Nonheme-iron absorption in first-degree relatives is highly correlated: a stable-isotope study in mother-child pairs

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ABSTRACT
Background: Iron absorption in humans is highly variable even after iron status and dietary components that influence iron absorption are controlled for. Inherited factors may help explain this variance.

Objective: Our objective was to compare nonheme-iron absorption from a noninhibitory, stable-isotope-labeled test meal in preschool-aged children and their mothers.

Design: We provided 72 test meals based on degermed maize flour and milk powder and fortified with [57Fe]ferrous fumarate or [58Fe] ferrous sulfate to healthy Mexican preschool children [n = 18; mean (±SD) age: 3.6 ± 1.0 y] and their mothers [n = 18; mean (±SD) age: 28.0 ± 5.2 y]. Iron absorption was calculated on the basis of incorporation of isotopes into erythrocytes after 14 d and was adjusted for differences in iron status.

Results: There was a wide variation in iron absorption from the test meals: in the mothers and children, the median fractional absorption of ferrous sulfate was 22.55% (range: 1.65–54.83%) and 5.51% (range: 2.23–17.20%), respectively (P < 0.0001). After adjustment for serum ferritin, the significant difference in absorption between mothers and their children disappeared. Despite this broad range of iron absorption, corrected fractional iron absorption from the ferrous fumarate–fortified (r² = 0.582) and the ferrous sulfate–fortified test meals (r² = 0.557) was strongly correlated in mothers and their children (P < 0.0001). There was a striking positive correlation between the mean corrected fractional iron absorption from both test meals in mothers and their children (r² = 0.782, P < 0.0001). In regression analyses that included age, sex, and hemoglobin, the only significant predictor of corrected fractional iron absorption in children was corrected fractional iron absorption in their mothers (standardized β = 0.884, P < 0.001).

Conclusions: Nonheme-iron absorption exhibits a strong familial tendency. After differences in meal matrix and serum ferritin are accounted for, these data suggest that inheritance and/or shared environmental factors explain most of the variance in dietary iron absorption. Am J Clin Nutr 2010;91:802–7.

INTRODUCTION
In humans, intersubject absorption of nonheme iron shows wide variation. In healthy young women, absorption of iron from a standardized test meals measured by using stable isotopes varies from 1% to 58% (1). Some of this variation is due to differences in iron status, because iron absorption is inversely related to body iron stores (1), and some of it is due to differences in meal matrix, because dietary components can strongly influence iron absorption (2). However, taken together, it has been estimated that iron status and dietary influences predict only ≈50% of the variance in iron absorption in a population (3). Cook et al (4) reported a positive correlation in body iron in iron-replete mothers and their young children and suggested this close correlation in iron status was due to a shared diet and/or possible genetic determinants of iron status such as shared iron-regulatory genes.

Iron absorption is modulated at least in part through varying concentrations of circulating hepcidin (1, 5). However, in 2 recent stable-isotope studies that used stepwise regression models to predict nonheme-iron absorption including plasma hepcidin as a covariate, the model that best predicted iron absorption contained serum ferritin (SF), and not hepcidin but explained only 40–43% of the variance of iron absorption (1, 5).

Thus, the available evidence suggests that as yet unidentified physiologic factors contribute to about half of variation in human dietary iron absorption (3), and it is likely that inheritance plays a role. Single nucleotide polymorphisms (SNPs) that affect iron absorption and/or utilization appear to be common in human populations (6). One example includes a polymorphism in the transferrin protein (G277S) associated with iron deficiency in women (7). The C282Y mutation of the HFE gene is associated with iron loading but shows varying penetrance, likely due to other modifier genes such as SNP rs884409 in CYBRD1 (8) and variants in the SLC40A1 gene (9).

To our knowledge, a familial tendency in iron absorption in the general population has not been studied by using iron isotopes. We hypothesized that there would be a positive correlation among first-degree relatives in nonheme-iron absorption from a standardized meal after correction for differences in iron status.
SUBJECTS AND METHODS

Subjects

Eighteen apparently healthy pairs of women and their preschool-aged children were recruited in Cuernavaca, Mexico. Exclusion criteria for the mothers were as follows: an SF ≥ 25 µg/L (to study women with low iron stores) and current pregnancy or lactation. The exclusion criterion for children was an age < 1.5 or > 6 y. Exclusion criteria for both mothers and children were an elevated C-reactive protein (CRP; > 3 mg/L), chronic diseases, chronic medication use, or recent/current use of iron supplements. The subjects were fully informed, and written consent was obtained from the mothers. The study protocol was approved by ethical committees at the National Institute of Public Health, Cuernavaca, Mexico, and the Swiss Federal Institute of Technology (ETH) Zürich (Zurich, Switzerland). The study was performed in the second half of 2005. After consideration of iron absorption and variation in previous studies (10, 11), we estimated that 18–20 mother-child pairs should be included to detect a 50% significant difference in log mean iron absorption between mothers and their children, with 90% power, a significance level of 0.05 (unpaired t test), and a type I error rate of 5%.

Study design

Women and their children were separately randomly assigned to receive the test meal labeled either with ferrous sulfate or with ferrous fumarate on consecutive days (days 1 and 2 of the study). The test meal was a sweetened drink based on the local Mexican maize-flour porridge drink atole. It was made from degermed maize flour (24.7%), whole-milk powder (11.7%), meals were identical except for the addition of 4 mg iron (to study women with low iron stores) and current pregnancy or lactation. The exclusion criterion for children was an age < 1.5 or > 6 y. Exclusion criteria for both mothers and children were an elevated C-reactive protein (CRP; > 3 mg/L), chronic diseases, chronic medication use, or recent/current use of iron supplements. The subjects were fully informed, and written consent was obtained from the mothers. The study protocol was approved by ethical committees at the National Institute of Public Health, Cuernavaca, Mexico, and the Swiss Federal Institute of Technology (ETH) Zürich (Zurich, Switzerland). The study was performed in the second half of 2005. After consideration of iron absorption and variation in previous studies (10, 11), we estimated that 18–20 mother-child pairs should be included to detect a 50% significant difference in log mean iron absorption between mothers and their children, with 90% power, a significance level of 0.05 (unpaired t test), and a type I error rate of 5%.

Stable-isotope labels

Isotopically labeled [57Fe]ferrous fumarate was produced by Dr Paul Lohmann GmbH (Emmerthal, Germany) from enriched elemental iron, and individual doses were preweighed into polyethylene tubes and stored. The [58Fe]ferrous sulfate, which were added to the test meal immediately before serving. Ferrous fumarate powder was added directly into the test meal, and ferrous sulfate was added as a solution. The meals were given to the subjects after an overnight fast. No food or drink was allowed for 3 h after the labeled meal. Five milliliters of venous blood were drawn into EDTA-treated tubes, and body weight and height were measured just before intake of the first test meal on day 1 and again on day 14.

Laboratory analysis

Whole-blood samples were collected for the measurement of hemoglobin concentration by using a portable HemoCue photometer (Hemocue Inc, Angelholm, Sweden) with the use of quality-control material from the manufacturer on each sampling day. Serum was separated from whole blood for SF and CRP measurements by automated immunoassay (Immule One; DPC, Los Angeles, CA) at ETH Zürich. Remaining whole blood was frozen for later analysis of isotopic composition. Anemia was defined as a hemoglobin concentration < 110 g/L in children aged ≤ 59 mo, < 115 g/L in children aged > 59 mo, and < 120 g/L in women (14). Iron deficiency was defined as SF < 12 µg/L in children < 5 y of age and SF < 15 µg/L in children ≥ 5 y of age and in women (14). Enriched whole-blood samples were mineralized by microwave digestion by using a mixture of concentrated HNO3 and H2O2 (30%) as oxidizing agents. Iron was separated from the matrix by using anion exchange chromatography and a liquid-liquid extraction step into diethyl ether (13). Chemical blanks were processed throughout the procedure. Isotopic composition of the separated iron was measured by NTIMS.

Data and statistical analysis

Data were analyzed by using Prism (version 3; GraphPad, San Diego, CA) and Excel (XP 2002; Microsoft, Seattle, WA). The amounts of 57Fe and 58Fe isotopic labels in blood 14 d after administration were calculated on the basis of the shift of iron isotopic ratios and the estimated amount of circulating iron in the body. Circulating iron was calculated on the basis of blood volume and hemoglobin concentration. Blood volume calculations were based on body weight and height according to Brown et al (15) for women and Linderkamp et al (16) for children. For calculations of fractional (%) iron absorption, 80% incorporation of the absorbed iron into erythrocytes was assumed for women (17) and 90% was assumed for children (18). Dietary iron absorption by each subject was adjusted to a value corresponding to an SF concentration of 40 µg/L by using the following equation (19):

\[
\ln[\text{Adj Abs} (\%)] = \ln[A_o (\%)] + \ln[F_o (\mu g/L)] - \ln[40 (\mu g/L)]
\]

where Adj Abs is adjusted iron absorption, \(A_o\) is measured iron absorption, and \(F_o\) is the SF concentration.

For the latter, all statistical tests were performed with the use of
log-transformed data. Student’s unpaired t test was used to compare the data between the age groups. Pearson’s correlations were used to look for associations between variables. Stepwise multiple regression was performed with iron absorption in the child (corrected for SF) as the dependent variable to test for associations with maternal iron absorption (corrected for SF), age, and hemoglobin as well as child age, sex, and hemoglobin. P values <0.05 were considered statistically significant.

RESULTS

The characteristics of the 18 mothers and their children who received the stable-iron-isotope–labeled test meals are shown in Table 1. The mothers had poorer iron status than did their children: SF concentrations were significantly lower in women, and 12 of 18 women were iron deficient (SF < 15 μg/L), whereas only one child was iron deficient (SF < 12 μg/L). All mothers and their children had CRP concentrations within the normal range, and there was no significant correlation between CRP and SF or hemoglobin in mothers or children. There was a significant inverse correlation between mean uncorrected iron absorption for both test meals and SF in both children (r = −0.47, P < 0.001) and mothers (r = −0.41, P < 0.001). Because of the marked influence of iron stores on iron absorption and the large differences in iron status between the subjects, the absorption data obtained for each subject were adjusted to a common amount of iron stores (19). After adjustment, the significant differences between groups disappeared (Table 1).

There was no correlation between SF or hemoglobin in the mother-child pairs. There were strong positive correlations between the log-corrected fractional iron absorption in mothers and their children (Figure 1): Figure 1A shows the values from the ferrous fumarate–fortified test meals (r² = 0.582); Figure 1B shows the values from the ferrous sulfate–fortified test meals (r² = 0.557); and Figure 1C shows the mean values from both test meals (r² = 0.782) (all P < 0.0001). The individual data points for the mean log-corrected fractional iron absorption values from the 2 test meals in mothers and children, and their associations, are shown in Figure 2.

Stepwise regression was performed with the mean log-corrected fractional iron absorption from the 2 meals in the child as the dependent variable; the model also included the mean log-corrected fractional iron absorption in the mother and maternal age and hemoglobin as well as child’s age, sex, and hemoglobin. The only significant predictor was the mean log-corrected fractional iron absorption in the mother (standardized β = 0.884, P < 0.001) but not the age or hemoglobin of the mother or the age, sex, or hemoglobin of the child; the adjusted r² of the model was 0.769.

DISCUSSION

Strong variation in iron-absorption data in human studies is common even after adjustment for reference-dose absorption, iron status, and dietary components that influence iron absorption. Reddy et al (3) examined the relative contribution of dietary components and iron status to the variation in dietary iron absorption. By using radioisotopes in 25 healthy human adults consuming different meals, they estimated that differences in SF concentration accounted for 32% of the overall variability in iron absorption. After each individual absorption value to a constant SF concentration (as in the present study) was adjusted for, only an additional 16% of the variation in iron absorption was explained by dietary components, mainly animal tissue and phytic acid. Thus, taken together, iron status and the relevant dietary factors predicted only ~50% of the variance in iron absorption. The authors concluded that unknown physiologic factors explain about half of variation in human dietary iron absorption (3); our findings suggest that these physiologic factors may be largely familial.

To our knowledge, this is the first study to convincingly show, by using isotope-labeled test meals in first-degree relatives, a marked familial tendency in iron absorption. The most plausible explanation for our findings is that iron absorption is at least

<table>
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<td>Characteristics of women and children who received the stable-iron-isotope–labeled test meals</td>
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1 Mean ± SD (all such values).
2 Median; range in parentheses (all such values).
3 Significantly different from mothers, P < 0.001 (t test).
4 Iron absorption in each subject corrected to a value corresponding to a serum ferritin concentration of 40 μg/L (19).
partially heritable. Alternatively, they could be due to unidentified shared environmental factors. Cook et al (4) measured iron status in 800 preschool children and their mothers in Bolivia and found a striking positive correlation in body iron (calculated from the ratio of SF to serum transferrin receptor): when the mothers and children were both iron replete, the slope of the regression curve between body iron in mothers and children was unity. The authors suggested that this close correlation in iron status indicated that 1) the bioavailability of iron from a shared diet is a key determinant of body iron and/or 2) the possible role of a genetic determinant of iron status and/or absorption such as shared iron-regulatory genes. The data in the present study point to a genetic determinant of iron absorption and suggest that inheritance at least partially explains the findings of Cook et al (4).

In the present study, both children and their mothers received an identical test meal to eliminate differences in meal matrix on iron absorption. Degermed maize flour was used to reduce the phytic acid content of the flour, an absorption inhibitor (20). From this simple noninhibitory matrix, the fractional and absolute iron absorption was significantly higher in women than in their children. This is likely explained by the lower iron status of the women, which up-regulated iron absorption in an attempt to maintain iron homeostasis, an effect modulated at least in part through circulating hepcidin (21). However, we did not measure hepcidin concentrations in this study, and there are limited data on the relation between hepcidin and iron absorption in children. Recent studies have clearly shown that other, as yet unidentified, genetic and/or physiologic factors influence iron absorption and/
adjusted between child and maternal iron absorption (described above; absorption. On the basis of our regression model on the relation plasma hepcidin) and explained only 40% of the variance in iron absorption included SF and total iron binding capacity (but not analyses showed that the model that best predicted iron ab-
sorption was apparent for both a water-soluble and a poorly water-soluble compound. Limitations of the study include the following: 1) we included only women with low iron stores, which may limit the generalizability of the findings; 2) because we used a simple noninhibitory meal, we did not test whether the effect of meal factors that enhance or inhibit iron absorption is the same between the mothers and their children; and 3) the lack of collection of DNA and direct assessment of candidate genes. If confirmed, our findings suggest a further search for SNPs in iron acquisition genes could be a fertile topic for future research. If common SNPs that influence iron absorption were identified, this might ultimately allow for personalized recom-
mendations for iron intake in human populations.

or utilization alongside hepcidin (1, 5). In young women without inflammation but with varying iron status who were given identical stable-isotope-labeled test meals (n = 196), there was a significant inverse correlation between iron bioavailability and plasma hepcidin (r² = 0.25) (1). But in regression analyses that included plasma hepcidin as a covariate, the model that best predicted iron absorption contained age, hemoglobin, and plasma ferritin (but not hepcidin) and explained 43% of the variance of iron absorption (1). Similar findings were reported by Roe et al (5) in men, who, with the use of stepwise regression analyses showed that the model that best predicted iron absorption included SF and total iron binding capacity (but not plasma hepcidin) and explained only 40% of the variance in iron absorption. On the basis of our regression model on the relation between child and maternal iron absorption (described above; adjusted r² = 0.769), our findings suggest that 77% of the variance in nonheme-iron absorption from a noninhibitory meal is explained by maternal-child relationships after adjustment for SF.

Our data suggest that a large portion of this remaining variance in human iron absorption, after meal matrix and iron status are controlled for, may be explained by inherited factors. There are estimated to be ~3 million SNPs in the human genome, and it is probable that some of them, passed from the mother to the child, affect the function of proteins involved in iron absorption and/or metabolism (6). For example, a common polymorphism in the transferrin protein (G277S) has been associated with iron deficiency in women (7). However, it has no effect on the iron-binding capacity of transferrin in vitro (22), and a stable-isotope study (23) comparing 25 nonanemic women who had either a heterozygous G277S/G277G (n = 10) or wild-type G277G/ G277G (n = 15) genotype did not find a significant difference in iron absorption. Interestingly, G277S carriers did not show the typical inverse correlation between iron stores and iron absorption (23). Another common mutation affecting iron metabolism, the C282Y mutation of the HFE gene, is carried by ~13% of individuals from white populations and is closely associated with hereditary hemochromatosis (8). Although the mutation is associated with iron loading, it does not appear to affect heme- or nonheme-iron absorption (24, 25), but its low penetrance makes it likely that there are other modifier genes (26, 27). In northern Europeans, SNP rs884409 in CYBRD1 is a putative genetic modifier of iron overload that may account for 11% of the variance in SF concentrations in C282Y homozygotes (8). An SNP in the SLC40A1 (ferroportin) gene, IVS1-24 C → G, may also modulate the severity of iron overload in these individuals (9). It is possible that other mutations, including the gene for the divalent metal transporter-1 (DMT1) (28) and for ferroportin (29) may also modulate iron absorption in the general population.

Thus, a strong familial correlation in iron absorption could be explained by single or, more likely, a combination of polymorphisms in these or other iron acquisition genes. A strength of this study is that we gave a fairly large number of labeled test meals (n = 72) and tested if the familial tendency in iron absorption was apparent for both a water-soluble and a poorly water-soluble compound. Limitations of the study include the following: 1) we included only women with low iron stores, which may limit the generalizability of the findings; 2) because we used a simple noninhibitory meal, we did not test whether the effect of meal factors that enhance or inhibit iron absorption is the same between the mothers and their children; and 3) the lack of collection of DNA and direct assessment of candidate genes. If confirmed, our findings suggest a further search for SNPs in iron acquisition genes could be a fertile topic for future research. If common SNPs that influence iron absorption were identified, this might ultimately allow for personalized recommendations for iron intake in human populations.

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The authors’ responsibilities were as follows—MH and SV: carried out the field work; MBZ and RFH: supervised the overall study; MBZ wrote the first draft of the manuscript; and all authors: contributed to editing of the manuscript. All authors contributed to the study design, data analysis and editing of the paper. The authors had no conflicts of interest or financial disclosures to make.

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