Maternal Hepcidin Is Associated with Placental Transfer of Iron Derived from Dietary Heme and Nonheme Sources1–4

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Abstract

The determinants of placental transport of dietary iron remain largely uncharacterized. The objective of this research was to elucidate determinants of fetal Fe transfer from maternally ingested dietary heme and non-heme Fe. The study was undertaken in 19 pregnant females (16–32 y) who ingested intrinsically labeled 58Fe-heme and a nonheme Fe source (57FeSO4) during the third trimester of pregnancy. At delivery, maternal and cord blood was obtained to assess neonatal 57Fe and 58Fe enrichment as a function of maternal/neonatal Fe status [serum ferritin (SF), transferrin receptor, hemoglobin (Hb), total body Fe, and hepcidin]. There was a greater percentage of maternally absorbed 58Fe tracer present in the neonates compared to the 57Fe tracer (5.4 ± 2.4 vs. 4.0 ± 1.6; P < 0.0001). Net dietary nonheme Fe (mg) and heme Fe (mg) transferred to the fetus were both inversely correlated with measures of maternal serum hepcidin (P = 0.002, r² = 0.43; P = 0.004, r² = 0.39) and SF (P = 0.0008, r² = 0.49; P = 0.003, r² = 0.41) and directly associated with neonatal Hb (P = 0.004, r² = 0.39; P = 0.008, r² = 0.35). The results of this study suggest that during pregnancy there appears to be preferential fetal use of maternally ingested Fe derived from a dietary, animal-based heme source compared to Fe ingested as ferrous sulfate. Maternal serum hepcidin and maternal/neonatal Fe status may play a role in placental uptake of dietary heme and nonheme Fe. J. Nutr. 142: 33–39, 2012.

Introduction

The neonatal Fe endowment at birth has been increasingly linked to subsequent health outcomes (1). During the third trimester of pregnancy, the fetus obtains the majority of its Fe stores and suboptimal placental Fe uptake may set the stage for increased risk of postnatal Fe insufficiency. The importance of maternal Fe status in the establishment of the neonatal Fe endowment at birth has been controversial. Previously, the fetus was thought to function as a “perfect parasite” and extract all necessary Fe from the mother largely independent of her Fe stores. However, there are now growing animal and human data to support a relationship between maternal anemia and suboptimal neonatal Fe status at birth (2–7). Moreover, there is a growing recognition of the long-term, irreversible effects of neonatal anemia on cognitive, motor, and social-emotional outcomes (8–10). We previously reported a relationship between maternal Fe status and transfer of nonheme Fe to the fetus (11). However, the regulation of heme Fe metabolism during pregnancy remains largely unexplored.

Hepcidin is a systemic regulator of Fe homeostasis and functions by binding to ferroportin and causing it to be internalized, thereby blocking Fe export from the enterocyte and Fe release from macrophages and the liver (12). At this time, there are limited human data on the role of hepcidin in the regulation of placental uptake of maternally ingested heme and nonheme Fe sources, although animal data has suggested a link between hepcidin and nonheme Fe homeostasis during pregnancy (13,14). The goal of this study was to evaluate placental Fe transfer of maternally ingested dietary heme and nonheme Fe during the third trimester of pregnancy in relation to maternal and neonatal Fe status and serum hepcidin.

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2 Author disclosures: M. Westerman is an officer of Intrinsic Lifesciences LLC and has ownership interest in the company. Intrinsic Lifesciences LLC is engaged in the commercial development of the hepcidin assay described in this manuscript. M. F. Young, I. Griffin, E. Pressman, A. W. McIntyre, E. Cooper, T. McNanley, Z. L. Harris, and K. O. O., no conflicts of interest.

3 Supplemental Figure 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

4 This trial was registered at clinicaltrials.gov as NCT01019096.

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Materials and Methods

Participants and Methods. Twenty pregnant study volunteers aged 16–32 y were recruited from the Strong Midwifery Group and the Rochester Adolescent Maternity Program in Rochester, NY. Only healthy, nonsmoking females with uncomplicated pregnancies were asked to participate. Women (≥19 y) and adolescents (≥18 y) were excluded if they had gestational diabetes, underlying malabsorption diseases, or medical problems known to affect Fe homeostasis at the time of enrollment. The study was approved by the Institutional Review Board of Cornell University and the University of Rochester Research Subjects Review Board and informed written consent was obtained from all participants. Data on Fe absorption in these study participants was previously published (15).

Isotope preparation. Fe stable isotopes provide a valuable tool for examining Fe bioavailability and metabolism, because these tracers are safe, nonradioactive forms of Fe that are naturally found in fixed amounts in our bodies and in the environment. Fe in nature is comprised of 4 stable isotopes: $^{54}\text{Fe}$, $^{56}\text{Fe}$, $^{57}\text{Fe}$, and $^{58}\text{Fe}$. Three of these forms of Fe ($^{56}\text{Fe}$, $^{57}\text{Fe}$, and $^{58}\text{Fe}$) are found at very low levels in our environment (0.287, 2.14, and 5.8%, respectively) and enriched sources of these stable isotopes can be utilized to trace maternal Fe absorption and placental Fe transfer (16). Fe isotopes for this study were purchased from Trace Sciences International as the metal ($^{57}\text{Fe}$ at 94.69% enrichment and $^{58}\text{Fe}$ at 93.34% enrichment). The nonheme Fe tracer ($^{56}\text{Fe}$) was converted into ferrous sulfate solution using a 2:1 molar ratio of ascorbic acid:Fe (Anazao Health). The Fe tracer used to intrinsically label heme ($^{58}\text{Fe}$) was converted into ferrous citrate and then analyzed for sterility by Analytical Research Labs. Fe isotopic composition of the final tracer solutions was assessed using a Thermofinni Scientific Triton TM Magnetic Sector TIMS (Thermo Fisher Scientific). Intrinsically labeled heme was undertaken at Baylor College of Medicine using a protocol approved by their Animal Use Committee. Methods were adapted from a previous study by Etcheverry et al. (17). Briefly, intrinsically labeling of the porcine muscle and RBC Fe was accomplished by 2 i.m. 75-mg doses of $^{57}\text{Fe}$ into a 3- to 4-month-old female piglet. The piglet was killed at 28 d of age at a USDA and State of Texas–approved facility at Texas A&M University. RBC, a rich source of Hb, and muscle, a rich source of myoglobin, were collected and used as the labeled heme Fe source for the study. The muscle ($^{58}\text{Fe}$ enrichment of 18.0 ± 0.80%) and RBC ($^{58}\text{Fe}$ enrichment of 17.3 ± 0.01%) were analyzed separately for enrichment of $^{58}\text{Fe}$ by TIMS as previously reported (15).

Study design and isotope dosing. The 2-d Fe absorption study took place during the third trimester of pregnancy (wk 32–35 of gestation) at Strong Memorial Hospital’s CTSI. All participants discontinued any Fe or prenatal supplementation for 3 d prior to the first day of Fe dosing. Fasting participants were asked to come to the CTSI on 2 consecutive days. Each individual’s height and weight were taken with the use of a stadiometer and a calibrated scale on the morning of the first study day. On each test day, a standard breakfast was provided and each research participant then remained in the CTSI for 2 h before receiving each Fe test meal. All participants consumed both an animal-based heme ($^{58}\text{Fe}$ intrinsically labeled ground pork) and nonheme ($^{56}\text{Fe}$ as ferrous sulfate) test meal in a random order on alternate days. The heme Fe dose included ~203 g of enriched ground pork and 3.9 g of enriched RBC cooked into a chili mix with tomato sauce (Hunts) and chili seasoning (McCormick). The net Fe load ingested from the heme and nonheme test meal in a random order on alternate days. The heme Fe dose included ~203 g of enriched ground pork and 3.9 g of enriched RBC cooked into a chili mix with tomato sauce (Hunts) and chili seasoning (McCormick). The net Fe load ingested from the heme and nonheme test meals were similar, with each containing ~8 mg of Fe. The heme meal provided 0.46 mg of labeled $^{58}\text{Fe}$ from intrinsically labeled pork muscle and 0.46 mg from intrinsically labeled RBC for a total dose of 0.92 mg $^{58}\text{Fe}$ (7.9 mg total Fe). The nonheme Fe dose consisted of an oral dose of 8.2 mg of $^{56}\text{Fe}$ as ferrous sulfate (8.6 mg total Fe) flavored with 2 mL of raspberry syrup (Humco) containing 0.391% ascorbic acid without food. The tracer dose of $^{58}\text{Fe}$ administered was lower than that given for $^{57}\text{Fe}$ because of the nearly 10-fold lower natural abundance of $^{58}\text{Fe}$ (0.287 vs. 2.14%). The Fe content of the heme meal and nonheme Fe dose was validated using atomic absorption spectrophotometry (PerkinElmer Analyst 800; PerkinElmer). Pregnant women and adolescents remained in the CTSI for 2 h after ingesting each test meal before being fed a standard lunch. Standard dinner and snacks were provided for the participants to take home and consume. Pregnant adolescents remained as inpatients overnight at the CTSI to avoid transportation difficulties and to ensure food intake was controlled. On the second test day, the same foods in the same amounts were consumed as those ingested on the first day.

All study participants were followed until delivery and their medical charts were flagged to ensure delivery samples would be obtained. At admission, a 15-mL maternal blood sample was obtained and at delivery, a 30-mL cord blood sample was taken.

Laboratory analysis. Whole blood samples collected at delivery were transported to Strong Memorial Hospital’s clinical laboratory for analysis of Hb using the Cell Dyn 4000 system. Hb measures were also obtained at bedside using a HemoCue. When clinical laboratory values for neonatal Hb were not available (due to logistical and technical problems with sample clotting, etc.), the HemoCue values were utilized. In other whole blood samples, serum was separated on site and shipped to Cornell on dry ice for analysis of Fe status indicators. SF and serum soluble TIR were measured with commercially available kits from Ramco Laboratories, Inc. From these 2 markers, TBI was calculated as described by Cook et al. (TBI [mg/kg] = $-\log$ (serum TIR/SF) - 2.8229/0.1207) (18) and recently validated in pregnant women (19). CRP, serum folate, vitamin B-12, and EPO were measured using the Immulite1000 immunoassay system. Intrinsic Life Sciences measured maternal and neonatal serum hepcidin using a competitive serum ELISA specific for the mature peptide (Intrinsic Life Sciences) (20). The lower limit of detection for this assay is 5 μg/L.

Isolation of Fe from samples/MS. Samples of whole blood (0.5 mL) were digested with 2 mL of nitric acid (Ultrex, JT Baker) and 0.1 mL hydrogen peroxide by heating in glass flasks on a hot plate. Digested samples were evaporated to dryness and reconstituted in 2 mL of ultrapure 6M hydrochloric acid (Ultrex, JT Baker). Fe was extracted from the digested blood samples using anion exchange chromatography. The eluate was dried on a hot plate and then reconstituted in 40 μL 3% nitric acid. Extracted Fe samples (8 μL), silica gel (3 μL) (Sigma-Aldrich), and 0.23 mol/L phosphoric acid (3 μL) were loaded onto degassed ultrapure zone-refined rhenium filaments (H. Cross). Fe isotope ratios ($^{57}\text{Fe}$/$^{56}\text{Fe}$, $^{58}\text{Fe}$/$^{56}\text{Fe}$, and $^{54}\text{Fe}$/$^{56}\text{Fe}$) were measured using TIMS (21). All acids used were ultrapure (Ultrex JT Baker).

Calculation of Fe transfer to fetus. The oral Fe tracer administered to the pregnant women has two primary fates once it crosses the enterocyte: it is either incorporated into the maternal RBC or used for maternal stores or it is transported across the placenta to be incorporated into fetal RBC or used for fetal Fe stores (Supplemental Fig. 1). The form of the absorbed $^{58}\text{Fe}$-heme tracer at the time of placental uptake is unknown; thus, the term Fe derived from a dietary heme source is used throughout.

To account for errors introduced by assumptions in the distribution of absorbed tracer, three approaches were used to assess the Fe isotopic data in the neonate at birth (Supplemental Fig. 1). The first approach directly examined the percent excess of $^{57}\text{Fe}$ and $^{58}\text{Fe}$ in cord blood which reflects the degree to which the natural abundance ratio was increased as a result of RBC incorporation of the stable Fe tracer as previously reported (11). The natural abundance Fe isotopic ratios utilized were 0.02317 for $^{57}\text{Fe}$ and 0.00307 for $^{58}\text{Fe}$. All measures used a dose-adjusted percentage of enrichment for both the $^{57}\text{Fe}$ and $^{58}\text{Fe}$ tracer. For this measure, the observed percent excess in cord blood and actual dose administered was used to obtain an expected percent excess using the mean tracer dose of $^{57}\text{Fe}$ administered (8.2 mg) and a corresponding mean dose of $^{58}\text{Fe}$ of 1.1 mg [to convert mg of Fe to mmol, divide the mg quantity by the atomic weight of Fe (55.847)].

The amount of Fe tracer in the neonate at birth was examined as a fraction of the tracer dose given to the mother. To obtain this measure,
the neonatal mass of circulating Fe was first estimated assuming a neonatal blood volume of 80 mL/kg (22,23) and an Fe content of Hb of 3.47 g/kg (24,25). The net mg of $^{57}$Fe present in the infant circulation from the dietary heme source and $^{57}$Fe present in the infant circulation (in mg) from the dietary FeSO$_4$ tracer was calculated and examined as a fraction of the total tracer dose ingested by each pregnant woman. Finally, the amount of Fe tracer in the neonate was examined as a fraction of the tracer dose absorbed by the mother based on the amount recovered in maternal and neonatal RBC.

**Data analysis.** All statistical analyses were completed using JMP 8.0 (SAS Institute). Variables that were not normally distributed were transformed prior to statistical testing (using the natural log). Linear regression analyses were used to examine the relationships among Fe status, hepcidin, and placental transfer. Multiple linear regression analyses using forward stepwise regression were used to identify significant determinants of placental Fe transfer of each ingested dietary Fe tracer. The significance of relationships between participants was assessed using Paired t tests (e.g. $^{57}$Fe vs. $^{58}$Fe transfer, neonatal Hb measures, and maternal-neonate Fe status measures). Differences between participant groups were compared using a t test or the Wilcoxon’s rank-sum test for nonparametric data (e.g., Fe transfer in participants with detectable vs. nondetectable serum hepcidin). Non-detectable serum hepcidin levels (<5 µg/L) were given a value of 2.5 µg/L for data analysis purposes. Normally distributed data are described by mean ± SD and median ± SD for non-normally distributed data. Results were analyzed with and without the two participants that developed PE. Unless specifically stated otherwise, the results and P values are given for the complete sample size (including PE participants). If inclusion of the PE participants affected the significance of the results obtained, this was highlighted. Our sample size of 20 participants was selected to detect a 1.1% difference in percent enrichment of $^{57}$Fe compared to $^{58}$Fe in cord blood with a power of 80% and an a level of 0.05 and an estimated attrition rate of 20% using the mean and SD in a prior study of placental nonheme transfer (11). Differences were considered significant if $P < 0.05$.

**Results**

**Participant characteristics.** General characteristics of the study participants were recorded (Table 1). Of the original pregnant adults ($n = 10$) and pregnant adolescents ($n = 10$), one pregnant adolescent was withdrawn from the study, because she did not finish the pork meal; therefore, only 19 study participants were followed to delivery. Of these 19 participants, one adult did not show up for the 2-wk blood draw, so Fe absorption data were not available for that participant, but maternal and cord blood samples were collected at delivery.

In total, 84% (16/19) of the participants had term deliveries (≥37 wk gestation) and 21% (4/19) gave birth to a LBW infant (<2500 g; mean birth weight of 2408 ± 74 g). Among the three preterm births, one woman had a spontaneous preterm birth and the other two were induced, because they developed PE (one adult/one adolescent). All preterm births resulted in LBW infants. One additional adult with a term pregnancy also gave birth to a LBW infant. The primary outcomes did not differ between the adult and teen participants.

Fe status indicators in mothers and their neonates at delivery were assessed (Table 2). No significant differences in the Hb values were evident between the HemoCue compared to the core laboratory value. At delivery, 42% of the participants had depleted Fe stores (Hb <110 g/L, SF <20 µg/L). Approximately 47% of the participants had nondetectable levels of serum hepcidin at delivery (<5 µg/L), whereas all of the neonates had detectable serum hepcidin at birth.

**Hepcidin and Fe status.** Maternal serum hepcidin concentrations were associated with multiple indicators of Fe status in the mother at delivery [SF ($P = 0.0001$, $r^2 = 0.59$); TBI ($P = 0.0001$, $r^2 = 0.59$); Hb ($P = 0.01$, $r^2 = 0.31$); and TfR ($P = 0.01$, $r^2 = 0.31$)]. Similarly, neonatal serum hepcidin was directly associated with both neonatal SF ($P < 0.0001$, $r^2 = 0.60$) and neonatal TBI ($P = 0.0001$, $r^2 = 0.60$). However, maternal serum hepcidin was not significantly related to neonatal serum hepcidin or other markers of Fe status in the neonate.

**Transfer of maternally ingested $^{57}$FeSO$_4$ to the fetus.** There was a greater dose-adjusted cord blood enrichment of the $^{58}$Fe tracer derived from the maternally ingested heme Fe compared to the $^{57}$Fe enrichment in cord blood derived from the maternally ingested $^{57}$FeSO$_4$ ($P = 0.02$) (Table 3). The differences in Fe transfer between the two isotopes remained significant if presented as percent of maternally ingested Fe tracer transferred or percent of maternal absorbed tracer present in the neonate at birth. Similar to our previous data on transfer of nonheme Fe to the fetus (11), no significant relationship was evident between neonatal enrichment of either of the Fe isotopic tracers and the number of days that had elapsed between dosing and delivery.

Relationships between the net amount (mg) of the $^{57}$Fe tracer from maternally ingested $^{57}$FeSO$_4$ that was present in the neonate at birth and maternal Fe status were explored. An inverse association was found between the net uptake of $^{57}$Fe in cord blood with maternal SF ($P = 0.0008$, $r^2 = 0.49$) and Hb ($P = 0.005$, $r^2 = 0.38$). Likewise, there was a strong inverse association between the net mg of $^{57}$Fe present in the neonate at birth and maternal TBI ($P < 0.001$, $r^2 = 0.64$) (Fig. 1). Net mg of $^{57}$Fe transferred to the fetus was directly associated with maternal TfR ($P = 0.0002$, $r^2 = 0.58$), maternal EPO ($P = 0.01$, $r^2 = 0.32$), and maternal absorption of the $^{57}$Fe non-heme tracer ($P = 0.002$, $r^2 = 0.47$).

Net transfer of the $^{57}$Fe tracer from maternally ingested $^{57}$FeSO$_4$ was also inversely associated with maternal serum hepcidin at delivery ($P = 0.002$, $r^2 = 0.43$). Pregnant participants with undetectable levels of serum hepcidin transferred a greater quantity of the maternally ingested $^{57}$FeSO$_4$ dose to their fetus compared to those with detectable levels of serum hepcidin ($P = 0.003$). These differences remained significant when percent of

**Table 1** General characteristics of mothers and neonates at delivery

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery, wk</td>
<td>39.9 [1.6] (36.0-41.6)</td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>19.0 [2.9] (16.0–32)</td>
</tr>
<tr>
<td>Adolescent (≤18 y), %</td>
<td>47</td>
</tr>
<tr>
<td>Adult (&gt;19 y), %</td>
<td>53</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0) [0–3]</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m$^2$</td>
<td>24.7 [7.0] (20.7-43.6)</td>
</tr>
<tr>
<td>Delivery BMI, kg/m$^2$</td>
<td>30.4 [7.1] (24.4-47.5)</td>
</tr>
<tr>
<td>Gestational weight gain, kg</td>
<td>18.0 ± 6.8 (8.6-34.5)</td>
</tr>
<tr>
<td>Infant weight, kg</td>
<td>3.17 ± 0.55 (2.3-3.9)</td>
</tr>
<tr>
<td>Race, %</td>
<td>53</td>
</tr>
<tr>
<td>African American</td>
<td>53</td>
</tr>
<tr>
<td>Caucasian</td>
<td>47</td>
</tr>
<tr>
<td>Ethnicity, %</td>
<td>37</td>
</tr>
<tr>
<td>Hispanic</td>
<td>37</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>63</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD (range) or median [SD] (range), n = 19, or percentage.
the maternal absorbed $^{57}$Fe tracer transferred to the fetus was used in place of the net mg of $^{57}$Fe transferred ($P = 0.03$). Likewise, mothers with depleted Fe stores (SF $<20$ mg/L) transferred more $^{58}$Fe tracer (mg) to the fetus than Fe-replete, nonheme sources. Similarities and differences in the determinants of placental uptake of each form of maternally ingested Fe were noted, which may provide additional insight into mechanisms of Fe partitioning during states of high Fe flux such as those that occur during pregnancy.

### Transfer of maternally ingested $^{58}$Fe-heme to the fetus.

Similar to the associations observed for neonatal enrichment of $^{57}$Fe, the net mg of $^{58}$Fe derived from maternally ingested, intrinsically $^{58}$Fe-heme Fe was also significantly associated with both maternal and neonatal Fe status. The net mg of $^{58}$Fe transferred to the fetus was inversely associated with maternal Hb ($P = 0.004$, $r^2 = 0.39$) and maternal ferritin ($P = 0.003$, $r^2 = 0.41$). Maternal TfR was likewise highly correlated with maternal absorption of intrinsically labeled $^{58}$Fe-heme Fe ($P = 0.01$, $r^2 = 0.35$) and with maternal TfR at delivery ($P = 0.01$, $r^2 = 0.33$). Maternal serum hepcidin at delivery was inversely associated with net $^{58}$Fe transfer ($P = 0.004$, $r^2 = 0.39$). Similar to the associations noted for $^{57}$Fe, women with depleted Fe stores ($P = 0.0003$) as well as those with nondetectable serum hepcidin at delivery ($P = 0.002$) transferred a significantly greater net quantity of $^{58}$Fe to the fetus. These differences remained significant if the percent of maternally ingested, intrinsically $^{58}$Fe-heme Fe tracer absorbed was used in place of net mg transferred ($P = 0.008$).

Net fetal transfer of $^{58}$Fe derived from maternally ingested $^{58}$Fe-heme Fe was directly associated with neonatal Hb ($P = 0.008$, $r^2 = 0.35$) and neonatal EPO ($P = 0.03$, $r^2 = 0.2$) but was not significantly related to other measures of Fe status. Using a multiple regression model, 79% of the variation in net fetal transfer of $^{58}$Fe derived from maternally ingested $^{58}$Fe-heme Fe could be explained by cord Hb, infant weight, and maternal TfR at delivery ($P < 0.0001$, $r^2 = 0.79$).

### Discussion

A significantly greater fraction of maternally absorbed, animal-based heme Fe was found in the neonate at birth compared to that observed for maternally absorbed ferrous sulfate. These data suggest that there may be differential partitioning or metabolism of Fe derived from dietary heme compared to nonheme sources. Similarities and differences in the determinants of placental uptake of each form of maternally ingested Fe were noted, which may provide additional insight into mechanisms of Fe partitioning during states of high Fe flux such as those that occur during pregnancy.

Both maternal and neonatal Fe status were associated with the net transfer of maternally ingested $^{57}$FeSO$_4$ to the fetus over

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mothers</th>
<th>Neonates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/L</td>
<td>111 ± 12</td>
<td>134 ± 30*</td>
</tr>
<tr>
<td>Anemic (&lt;110 g/L), %</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>SF, μg/L</td>
<td>25.4 [20.6]</td>
<td>135 [110]*</td>
</tr>
<tr>
<td>Fe-deficient (&lt;12 μg/L), %</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Fe-depleted (&lt;20 μg/L), %</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Serum TfR, mg/L</td>
<td>5.5 [3.3]</td>
<td>8.4 ± 2.1</td>
</tr>
<tr>
<td>&gt;0.5 mg/L, %</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>TBI, mg/kg</td>
<td>3.4 ± 3.9</td>
<td>8.6 ± 2.7*</td>
</tr>
<tr>
<td>&lt;0 mg/kg, %</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Serum hepcidin, μg/L</td>
<td>9.30 [50.1]</td>
<td>61.7 [77.0]*</td>
</tr>
<tr>
<td>Serum C-reactive protein, mg/L</td>
<td>6.1 [14.7]</td>
<td>0.20 [0.15]*</td>
</tr>
<tr>
<td>Serum folate, nmol/L</td>
<td>38.0 [19.5]</td>
<td>69.4 ± 26.6*</td>
</tr>
<tr>
<td>Serum vitamin B-12, pmol/L</td>
<td>262 ± 123</td>
<td>540 [547]*</td>
</tr>
<tr>
<td>Serum EPO, IU/L</td>
<td>25 [23]</td>
<td>30 [74]</td>
</tr>
<tr>
<td>Serum leptin, μg/L</td>
<td>41.0 ± 22.2</td>
<td>7.3 [9.6]*</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD or median [SD], $n = 19$, or percentage. *Different from maternal, $P < 0.05$. EPO, erythropoietin; Hb, hemoglobin; SF, serum ferritin; TBI, total body Fe; TfR, transferrin receptor.

2 To convert mg of Fe to mmol, divide the mg quantity by the atomic weight of Fe (55.847).

3 Quantity of tracer dose in neonate as a fraction of the maternally absorbed tracer dose.

4 Quantity of tracer dose in neonate divided by average tracer dose administered to mother.

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonheme ($^{57}$Fe)</th>
<th>Heme ($^{58}$Fe)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% enrichment in cord blood (dose adjusted)</td>
<td>6.9 ± 3.2</td>
<td>8.4 ± 3.2</td>
<td>0.02</td>
</tr>
<tr>
<td>% of Maternal ingested Fe tracer present in neonate at birth</td>
<td>2.2 ± 1.4</td>
<td>2.7 ± 1.3</td>
<td>0.02</td>
</tr>
<tr>
<td>% of Maternal absorbed Fe tracer present in neonate at birth</td>
<td>4.0 ± 1.6</td>
<td>5.4 ± 2.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD, $n = 19$.

2 Percent excess was adjusted for the natural abundance of isotope administered and for small differences in dose received.

3 Quantity of tracer dose in neonate divided by average tracer dose administered to mother.

4 Quantity of tracer dose in neonate as a fraction of the maternally absorbed tracer dose.

36 Young et al.
the last 1.4–9.6 wk of pregnancy. The average percent of maternally absorbed $^{57}$FeSO$_4$ present in the neonate at birth (4.1%) was slightly lower than we previously reported among neonates born to Fe supplemented (7.0%) and unsupplemented (8.6%) Peruvian women in a similarly designed $^{57}$FeSO$_4$ isotope study (11). Likewise, an early radioisotope study noted that 7–10% of the Fe absorbed by the mother was present in the neonate at birth (26). In our current study, 64% of the variation observed in the transfer of maternally ingested $^{57}$FeSO$_4$ to the fetus during late pregnancy was explained solely by maternal TBI at delivery. Although hepcidin has been demonstrated to affect placental transport of nonheme Fe in animal models (13,14), maternal hepcidin explained only 43 or 39% of the variation in placental transfer of maternally ingested $^{58}$Fe or $^{57}$Fe$heme$, respectively, and the predictive ability of this hormone was no greater than that found using traditional Fe status markers. It is interesting to note that hepcidin explained more variability in the neonatal enrichment of maternally ingested $^{57}$FeSO$_4$ than the 25–30% variability that we (27) and others (28,29) have reported between hepcidin and nonheme Fe absorption in nonpregnant adults. Our data support current findings reported in transgenic mice overexpressing hepcidin. These mice die shortly after birth due to severe Fe deficiency, presumably due to insufficient Fe endowment of birth caused by a deficit in placental Fe flux to the developing fetus (14). Although neonatal hepcidin was not significantly associated with placental Fe transfer, a significant correlation between placental Fe transfer and neonatal Hb was detected in this study. Because maternal and neonatal Fe status are inherently linked, it is difficult to tease apart their individual contributions to Fe partitioning during pregnancy. In previous research by Gambling et al. (30), fetal but not maternal liver Fe levels were associated with expression of liver TfR and hepcidin in the mother. We (31) and others (32–34) previously found that both maternal and neonatal Fe status are associated with protein expression of key placental Fe transporters, suggesting both may play a role in Fe homeostasis during pregnancy.

Use of both $^{58}$Fe-intrinsically labeled heme and $^{57}$FeSO$_4$ allows for similarities and differences in the fate of these two forms of ingested dietary Fe to be explored during pregnancy. Fe status of both the mother and neonate were significantly associated with net fetal transfer of the two forms of maternally ingested Fe, although relationships were stronger for maternally ingested $^{57}$FeSO$_4$ compared to maternally ingested $^{58}$Fe-heme. A greater fraction of Fe derived from dietary heme Fe was observed in the neonate at birth, which may be a consequence of greater intestinal heme Fe uptake in the mother. However, this did not appear to explain the differences noted in our study, because the dose-adjusted percent excess, the net mg transferred as a fraction of the maternally ingested tracer, or as a fraction of the amount of Fe absorbed by the mother all supported a preferential fetal uptake of Fe derived from maternally ingested heme. It should be noted that correcting for maternal Fe absorption in our placental transfer calculations cannot account for all factors that contribute to the observed differences in the neonatal enrichment values and additional time course studies are needed to fully explore these relationships. Absolute values for mg of Fe transferred to the fetus are affected by assumptions used in our calculations. For example, fetal blood volume was estimated, but because it is a constant factor in both calculations, it would not influence relative differences observed between the two forms of Fe. Moreover, our study conclusions were comparable if direct measures of neonatal enrichment (no assumptions of blood volume or RBC Fe incorporation) were utilized in place of absolute mg or percent of dose values. We are only able to assess tracer
enrichment in neonatal RBC and not that transferred to other non-Hb associated pools. Because the majority (75–80%) of Fe in the neonate is found as circulating Hb (35), this limitation is unlikely to account for the observed preferential placental transfer of maternally ingested heme Fe.

Cellular mechanisms of heme uptake and export are not well characterized. Absorbed heme may be degraded within the cell into inorganic Fe, or it has been proposed that some fraction of absorbed heme may be exported intact through known heme export proteins in the enterocyte (such as Feline leukemia virus subgroup C receptor-FLVCR) (36). Recent data by Yang et al. (37) have demonstrated that both FLVCR and hemopexin (a heme scavenger protein) are integral to whole body Fe partitioning and metabolism. Interestingly, both FLVCR1 (38) and the hemopexin receptor (39) are highly expressed in the placenta, which may suggest a metabolic role for heme Fe in the placenta. This is also supported by our recent data demonstrating a relationship between placental FLVCR1 protein expression and maternal anemia in pregnant adolescents (40). Further research on both heme and nonheme placental Fe transporters in the placenta in relation to Fe flux during pregnancy is needed to fully elucidate mechanisms controlling placental Fe utilization.

We are unable to distinguish the form of the 57Fe or 58Fe at the time it crosses the placenta or possible early differences in Fe partitioning prior to incorporation into fetal or maternal RBC. Our findings may suggest that at least a small fraction of the dietary heme Fe was exported intact or these findings may be indicative of differential clearance kinetics of these different forms of Fe from the enterocyte. Previous research in humans and dogs has suggested that the majority (90 to >95%) but not all administered heme Fe enters the circulation as elemental Fe (41,42). Questions remain on the mechanisms of cellular heme flux, particularly during pregnancy when ~3–8 mg of Fe is trafficked to the fetus daily (43) from maternal diet or existing maternal stores.

Systemic Fe homeostasis may differentially affect the enterocyte and the placenta, because previous research has suggested that mechanisms of hepcidin regulation may be cell-type dependent (44). It is interesting to note that although we observed correlations between neonatal 57Fe or 58Fe enrichment derived from maternally ingested 57Fe-heme and maternal serum hepcidin, in these same women we previously found no significant association between maternal hepcidin and maternal utilization of dietary 57Fe-heme (15). To our knowledge, this is the first study to examine relationships between maternal and neonatal hepcidin and placental Fe transfer during pregnancy. Our hepcidin concentrations are slightly lower than the geometric means reported in a population of nonanemic mothers and neonates (12.4 μg/L, n = 116 vs. 78.4 μg/L, n = 137, respectively) (45). Lower serum hepcidin may be expected in our participants because of the high rates of Fe depletion typically observed in pregnant teens (46). Adolescents with undetectable serum hepcidin transferred significantly more Fe (both 57FeSO4 and 58Fe-heme) to their fetus compared to those with detectable hepcidin. The differences in 57Fe compared to 58Fe transfer were more pronounced in women with detectable serum hepcidin, whereas women with nondetectable serum hepcidin upregulated Fe transfer of the two tracers to a similar extent.

In summary, maternal hepcidin and maternal and neonatal Fe status are significantly associated with placental transport of Fe from maternally ingested heme and nonheme Fe. Fe derived from maternally ingested, animal-based heme may be preferentially transported to the fetus during the third trimester of pregnancy. Further research on mechanisms of placental Fe uptake of dietary heme and nonheme Fe are warranted.

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