Maternal iron status influences iron transfer to the fetus during the third trimester of pregnancy[1–3]

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ABSTRACT

Background: The effect of maternal iron status on fetal iron deposition is uncertain.

Objective: We used a unique stable-isotope technique to assess iron transfer to the fetus in relation to maternal iron status.

Design: The study group comprised 41 Peruvian women. Of these women, 26 received daily prenatal supplements containing iron and folate (n = 11; Fe group) or iron, folate, and zinc (n = 15; Fe+Zn group) from week 10–24 of pregnancy to 1 mo postpartum. The remaining 15 women (control group) received iron supplementation only during the final month of pregnancy. During the third trimester of pregnancy ($\bar{x} \pm$ SD: 32.9 ± 1.4 wk gestation) oral ^{57}Fe (10 mg) and intravenous ^{58}Fe (0.6 mg) stable iron isotopes were administered to the women, and isotope enrichment and iron-status indicators were measured in cord blood at delivery.

Results: The net amount of ^{57}Fe in the neonates' circulation (from maternal oral dosing) was significantly related to maternal iron absorption ($P < 0.005$) and inversely related to maternal iron status during the third trimester of pregnancy: serum ferritin ($P < 0.0001$), serum folate ($P < 0.005$), and serum transferrin receptors ($P < 0.02$). Significantly more ^{57}Fe was transferred to the neonates in non-iron-supplemented women: 0.112 ± 0.031 compared with 0.078 ± 0.042 mg in the control group (n = 15) and the Fe and Fe+Zn groups (n = 24), respectively ($P < 0.01$). In contrast, ^{58}Fe tracer in the neonates' circulation was not significantly related to maternal iron status.

Conclusion: The transfer of dietary iron to the fetus is regulated in response to maternal iron status at the level of the gut. *Am J Clin Nutr* 2003;77:924–30.

KEY WORDS Iron, iron status, anemia, prenatal supplements, stable isotopes, neonates, cord blood, women, Peru

INTRODUCTION

Iron deficiency anemia is the most common nutritional deficiency in the world; estimates suggest that 2 billion persons worldwide are iron deficient (1). Because of the increased iron requirements of pregnancy and growth, pregnant women and infants are recognized as the groups most vulnerable to iron deficiency anemia.

Symptomatic iron deficiency during pregnancy has deleterious effects on maternal and perinatal health (2). Iron deficiency anemia during pregnancy is associated with higher rates of premature birth and low birth weight (3, 4). Severe maternal anemia increases the risk of reproduction-related mortality at delivery and during the perinatal period (5). Iron deficiency in infants may also adversely influence cognitive development (6, 7) and may have long-term consequences. Severe iron deficiency anemia in infants has been associated with impaired psychomotor development and developmental delays > 10 y after the treatment of iron deficiency during infancy (8).

The total iron requirements over pregnancy in a 55-kg woman are ≈1040 mg (9). Most of this iron is required during the third trimester, at which time daily iron needs increase from prepregnancy requirements of ≈1–1.5 mg/d to ≤6 mg/d (5, 9). The magnitude of this demand is difficult to meet from dietary sources alone, especially in developing countries where the diets are often limited in iron content and bioavailability is generally low or moderate as the result of high intakes of dietary fiber and phytates.

In Peru, mineral deficiencies are significant public health concerns as documented by biochemical, clinical, and nutritional surveys in this country (10, 11). Dietary studies in pregnant Peruvian women from our study population have reported a prevalence of dietary iron inadequacy in this population of 93% (12). Studies have shown that these low iron intakes are associated with reduced iron status in pregnant women from this community (13).

Many studies have found that the fetus can accumulate sufficient iron even in the face of mild or moderate maternal iron deficiency (14–16). By contrast, other findings indicate that maternal iron deficiency anemia during pregnancy compromises fetal iron reserves (17–20). To pursue maternal-fetal iron transfer, recent studies have focused on characterizing the physiologic adaptations that occur at the level of the placenta to support fetal iron demands in both iron-replete women and in women with dietary intakes or medical situations that increase the risk of iron deficiency (21–23). At this time, many questions still exist concerning the mechanisms by which iron is transferred to the fetus, and the predictors of this process have been difficult to determine in vivo with the use of standard approaches.

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The purpose of our study was to characterize the transfer of iron to the fetus during the third trimester of pregnancy in relation to maternal iron status. To accomplish this aim, we carried out dual stable-isotope studies of iron absorption in pregnant Peruvian women during the third trimester of pregnancy and followed the transfer of this iron to the fetus by measuring the net transfer of stable iron isotopes in cord blood and in samples of neonatal blood obtained at delivery.

SUBJECTS AND METHODS

Subjects

Pregnant women aged 18–35 y were recruited from a maternity hospital (Cesar Lopez Silva Hospital) in Villa El Salvador, a peri-urban, low-income community in Lima, Peru. To avoid the potential influence of high altitude on iron dynamics, all women recruited had resided in Lima (a sea-level community) for ≥1 y before their inclusion in this study. Subjects recruited into the study were in good health, had a parity between 0 and 3, and had experienced no medical complications during pregnancy. Three groups of women were recruited when they were between 30 and 36 wk of gestation. Two of these groups were recruited from an ongoing study of prenatal supplementation involving ≈1300 women from this community (24). These women consumed daily prenatal supplements containing 60 mg Fe (as ferrous sulfate) and 250 μg folate (Fe group) with or without the addition of 15 mg Zn (as zinc sulfate; Fe+Zn group). Women received prenatal supplements starting at 10–24 wk of gestation and continued supplementation to 1 mo postpartum.

Women in the unsupplemented control group were recruited from the same study community but did not receive prenatal supplements because they did not enter prenatal care until late in the third trimester. Women in the control group were provided with daily iron supplements (60 mg Fe and 250 μg folate) after the iron-absorption trial was completed (2 wk after they were recruited into the study). These women therefore received iron supplementation for ≈4 wk before the delivery of their infants.

The study was approved by the Committee for Human Research at the Johns Hopkins School of Hygiene and Public Health and by the Ethical Committee at the Instituto de Investigación Nutricional, Lima, Peru. Written informed consent was obtained from each woman before the start of the study. Data on maternal iron and zinc absorption in these women during the third trimester of pregnancy were previously reported (25, 26).

Isotope preparation

Iron isotopes of Russian origin were purchased as the metal (^{57}Fe at 94.67% enrichment and ^{58}Fe at 93.13% enrichment). The oral ^{57}Fe tracer was converted into ferrous sulfate according to the procedure of Kastenmayer et al (27), except that no ascorbic acid was added during tracer preparation to avoid the influence of this vitamin on iron absorption. The intravenous ^{58}Fe isotope was converted from the metal into a sterile and pyrogen-free solution of ferrous citrate by Merck Frosst Canada Inc (Quebec). The isotopic composition of the final tracer solutions was validated by using magnetic sector thermal ionization mass spectrometry (MAT 261; Finnigan, Bremen, Germany).

Study design and isotope dosing

On the day the isotopes were administered, fasted (for ≥1.5 h) pregnant women came to the Cesar Lopez Silva hospital, a baseline venous blood sample (10 mL) was taken, and an intravenous ^{58}Fe tracer (0.6 mg as ferrous citrate) was infused over a 10-min interval. On this day each woman also consumed 10 mg ^{57}Fe (as ferrous sulfate) in 60–90 mL of a non–ascorbic acid–containing flavored drink. Women in the Fe and Fe+Zn groups also consumed their regular prenatal supplement at this time. The prenatal supplement ingested on this day was identical to the supplement normally consumed except that the total iron content was reduced by 10 mg to keep the total dose of supplement and tracer constant at 60 mg Fe. Women remained fasting for 1.5 h after dosing. Two weeks after dosing, a 5-mL blood sample was obtained for analyses of iron isotopes and determination of maternal iron absorption and red blood cell iron incorporation. Details of this study were previously reported (25).

When the women went into labor, a fieldworker accompanied them to the hospital. At delivery, samples of venous cord blood (5 mL, reflecting the isotopic enrichment of the baby and placental unit) were obtained and heel stick samples of blood (≈200 μL) were collected from each neonate. Although the expected enrichment of oral and intravenous iron tracer should be identical between the cord and heel stick samples, data from both were collected to confirm this relation and to provide us with sufficient sample volume to analyze all iron-status indicators in the neonates.

Isolation of iron from samples

Whole blood from neonatal cord (1 mL) and heel stick (200 μL) blood samples was digested with 15 mL nitric acid in a 25-mL Erlenmeyer flask by heating overnight on a hot plate. After each digest was clear, it was transferred to a beaker and evaporated to dryness. The digested residue was reconstituted in 2–4 mL of 6 N hydrochloric acid and was covered and heated slightly until the residue went into solution. Samples were cooled before the chromatography process.

Iron was extracted from the digested whole blood by using an anion exchange chromatography method and was reconstituted in 10–30 μL of 3% nitric acid (28). All acids used were ultrapure (Ultrex; JT Baker, Phillipsburg, NJ)

Mass spectrometry

Extracted blood samples (10 μL) were processed as previously reported (29), and isotope ratios were measured by using magnetic sector thermal ionization mass spectrometry (Finnigan). Typical relative SDs and precision with the use of this technique are 0.5% or better. Maternal iron absorption was measured by measuring the fraction of the oral ^{57}Fe dose incorporated into red blood cells after adjustment for the fraction of intravenous ^{58}Fe incorporated into red blood cells over the same interval. In brief, the amount of ^{57}Fe that was absorbed from the oral dose was determined as

$$\text{Iron absorption} = (\text{percentage of oral } ^{57}\text{Fe incorporated into RBCs})/(\text{percentage of intravenous } ^{58}\text{Fe incorporated into RBCs}) \times 100 \quad (1)$$

where RBCs is red blood cells.

Detailed calculations for determination of iron absorption and red blood cell iron incorporation in these women were reported previously (25).

Calculation of tracer enrichment in the fetus

The degree to which the iron isotope ratios in cord or heel stick blood were increased over the natural abundance ratios at baseline was determined as

$$\text{Change in percent excess } {}^{57/56}\text{Fe} = ({}^{57/56}\text{Fe observed} - {}^{57/56}\text{Fe baseline})/({}^{57/56}\text{Fe baseline}) \times 100 \quad (2)$$

The same equation was used for the $^{58/56}$Fe change in percent excess, substituting the $^{58/56}$Fe baseline and observed ratios into the equation. The natural abundance ratios of the $^{57/56}$Fe and $^{58/56}$Fe tracers used were 0.02326 and 0.00311, respectively.

Calculation of iron transfer to the fetus

The total circulating iron pool in the neonates was determined by assuming a neonatal blood volume of 80 mL/kg (30, 31) and the iron content of hemoglobin (3.47 g/mL) with the following equation (28, 32):

$$\text{Circulating Fe pool (mg)} = 80 \text{ mL/kg} \times \text{cord hemoglobin (g/mL)} \times 3.47 \quad (3)$$

The total amount of naturally occurring ^{58}Fe and ^{57}Fe in the neonates' blood was determined by multiplying the total circulating iron pool by the natural abundance level for each of the ^{57}Fe and ^{58}Fe tracers administered (0.0214 and 0.00287, respectively). The total milligram quantities of oral iron tracer transferred to the neonates' circulation were calculated as

$$^{57}\text{Fe tracer transferred to neonatal circulation (mg)} = (\text{change in percent excess } {}^{57}\text{Fe}/100) \times (\text{quantity of naturally occurring } {}^{57}\text{Fe in neonatal circulation}) \quad (4)$$

The same equation was used to estimate the total quantity of the intravenously administered maternal ^{58}Fe transferred to the neonatal circulation by substituting the change in percent excess of ^{58}Fe and the estimated naturally occurring ^{58}Fe in the neonate.

The net daily transfer of isotope to the fetus was determined by dividing the total quantity of tracer transferred (mg) to the fetus by the number of days that had elapsed between maternal dosing and parturition. The percentage of iron transferred to the fetus in relation to the net amount of iron tracer absorbed by the mother was then calculated as

$$\text{Amount of } {}^{57}\text{Fe tracer in the neonate (mg)}/\{(\text{percentage of Fe absorption in mother}/100) \times [{}^{57}\text{Fe dose}/(\text{percentage RBC incorporation}/100)]\} \times 100 \quad (5)$$

Iron-status indicators in cord blood

Total iron was measured in cord blood samples by using a colorimetric procedure. Hemoglobin was analyzed by using the cyanomethemoglobin method, and packed cell volume was analyzed by using the microhematocrit method. Serum ferritin was measured by enzyme-linked immunosorbent assay with human antiferritin and antiferritin peroxidase antibodies purchased from DAKO (Santa Barbara, CA). Serum transferrin receptors were measured with a commercially available enzyme-linked immunosorbent assay (Quantikine R&D Systems, Minneapolis). Serum folate and vitamin B-12 were measured in the same sample by radioimmunoassay (Diagnostic Products Corporation, Los Angeles).

Statistical analyses

Analysis of variance was used to detect significant differences in measured variables among supplementation groups. Scheffe's test was used for post hoc comparisons. All data are expressed as means ± SDs. Linear regression analysis was used to examine the relations between iron-status indicators and enrichment of the tracers in cord blood. For statistical purposes, ferritin values were transformed by using a natural logarithm. Data are presented as the nontransformed values for interpretation purposes. Stepwise regression was used to examine the relations between maternal and neonatal iron-status variables and enrichment of oral and intravenous tracer in the neonates at birth. Statistical analyses were completed by using the STATVIEW 5.0.1 software program (SAS Institute Inc, Cary, NC). All differences were considered significant at $P < 0.05$.

RESULTS

The physical characteristics of the study population are presented in **Table 1**. No significant differences were observed in maternal anthropometric measures between the 3 study groups. Mothers in the Fe and Fe+Zn groups entered the supplementation study at similar stages of gestation (15.7 ± 4.9 and 15.0 ± 4.6 wk of gestation, respectively). Compliance (determined from twice-weekly pill counts) did not significantly differ between the Fe and Fe+Zn groups (140 ± 43 and 166 ± 34 tablets, respectively).

As previously reported, iron status was significantly improved in women consuming prenatal supplements during pregnancy compared with that in the unsupplemented group (25). Despite differences in iron status, however, maternal absorption of nonheme iron during the third trimester of pregnancy did not significantly differ between the 3 study groups and averaged 11.5 ± 8.3% in the Fe group, 12.2 ± 5.9% in the Fe+Zn group, and 12.2 ± 4.6% in the control group.

All neonates had birth weights > 2500 g and no significant differences were present in neonatal birth weight or length between groups. Neonatal anthropometric and hematologic data are presented in **Table 2**. Hematocrit was significantly higher and total-iron-binding capacity was significantly lower in cord blood from the control group than in cord blood from the Fe or Fe+Zn group.

The mean change in percent excess of ^{57}Fe and ^{58}Fe in neonatal cord or heel stick blood samples obtained at birth and the net

TABLE 1
Characteristics of the maternal study population during the third trimester of pregnancy[1]

	Fe group (n = 15)	Fe+Zn group (n = 11)	Control group (n = 15)
Age (y)	22.7 ± 4.3 (18–30)	23.6 ± 4.1 (18–30)	23.1 ± 4.2 (18–30)
Weight (kg)	62.2 ± 7.8 (49.8–74.7)	62.3 ± 7.7 (49.3–71.2)	61.2 ± 4.2 (52.8–67.2)
Height (cm)	154.0 ± 4.3 (146.0–162.0)	153.9 ± 4.9 (143.5–160.4)	153.0 ± 4.8 (144.7–162.8)
BMI (kg/m^2)	26.4 ± 3.4 (21.7–31.4)	26.2 ± 2.5 (22.8–30.5)	26.2 ± 2.0 (23.4 – 30.6)
Gestation (wk)	33.1 ± 1.7 (30–36)	33.4 ± 1.4 (32–36)	32.3 ± 0.7 (32–34)
Iron absorption (%)[2]	11.5 ± 8.3[3] (1.0–32.7)	12.2 ± 5.9 (1.2–19.1)	12.2 ± 4.6 (5.1–23.8)

[1] $\bar{x}$ ± SD; range in parentheses. There were no significant differences between the groups for the variables presented.
[2] Measured during the third trimester of pregnancy with the use of oral (^{57}Fe) and intravenous (^{58}Fe) stable iron isotopes.
[3] n = 14.

TABLE 2
Birth weight and hematologic status in the neonates[1]

Variable	Fe group (n = 7 M, 8 F)	Fe+Zn group (n = 5 M, 6 F)	Control group (n = 8 M, 7 F)
Birth weight (g)	3156 ± 418 [15]	3087 ± 282 [11]	3109 ± 504 [15]
Cord hemoglobin (g/L)	155.0 ± 15.0 [13]	163.2 ± 14.0 [11]	150.4 ± 16.0 [15]
Cord hematocrit (%)	37.5 ± 4.7[a] [13]	37.1 ± 3.6[a] [11]	43.7 ± 8.8[b] [15]
Cord ferritin (μg/L)	201.5 ± 107.6 [6]	187.5 ± 66.9 [4]	170.1 ± 70.7 [15]
Cord TIBC (μg/dL)	610.5 ± 97.1[a] [13]	615.6 ± 134.3[a] [9]	465.4 ± 99.8[b] [15]
Cord sTFR (nmol/L)	23.0 ± 7.6 [11]	27.5 ± 7.0 [10]	25.6 ± 6.3 [14]
Cord serum iron (μg/dL)	158.4 ± 40.0 [14]	138.0 ± 37.4 [10]	143.1 ± 48.0 [15]
Cord folate (ng/mL)	10.1 ± 3.1 [8]	10.6 ± 3.4 [10]	11.8 ± 5.4 [5]
Cord vitamin B-12 (pg/mL)	152.6 ± 52.2 [8]	284.9 ± 180.1 [10]	240.5 ± 106.6 [6]

[1] $\bar{x}$ ± SD; n in brackets. TIBC, total-iron-binding capacity; sTFR, serum transferrin receptor. Means with different superscript letters are significantly different, $P < 0.05$ (Scheffe's correction for multiple comparisons).

transfer of these tracers to the fetus are presented in **Table 3**. Stable-isotope measures in neonatal cord and heel stick blood samples were significantly positively correlated for both ^{57}Fe ($R = 0.807$, $P < 0.0001$; $n = 32$) and ^{58}Fe ($R = 0.440$, $P < 0.02$; $n = 27$). Despite no significant differences in the percentage of iron absorption in the mothers, the net transfer of the oral nonheme iron tracer (^{57}Fe) to the neonates was significantly greater in neonates born to women in the control group than in those born to women in the Fe or Fe+Zn group (Table 3). A significant positive relation was seen between the percentage of iron absorption in the mother and the net transfer of the oral ^{57}Fe tracer to the fetus ($R = 0.476$, $y = 0.54 + 0.003x$, $P < 0.005$; $n = 39$).

After the data from all study groups were pooled, a significant linear relation was observed between maternal serum transferrin receptor concentration and the transfer of the oral iron tracer (^{57}Fe) to the fetus ($R = 0.379$, $y = 0.059 + 0.002x$, $P < 0.02$; $n = 38$). A highly significant inverse relation was also observed between the natural log of maternal serum ferritin concentrations and the transfer of the oral iron tracer to the fetus ($R = 0.578$, $y = 0.189 - 0.037x$, $P < 0.0001$; $n = 48$; **Figure 1**). The amount of the ^{57}Fe tracer in the fetus was also inversely related to maternal folate concentrations during the third trimester of pregnancy ($R = 0.359$, $y = 0.14 - 0.009x$, $P = 0.005$; $n = 32$).

Relations between hematologic measures in cord blood (including serum transferrin receptors, ferritin, vitamin B-12, folate, hemoglobin, hematocrit, and total-iron-binding capacity) and the net transfer of ^{57}Fe to the neonate were examined. Ferritin concentrations in cord blood were significantly inversely related (P = 0.04, R = −0.432; n = 24) and cord hematocrit values were significantly linearly related (P = 0.02, R = 0.367; n = 38) to the amount of ^{57}Fe transferred to the fetus. Higher cord serum transferrin receptor concentrations were associated with a higher transfer of ^{57}Fe to the fetus, although this relation was not significant (P = 0.07, R = 0.311; n = 35). The amount of the intravenous iron tracer (^{58}Fe) in the neonate at birth was not significantly related to maternal serum ferritin, maternal iron absorption, or maternal red blood cell iron incorporation or cord blood iron-status indicators.

DISCUSSION

It is unknown whether the amount of iron transferred to the fetus is proportional to the amount of iron available in the mother or whether the fetus preferentially receives iron from maternal reserves in response to maternal iron status or fetal requirements. Our data suggest that the transfer of dietary iron to the fetus is significantly related to maternal and neonatal iron status. Transfer of intravenously administered iron to the fetus was not regulated in response to maternal or fetal iron status.

Maternal iron stores, as determined by serum ferritin concentrations, were the strongest predictor of the degree of enrichment

TABLE 3
Change in percentage excess of orally (^{57}Fe) and intravenously (^{58}Fe) administered stable iron isotopes and net transfer of oral isotope to the fetus[1]

Variable and sample analyzed	Fe group	Fe+Zn group	Control group
Excess of ^{57}Fe (%)			
Cord blood	2.72 ± 1.42[a] [14]	2.36 ± 1.42[a] [11]	4.08 ± 0.96[b] [15]
Heel stick blood	2.54 ± 1.15[a] [13]	3.07 ± 1.25[a] [9]	4.64 ± 1.49[b] [11]
Excess of ^{58}Fe (%)			
Cord blood	11.64 ± 4.27 [14]	12.58 ± 3.70 [11]	12.82 ± 2.43 [14]
Heel stick blood	12.27 ± 3.31 [11]	11.78 ± 1.42 [9]	13.43 ± 2.86 [9]
Net ^{57}Fe transferred to fetus (mg)[2]			
Cord blood	0.085 ± 0.045 [13]	0.070 ± 0.039 [11]	0.112 ± 0.031 [15]
Net ^{58}Fe transferred to fetus (mg)			
Cord blood	0.049 ± 0.021 [13]	0.050 ± 0.015 [11]	0.048 ± 0.012 [14]
^{57}Fe absorbed by mother and transferred to fetus (%)			
Cord blood	7.02 ± 3.6 [13]	6.40 ± 6.9 [11]	8.62 ± 4.5 [15]

[1] $\bar{x}$ ± SD; n in brackets. Means in the same row with different superscript letters are significantly different, $P < 0.01$ (Scheffe's correction for multiple comparisons).

[2] Mean for the control group significantly different from the combined mean of the iron-supplemented groups (Fe and Fe+Zn groups), $P < 0.01$ (Scheffe's correction for multiple comparisons).

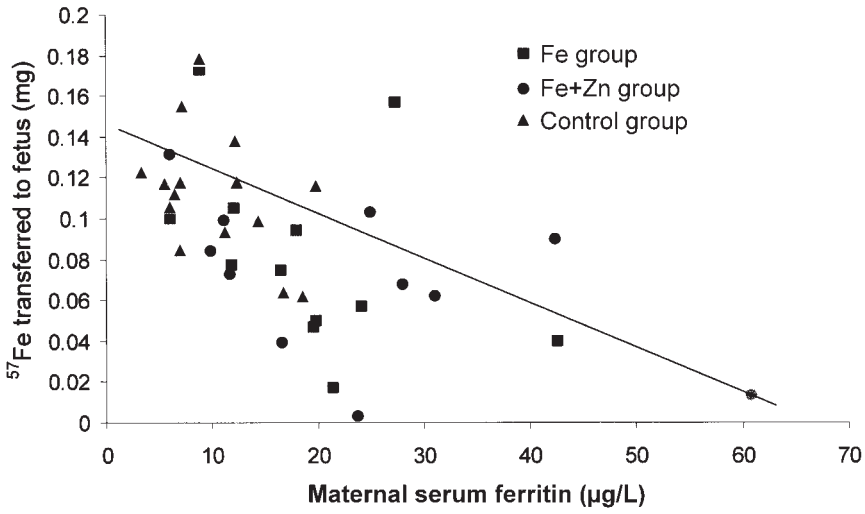

FIGURE 1. Transfer of nonheme dietary iron to the fetus was measured in 41 Peruvian women who received 10 mg ^{57}Fe orally during the third trimester of pregnancy ($\bar{x}$ ± SD: 33 ± 1 wk gestation). The amount of the stable isotope in the neonatal circulation was measured in cord blood samples obtained at delivery. Women in the intervention groups had received prenatal supplements containing 60 mg Fe and 250 μg folate with (Fe+Zn group) or without (Fe group) 15 mg Zn from week 10–24 of pregnancy to 1 mo postpartum. Women in the control group received iron supplementation only (60 mg Fe and 250 μg folate) over the final 32 ± 15 d of their pregnancy. The net amount of the stable iron isotope transferred to the fetus was inversely related to maternal ferritin concentrations during the third trimester of pregnancy. A significantly greater net amount of ^{57}Fe was transferred to the fetus in women with depleted ferritin stores ($y = 0.123 - 0.002x$, $R = 0.544$, $P < 0.001$; $n = 38$).

of the oral iron tracer in the neonates at birth. A significantly higher amount of the oral tracer was present in neonates born to mothers with depleted iron reserves. Serum transferrin receptors are also an indicator of tissue iron sufficiency. This receptor, found on the cell membrane, mediates cellular iron uptake. A fraction of this receptor is released into the circulation such that elevations in serum transferrin receptor are indicative of tissue iron depletion (33). In our study, serum transferrin receptor was also linearly related to the net transfer of the oral tracer to the neonate. Iron status in the neonate as determined by cord ferritin and hematocrit values was also significantly related to the net amount of ^{57}Fe present in the neonate at delivery.

In addition to the relations found between maternal and neonatal iron-status indicators and transfer of the oral iron tracer to the fetus, a significant correlation was evident between maternal folate concentrations and the amount of oral iron tracer in the neonate. This finding may in part be due to the presence of folate in the iron supplements, because there was a strong correlation between maternal folate status and serum ferritin concentrations. This finding may also be related to relations between maternal folate status and placental integrity, because folate deficiency increases apoptosis of human placental trophoblastic cells (34) and has been related to increased risk of low birth weight and fetal growth retardation (35, 36).

Increased transfer of dietary iron to the fetus might be related to changes in the expression of placental iron transport proteins, but these were not measured in our study. It has been shown that increased fetal iron demand or maternal iron insufficiency is related to both an increase in the expression of placental transferrin receptor on the syncytiotrophoblast (37) and an increase in the expression of the ferritin receptor in the placental microvilli membrane (38). Expression of the endosomal membrane iron transporter, divalent metal ion transporter (DMT-1), has also been shown to be involved in the transfer of iron from the syncytiotrophoblastic endosome into the cytoplasm (22). Moreover, placental iron regulatory protein 1 activity has been directly related to transferrin receptor messenger RNA concentrations in human placenta, and expression of this protein has been found to be related to the iron content of the placenta (23).

No significant relation was evident between the number of days between maternal isotope dosing and parturition and the amount of tracer present in the neonate's circulation across the range of time elapsed between maternal dosing and delivery (7–69 d). This indicates that most of the dietary iron was transferred to the fetus relatively rapidly and that little iron tracer in the maternal circulation was remobilized and transferred to the fetus over the subsequent course of gestation. This finding is consistent with early radiotracer data showing the transfer of an oral ^{59}Fe radiotracer to fetal circulation within 40 min of administration in a pregnant woman in active labor (39). Another early ^{59}Fe radiotracer study found that ^{59}Fe was measurable in the fetal circulation when administered orally to pregnant women 1.5–21 d before parturition (40). Transfer of intravenous ^{59}Fe radiotracer to the fetus also appears to be very rapid; early studies detected radiolabeled iron in the fetus within 12 min of introducing the ^{59}Fe into the mother's blood (41).

Rapid transfer of the oral iron tracer to the fetus suggests that the tracer was transferred before incorporation in maternal hemoglobin. A limitation with stable-isotope studies is that the mass of iron administered can be large in relation to the size of the miscible iron pool. The amount of oral iron tracer absorbed by both groups was ≈1 mg. Additional iron absorbed from the dietary supplements would contribute ≈6 mg Fe in the iron-supplemented groups. Although these amounts of absorbed iron are consistent with requirements for absorbed iron during the third trimester of pregnancy (3.54–8.8 mg/d) (5), it is not known whether these

differences in the total mass of iron absorbed would have affected the placental handling of iron between these groups.

The relatively rapid transfer of dietary iron to the fetus indicates that the isotope method used to measure iron absorption during pregnancy underestimates true maternal iron absorption, because this technique assumes all absorbed iron is incorporated into the maternal red blood cell. Our data show that there is a transfer of dietary iron to the fetus and that the degree of this transfer is not reflected by differences in the incorporation of dietary iron into the maternal red blood cell. The average amount of ^{57}Fe tracer found in the neonate's circulation at birth was 0.09 mg, which corresponds to an average of 7.5% of the absorbed maternal oral dose. Although this amount is relatively small, it would underestimate true maternal iron absorption on average by 5%. The degree of transfer of dietary iron to the fetus that we observed in our study is consistent with early radiotracer studies undertaken by Hahn et al (42) in 68 pregnant women. In that study, neonatal blood at delivery contained an amount of radioactivity that was comparable to 7–10% of the amount of radioactive iron that the mother absorbed and incorporated into her red blood cells irrespective of the time of gestation or the dose of iron administered (42). Iron isotope methods used in iron absorption studies during pregnancy therefore measure maternal iron utilization but underestimate true iron absorption. Our data indicate that the degree of this underestimation would be larger in women with depleted iron reserves.

Maternal iron status influenced the stable iron isotope enrichments in the neonates' circulation, but we were unable to address the partitioning of isotopic tracer into fetal iron reserves or the fraction of isotope that might have been retained in the placenta. Moreover, it is unknown whether the degree of increase in transfer of dietary iron to the fetus would lead to an increase in fetal iron stores or whether this allowed the fetus to acquire iron stores similar to those accumulated by iron-replete groups. Studies by Georgieff et al (23) determined that up-regulation of the placental transferrin receptor does not result in an increase in iron transfer to the fetus sufficient to restore iron pools to normative levels, and studies in diabetic women found that up-regulated placental iron uptake mechanisms do not prevent tissue iron deficits in infants born to diabetic women (43). Despite the magnitude of the difference in iron transfer found in our study, only subtle differences in iron status were evident in the neonates, and cord hemoglobin differences between the unsupplemented and iron-supplemented groups were not significant.

In conclusion, transfer of dietary iron to the fetus is regulated in response to maternal iron status. Transfer of intravenously administered iron (^{58}Fe) from the mother to the fetus was not significantly regulated in response to maternal iron status. This finding indicates that most of the physiologic regulation of iron transfer to the fetus occurs at the level of the gut and suggests that the iron needs of the fetus take priority over maternal requirements. The signals responsible for the up-regulation of iron uptake and transfer mechanisms at the level of the placenta have not been fully characterized. More research is needed to characterize these mechanisms as well as to address the effect of these differences in iron transfer on the ability of the neonate to maintain iron status during the first 6 mo of life.

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KOO was responsible for study design and article preparation. NZ assisted in the design of the study and implemented the clinical studies in Peru. LEC was responsible for the design of the larger supplementation study from which the supplemented women were recruited and assisted in the study implementation and statistical analyses. SAA was responsible for the isotopic analyses and assisted in the study design. No authors had conflicts of interest with any company or organization sponsoring this research.

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