td>18.7 ± 6.6 (50.9–77.4)</td>
</tr>
<tr>
<td>Plasma transferrin receptor (nmol/L)</td>
<td>18.5 ± 3.4 (13.2–24.7)</td>
<td>18.1 ± 2.9 (13.2–25.4)</td>
<td>16.3 ± 2.3 (13.1–18.4)</td>
<td>18.1 ± 3.0 (13.1–25.4)</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>31.9 ± 7.1 (20.0–46.9)</td>
<td>36.2 ± 8.6 (22.3–49.2)</td>
<td>38.9 ± 8.9 (26.0–46.1)</td>
<td>34.6 ± 8.1 (20.0–49.2)</td>
</tr>
<tr>
<td>Hepcidin-25 (nmol/L)</td>
<td>2.1 ± 1.9 (0.1–7.8)</td>
<td>2.4 ± 1.3 (0.4–6.0)</td>
<td>3.2 ± 1.9 (0.8–4.9)</td>
<td>2.3 ± 1.7 (0.1–7.8)</td>
</tr>
<tr>
<td>Iron absorption from fortified cereal products (%)</td>
<td>4.9 ± 2.0 (2.9–9.2)</td>
<td>5.4 ± 1.4 (3.9–8.5)</td>
<td>5.4 ± 2.7 (3.5–9.3)</td>
<td>5.2 ± 1.8 (2.9–9.3)</td>
</tr>
<tr>
<td>Iron absorption from high-bioavailable iron meals (%)</td>
<td>6.8 ± 6.8 (1.9–26.8)</td>
<td>7.6 ± 3.3 (2.4–12.7)</td>
<td>9.9 ± 10.7 (1.6–26.0)</td>
<td>7.5 ± 6.2 (1.6–26.8)</td>
</tr>
</tbody>
</table>
absorption is unclear. It binds to ferroportin at the basolateral membrane, causing its internalization and degradation (3), but has also been shown to inhibit apical iron uptake by inhibiting DMT1 transcription (4, 15). In rats, iron absorption is inversely related to hepatic hepcidin gene expression (16). In mice injected with synthetic hepcidin, iron uptake into the duodenum is reduced; however, interestingly, the proportion transferred to the circulation is not reduced (17). This finding suggests that an additional factor is involved. Our observation that only ≈40% of the interindividual variance could be explained by a model that included both ferritin and hepcidin is either the result of measurement imprecision or, more likely, the presence of as yet unidentified physiologic factors that modulate iron absorption.

In this study in humans, we observed a significant relation between iron absorption and plasma hepcidin that, in the apparently healthy male population, is explained by iron stores (serum ferritin concentration). It should, however, be noted that the individuals participating in the iron absorption studies were specifically selected in relation to their genotype. Nevertheless, the results presented illustrate that within the range of hepcidin concentrations measured at the time of the iron absorption tests (0–7.8 nmol/L), a model with plasma hepcidin concentration (and total-iron-binding capacity) significantly explained 36% of the interindividual variation in iron absorption. The range of hepcidin concentrations found in this study was within the reference range for serum hepcidin concentrations measured by ionization time-of-flight mass spectrometry (mean ± SD: 4.5 ± 2.8; range: 0.5–13.9 nmol/L; n = 24; J Kroot and DW Swinkels, unpublished observations, 2008; www.hepcidinanalysis.com).

The relation between plasma hepcidin concentration and iron absorption in men found in our study confirms the relation found by Young et al (10), who showed that hepcidin concentration in serum collected from healthy young women 14 d after a test meal was significantly correlated with iron absorption from food and supplemental sources. Interestingly, although there was a significant relation between serum hepcidin concentrations and iron absorption, in contrast with our study, there was no significant relation between serum hepcidin and serum ferritin.

A model that contained serum ferritin (and total-iron-binding capacity) explained 40% (P < 0.001) of the variance in iron absorption, which is in agreement with the well-established relation between iron stores and efficiency of iron absorption (18). However, there was no indication of a deviation from the linear relation at higher serum ferritin concentrations (>60 μg/L), as previously shown by Hallberg et al (19). The main conclusions

![FIGURE 1. Correlation between plasma hepcidin and iron absorption (n = 33; r = −0.55, P < 0.001).](image1)

![FIGURE 2. Correlation between plasma hepcidin and serum ferritin (n = 33; r = 0.82, P < 0.001).](image2)
from our study were that plasma hepcidin and serum ferritin concentrations are highly correlated ($r = 0.82, P < 0.001$), and that, in the normal range of plasma hepcidin values, 36% of the interindividual variance in iron absorption is explained by differences in circulating plasma hepcidin. This new information may be instrumental in the rational design of iron supplementation programs, but further research is required to identify other physiologic factors that control iron absorption.

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The authors’ responsibilities were as follows—MAR: supervised the study, conducted the laboratory work, analyzed the data, and contributed to the draft and revision of the manuscript; RC: conducted the laboratory work and contributed to the revision of the manuscript; JRD: carried out the statistical analysis and contributed to the draft and revised manuscript; DWS: supervised the laboratory measurements and contributed to the draft and revised manuscript; and SJF-T: designed and supervised the study, prepared the first draft, and contributed to the revision and finalized the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES