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Placental Iron Content Is Lower than Previously Estimated and Is Associated with Maternal Iron Status in Women at Greater Risk of Gestational Iron Deficiency and Anemia

Alexa Barad,[1] Ronnie Guillet,[2] Eva K Pressman,[3] Philip J Katzman,[4] Richard K Miller,[3,4] Thomas H Darrah,[5,6] and Kimberly O O'Brien[1]

[1]Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA; [2]Department of Pediatrics, Division of Neonatology, University of Rochester School of Medicine, Rochester, NY, USA; [3]Department of Obstetrics and Gynecology, University of Rochester School of Medicine, Rochester, NY, USA; [4]Department of Pathology and Clinical Laboratory Medicine, University of Rochester School of Medicine, Rochester, NY, USA; [5]School of Earth Science, The Ohio State University, Columbus, OH, USA; and [6]Global Water Institute, The Ohio State University, Columbus, OH, USA

ABSTRACT

Background: Based on limited data, it is estimated that the placenta retains 90 mg of iron. Little is known about determinants of placental iron content. Animal data indicate that the placenta prioritizes iron for its own needs, but this hypothesis has not been evaluated in humans.

Objectives: To characterize placental iron content and placental iron concentration (p[Fe]) in pregnant women at risk of iron insufficiency and identify determinants of p[Fe].

Methods: Placentas were collected from 132 neonates born to teens carrying singletons (≤18 y) and 101 neonates born to 48 women carrying multiples (20–46 y). Maternal and neonatal iron status indicators [hemoglobin, serum ferritin (SF), soluble transferrin receptor (sTfR), serum iron, total body iron (TBI)] and hormones (erythropoietin, hepcidin) were measured. p[Fe] was measured using inductively coupled plasma–mass spectrometry. Correlation analyses and mixed-effects models were constructed to identify determinants of p[Fe].

Results: Mean placental iron content was 23 mg per placenta (95% CI: 15, 33 mg) in the multiples and 40 mg (95% CI: 31, 51 mg) in the teens ($P = 0.03$). Mean p[Fe] did not differ between the cohorts. p[Fe] was higher in anemic (175 μg/g; 95% CI: 120, 254 μg/g) compared with nonanemic (46 μg/g; 95% CI: 26, 82 μg/g) women carrying multiples ($P = 0.009$), but did not differ between anemic (62 μg/g; 95% CI: 40, 102 μg/g) and nonanemic (73 μg/g; 95% CI: 56, 97 μg/g) teens. In women carrying multiples, low maternal iron status [lower SF ($P = 0.002$) and lower TBI ($P = 0.01$)] was associated with higher p[Fe], whereas in teens, improved iron status [lower sTfR ($P = 0.03$) and higher TBI ($P = 0.03$)] was associated with higher p[Fe].

Conclusions: Placental iron content was ∼50% lower than previously estimated. p[Fe] is significantly associated with maternal iron status. In women carrying multiples, poor maternal iron status was associated with higher p[Fe], whereas in teens, improved iron status was associated with higher p[Fe]. More data are needed to understand determinants of p[Fe] and the variable iron partitioning in teens compared with mature women. *J Nutr* 2022;152:737–746.

Keywords: placenta, iron, anemia, iron deficiency, placental content, high-risk pregnancy

Introduction

The placenta is an ephemeral organ that transports ∼270 mg iron to the developing fetus (1). Placental transfer of iron varies across gestation in relation to fetal acquisition rates, typically peaking in the third trimester (1), and this transfer is unidirectional (2, 3). The relative use of iron by the placenta and/or fetus can vary across pregnancy as the placental to body weight ratio (PW:BW) decreases considerably, with the fetus weighing 2–3 times more than the placenta at 24 wk of gestation and 5–7 times more at birth (4). At term, ∼90 mg iron is retained by the placenta, but this value is based on limited data (5).

Unlike the enterocyte, which can store excess iron until cells are sloughed off into the gut lumen, the placenta must either restrict iron uptake or store this mineral throughout pregnancy if not transferred to the fetus. The placenta is exposed to both maternal and fetal signals, and fetal iron acquisition is dependent on maternal iron stores and the

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capacity of the placenta to transport iron (6). Under normal circumstances, maternal diferric-transferrin is taken up by placental transferrin receptor 1 (TfR1) on the apical membrane of the syncytiotrophoblast (STB). Iron is released from the basolateral side of the STB via ferroportin (FPN) and then crosses the fetal vascular endothelium into fetal circulation (6). Maternal iron deficiency has been associated with increased expression of placental TfR1 in humans (7, 8) and animals (9), and decreased expression of FPN in animals (10), suggesting that the placenta responds to changes in maternal iron status to protect its own iron reserves or to support fetal iron demands.

A greater understanding of factors that influence partitioning of iron between the mother, placenta, and fetus is emerging. Animal data indicate that the murine placenta prioritizes iron in support of its own metabolic demands even at the expense of the mother and fetus (10). Human data are challenging to obtain because maternal iron intake cannot ethically be manipulated outside recommended ranges. Women who habitually consume a low iron diet are also likely ingesting insufficient amounts of other nutrients needed to support placental function, making the task of isolating the effects of low maternal iron difficult. A growing body of literature evaluating placental iron content and placental iron concentration (p[Fe]) provides opportunities to re-examine these assumptions. The goal of our study was to characterize total placental iron content and p[Fe] in 2 large cohorts of pregnant women who were at increased risk of iron insufficiency and to identify determinants of p[Fe]. A secondary goal was to undertake a literature search to evaluate published data on placental iron content and p[Fe].

Methods

Study participants

Placental samples were obtained from healthy pregnant adolescents (≤18 y) carrying singletons (teen cohort) ($n = 132$) and healthy pregnant women carrying multiples (multiples cohort) ($n = 48$) from Strong Memorial and Highland Hospitals of the University of Rochester, New York. Studies were approved by the Institutional Review Boards at the University of Rochester and Cornell University. Informed written consent was obtained from all participants aged >14 y, and parental consent and adolescent assent were obtained from participants aged ≤14 y.

Details on demographic data collection have been published (11, 12). Prepregnancy BMI (ppBMI, in kg/m^2) was calculated based on self-reported weight and height, and categorized as underweight (<18.5), normal weight (18.5–24.9), overweight (25.0–29.9), or obese (≥30) (13). All mothers were prescribed prenatal supplements containing 27 mg Fe (ferrous fumarate) as part of their prenatal care. Teens reporting intolerance to this supplement were prescribed 2 pediatric chewable supplements (Flintstones, Bayer Corporation, Whippany, NJ) to increase compliance. Those who were anemic received additional ferrous sulfate supplements providing 60–120 mg/d iron. Information on self-reported prenatal supplement use was collected (11, 12).

Serum collection and biochemical analyses

Sample collection flowcharts are presented in **Supplemental Figure 1**. Maternal and neonatal iron status [hemoglobin, serum ferritin (SF), serum soluble transferrin receptor (sTfR), and serum iron], hormones [hepcidin and erythropoietin (EPO)], and inflammatory markers [C-reactive protein (CRP) and IL-6] were evaluated as described (11, 12). Briefly, hemoglobin was analyzed with a Cell-Dyn 4000 hematology analyzer (Abbott Laboratories). SF and serum sTfR were measured using ELISA (Ramco Laboratories). Serum iron was measured with a graphite furnace atomic absorption spectrophotometer (PerkinElmer AAnalyst 800). Serum EPO was analyzed by immunoassay (Siemens Immulite 2000) in both cohorts. Hepcidin, CRP, and IL-6 were measured with different assays between the cohorts. In the teen cohort, serum hepcidin was measured by ELISA (Intrinsic Life Sciences). Serum CRP was analyzed by immunoassay (Siemens Immulite 2000) and serum IL-6 by Magnetic Multiplex assay (Millipore). In the multiples cohort, serum hepcidin was measured by ELISA (Bachem) and serum CRP and IL-6 were also measured by ELISA (R&D Systems). Data on maternal (11, 12) and neonatal (14–17) iron status in these cohorts have been published.

Maternal anemia was defined as hemoglobin <11.0 g/dL in both the first and third trimesters, and <10.5 g/dL in the second trimester (18). The hemoglobin cutoff for anemia in black mothers was adjusted downwards by 0.8 g/dL in each trimester (19), and both adjusted and nonadjusted values for anemia are reported. Severity of maternal anemia was further categorized as severe (hemoglobin <7.0 g/dL), moderate (hemoglobin 7.0–9.9 g/dL), or mild (hemoglobin 10.0–10.9 g/dL) (20), and these were adjusted for trimester. Maternal iron deficiency was defined as SF <12.0 μg/L (21), sTfR >8.5 (22), or total body iron (TBI) <0 mg/kg (23). Neonatal anemia was defined as umbilical cord hemoglobin <13.0 g/dL (24).

Placental collection and total iron analysis

All placentas were collected after delivery. Weights of placentas were recorded before removing the membranes and superficial tissue on the maternal surface. Weights of fused placentas were calculated by dividing total placental mass by the number of placentas. Placental area was calculated with the equation:

$$\text{Area}(\text{cm}^2) = \text{height}(\text{cm}) \times \text{width}(\text{cm}). \quad (1)$$

In the multiples cohort, placental area was only calculated for discrete placentas. Aliquots of tissue were collected from the interior of the placental parenchyma in different quadrants of the placenta. Tissue was rinsed to remove adherent blood, minced, and frozen at −80°C. Samples were shipped to Ohio State University for quantification of iron using inductively coupled plasma–mass spectrometry as described (25). Reports on placental expression of iron trafficking proteins in the adolescent cohort including TfR1, FPN, feline leukemia virus subgroup C receptor 1, proton-coupled folate transporter, low density lipoprotein receptor–related protein 1, breast cancer resistance protein, and heme-responsive gene 1 have been published (7, 8, 26, 27).

Statistical analyses

Descriptive placental, maternal, and neonatal statistics are presented as means ± SE for continuous variables, and percentages for categorical variables. Nonnormally distributed variables were logarithm transformed and back transformed to geometric means and 95% CIs. For continuous variables, Student t tests and ANOVA were used to assess differences between the groups, and Tukey honest significance difference was used as a follow-up to ANOVA. For categorical variables, χ^2 tests or logistic regression analyses were used to assess differences between the groups. To account for nonindependent observations in the multiples cohort, a mom identification number was added to the

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Address correspondence to KOO (e-mail: koo4@cornell.edu).
Abbreviations used: BW, birth weight; CRP, C-reactive protein; EPO, erythropoietin; FPN, ferroportin; GA, gestational age; p[Fe], placental iron concentration; ppBMI, prepregnancy body mass index; PW:BW, placental weight to birth weight ratio; SF, serum ferritin; STB, syncytiotrophoblast; sTfR, soluble transferrin receptor; TBI, total body iron; TfR1, transferrin receptor 1.

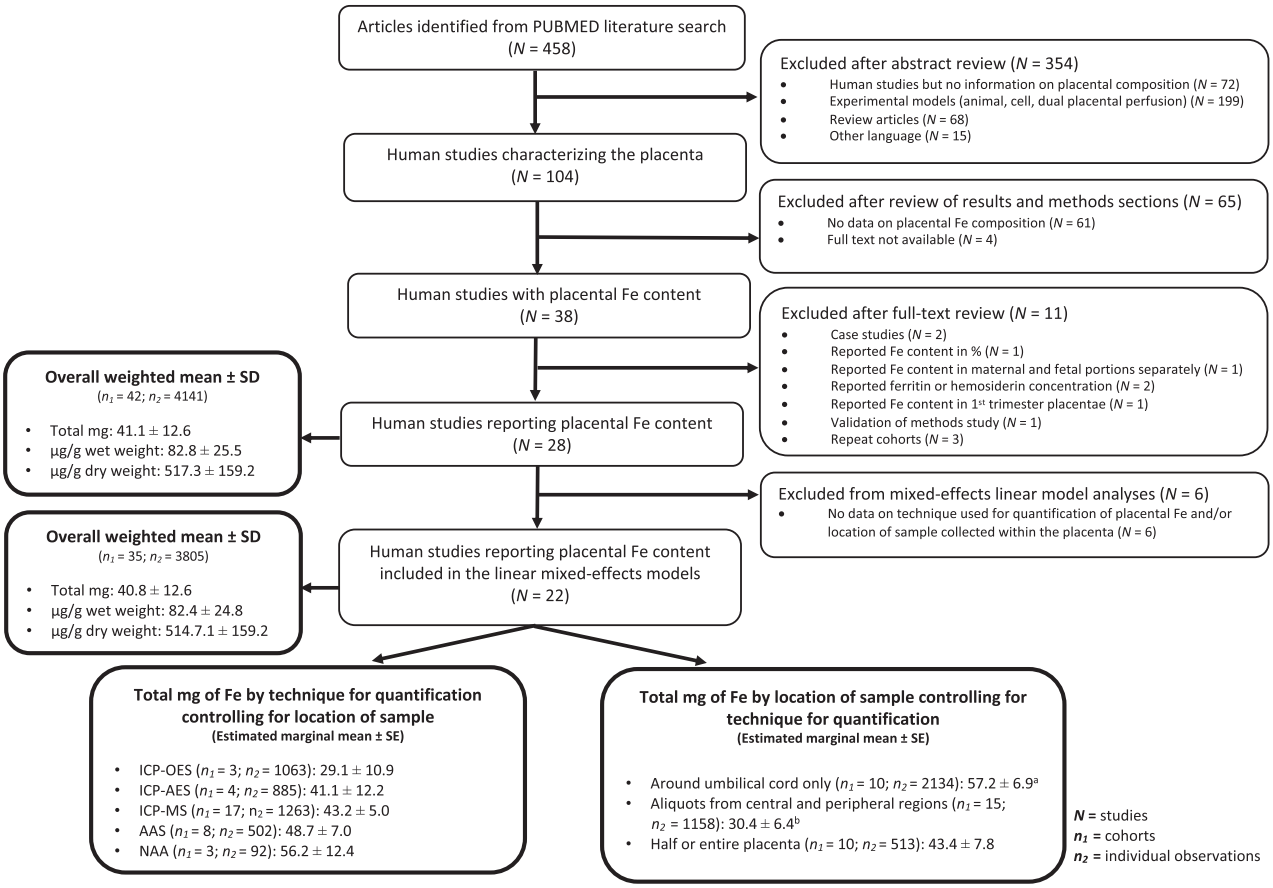

FIGURE 1 Literature search of the iron content of the human placenta. Flowchart of selection process from all identified articles from a PubMed search and main results of review. Estimated marginal means (or least square means) obtained from linear mixed-effects models. Different letters indicate significant differences between groups, $P < 0.05$. AAS, atomic absorption spectrometry; ICP-AES, inductively coupled plasma–atomic emission spectrometry; ICP-MS, inductively coupled plasma–mass spectrometry; ICP-OES, inductively coupled plasma–optical emission spectrometry; NAA, neutron activation analysis.

models as a random-effect. Paired *t* tests were used to assess longitudinal differences in variables from mothers that provided samples at mid-gestation and delivery. Pearson correlation coefficients were used to assess correlations between continuous variables. In the multiples cohort, the average p[Fe] of placentas obtained from the same mother was used in correlation analyses. Unadjusted and adjusted mixed-effects interaction models were constructed to identify determinants of p[Fe], and mixed-effects additive models for analyses performed separately by cohort. Models including maternal anemia as the independent variable were analyzed using ethnicity-adjusted and nonadjusted data. Because the significant variables identified from these models were not different, the nonadjusted values were reported. Results of statistical tests were considered significant at $P < 0.05$. Statistical analyses were conducted using JMP Pro 15.0 (SAS Institute) or R 4.0.4 (The R Foundation for Statistical Computing).

Literature review on the iron content of the human placenta

A PubMed search for articles from any geographical region written in English was conducted using the search phrase: ["placenta"(MeSH Terms) OR "placenta"(All Fields) OR "placental"(All Fields) OR "placentae"(All Fields)] AND ["iron"(MeSH Terms) OR "iron"(All Fields) OR "Fe"(All Fields) OR "Fe2+"(All Fields) OR "Fe3+"(All Fields) OR "minerals"(All Fields)] AND ["content"(All Fields) OR "composition"(All Fields) OR "concentration"(All Fields) OR "concentrations"(All Fields)]. Figure 1 outlines the article selection process and main results of the review. To compare p[Fe] between publications, all values were converted to the same units (micrograms per gram) and dry values were converted to wet values using 2 equations:

$$\mu g\,Fe\,per\,g\,wet\,weight = \mu g\,Fe\,per\,g\,dry\,weight\,(100 - \%water)/100 \quad (2)$$

assuming 84% water in an average human placenta (28); and

$$\mu g\,Fe\,per\,g\,wet\,weight = \mu g\,Fe\,per\,g\,dry\,weight/6 \quad (3)$$

(29). Both formulas gave similar values (±1–5%) therefore, values obtained with Equation 2 were used. Total milligrams of iron was estimated assuming an average placental weight of 496 g using the formula:

$$total\,mg = (\mu g\,Fe\,per\,g\,wet\,weight \times 496\,g)/1000 \quad (4)$$

This estimated placental weight was obtained by averaging placental weights reported in the studies ($n = 13$). Overall weighted means and SDs were calculated using the package Hmisc in R (30). To explore possible effect of tissue location within the placenta or analytical technique on placental iron content, a linear mixed-effects model was constructed. Estimated marginal means of placental iron content by location of sample and analytical technique were calculated using the package emmeans in R (31). The models included location of sample and analytical technique as fixed effects. Groups within each study that had different placental iron content values were included in the analyses as individual observations, and the study from which the data were collected was added as random effect. The sample size of each group was added as weight. Articles included in the analyses are presented in **Supplemental Table 1.**

TABLE 1 Placental, maternal, and neonatal characteristics and placental iron composition in women carrying multiple fetuses and their neonates and pregnant teens and their neonates[1]

	Multiples cohort			Teen cohort
	All ($n = 101$)	Twins ($n = 68$)	Triplets ($n = 33$)	Singletons ($n = 132$)
Placental characteristics				
Fused placentas, %	83 (84)	84 (57)	81 (27)	—
Placental weight				
Discrete, g	296 ± 59.2 (17)*	298 ± 72.0 (11)[a]	293 ± 29.3 (6)[a]	619 ± 149.4 (121)[b]
Fused, g	628 ± 178 (42)	616 ± 156 (30)	657 ± 230 (12)	—
Weight/fetus,[2] g	294 ± 75.4 (101)*	309 ± 81.1 (68)[a]	268 ± 50.9 (33)[a]	619 ± 149 (121)[b]
Total weight/mom, g	659 ± 169 (47)	609 ± 147 (35)[b]	804 ± 145 (12)[a]	619 ± 149 (121)[b]
PW:BW	0.13 ± 0.03 (101)*	0.13 ± 0.03 (68)[a]	0.13 ± 0.03 (33)[a]	0.19 ± 0.04 (120)[b]
Placental area,[3] cm2	137 ± 33.0 (17)	139 ± 38.2 (11)	135 ± 23.6 (6)	134 ± 28.6 (114)
Placental iron composition				
μg/g wet weight	82.6 [56.5, 121] (101)	84.6 [52.1, 137] (68)	78.6 [41.8, 148] (33)	71.1 [56.6, 89.4] (132)
Total mg[4]	23.4 [15.9, 34.4]* (101)	25.1 [15.3, 41.3] (68)	20.3 [11.0, 37.5] (33)	40.3 [31.5, 51.5] (123)
Maternal characteristics	($n = 48$)	($n = 36$)	($n = 12$)	($n = 132$)
GA at delivery, wk	35.3 ± 2.3 (48)*	35.7 ± 2.4 (36)[a]	34.3 ± 2.3 (12)[b]	39.8 ± 1.2 (132)[c]
Age at delivery, y	30.6 ± 5.5 (48)*	29.6 ± 5.7 (36)[b]	33.7 ± 3.4 (12)[a]	17.4 ± 1.1 (130)[c]
Black mothers, %	27 (13)*	33 (12)[a]	8 (1)[a]	67 (88)[b]
Delivery hemoglobin, g/dL	11.1 ± 1.7 (48)	11.0 ± 1.7 (36)[a]	11.6 ± 1.7 (12)[a,b]	11.6 ± 1.3 (126)[b]
Delivery anemia, % (ethnicity-adjusted)[5]	38 (18)*	36 (13)[a]	42 (5)[a]	17 (21)[b]
Delivery anemia, % (not ethnicity-adjusted)[5]	44 (21)	44 (16)	42 (5)	30 (38)
Delivery SF <12 μg/L, %	21 (8/38)	27 (8/30)	0 (0/8)	24 (30/127)
Parity ≥1, %	56 (27)*	61 (22)[a]	42 (5)[a,b]	23 (31)[b]
Supplement use[6]				
Every day, %	87 (39/45)*	85 (29/34)[a]	90 (10/11)[a]	61 (80/131)[b]
≤2–5 times/wk, %	13 (6/45)	15 (5/34)	10 (1/11)	39 (51/131)
Neonatal characteristics	($n = 101$)	($n = 68$)	($n = 33$)	($n = 132$)
Sex (female), %	54 (55)	60 (41)	42 (14)	52 (67/130)
Birth weight, g	2269 ± 542 (101)*	2364 ± 550 (68)[a]	2073 ± 477 (33)[a]	3257 ± 448 (131)[b]
Birth length, cm	45.6 ± 3.6 (90)*	46.6 ± 3.2 (60)[a]	43.6 ± 3.5 (30)[b]	51.2 ± 2.6 (125)[c]
Hemoglobin, g/dL	15.1 ± 2.7 (87)*	15.0 ± 2.6 (57)	15.3 ± 3.0 (30)	13.9 ± 2.8 (82)
Anemia, %	14 (12/87)*	12 (7/57)	17 (5/30)	26 (21/82)

[1] Values are reported as mean ± SE, geometric mean [95% CI] for nonnormal data, or % (n). ANOVA tests and Tukey honest significance difference were used to test for differences in continuous variables between groups. χ^2 analyses or logistic regression analyses for binary outcomes were used to test for differences in categorical variables between groups. To account for nonindependence of siblings or placentas in the multiples cohort, a mom identification variable was included in the models as a random effect. Labeled values in a row without a common letter differ, $P < 0.05$.*Different from the teen cohort, $P < 0.05$. GA, gestational age; PW:BW, placental weight to birth weight ratio; SF, serum ferritin.

[2] Average weights of fused placentas were obtained by dividing total placental mass by the number of placentas.

[3] Placental area was calculated for discrete placentas only in the multiples cohort.

[4] Total milligrams of iron was calculated using the following equation: Total mg = μg/g wet weight(Placental weight per fetus in g)/1000.

[5] Maternal anemia was defined as hemoglobin concentrations <11.0 g/dL in the first and third trimesters, and <10.5 g/dL in the second trimester. For the ethnicity-adjusted data, the cutoff in black mothers was adjusted downwards by 0.8 g/dL in each trimester.

[6] Supplement use refers to prenatal supplements containing 27 mg Fe/d or 2 chewable supplements containing an equivalent amount of iron per day.

Results

Placental characteristics and iron content

Placental characteristics and placental iron content (total milligrams) and p[Fe] (micrograms per gram wet weight) are shown in **Table 1**. Most twin (84%) and triplet (81%) placentas were fused. Average placental weight per fetus was lower in the triplets and twins compared with the singletons, even after controlling for delivery gestational age (GA). Mean p[Fe] did not differ between cohorts. Mean total milligrams of iron per placenta was significantly lower in the multiples compared with the teens ($P = 0.03$), but this difference disappeared after controlling for delivery GA ($P = 0.1$). We did not have the statistical power to detect a pairwise difference in total milligrams of iron when the multiples were divided into triplets and twins (Table 1).

Maternal and neonatal characteristics

Women carrying multiples were on average 13.2 y older and gave birth 4.4 wk earlier than the teens (Table 1). Neonatal birth weight was significantly higher in the singletons compared with the twins and triplets, but this difference disappeared after controlling for GA. Neonatal length at birth was significantly different between triplets, twins, and singletons, and this difference also disappeared after controlling for GA (Table 1). Additional data on maternal and neonatal characteristics are provided in **Supplemental Tables 2** and **3**.

A higher proportion of women carrying multiples had anemia at delivery (38%) compared with the teens (17%) ($P = 0.003$) but a lower proportion of neonates in the multiples cohort (14%) had anemia compared with neonates born to the teens (26%) ($P = 0.05$). These differences, using ethnicity-adjusted maternal data, were also evident in the larger

TABLE 2 Bivariate correlations between maternal and neonatal iron status biomarkers and regulatory hormones and placental iron concentration in women carrying multiple fetuses and their neonates and pregnant teens and their neonates[1]

	Multiples cohort			Teen cohort		
	n	*r*	*P* value	*n*	*r*	*P* value
Mid-gestation						
Hemoglobin	40	−0.073	0.66	66	0.093	0.46
SF	41	0.032	0.84	81	0.144	0.20
sTfR	41	−0.133	0.40	81	0.043	0.70
TBI[2]	41	0.100	0.54	81	0.089	0.43
Serum Fe	41	0.091	0.57	81	0.032	0.78
Hepcidin	42	−0.168	0.29	81	−0.107	0.34
EPO	40	0.147	0.36	79	−0.254	0.02
Delivery						
Hemoglobin	48	−0.239	0.10	126	0.066	0.46
SF	38	−0.490	0.002	127	0.131	0.14
sTfR	38	0.188	0.26	128	−0.189	0.03
TBI[2]	38	−0.406	0.01	127	0.195	0.03
Serum Fe	37	−0.133	0.43	124	0.137	0.13
Hepcidin	38	−0.191	0.25	126	0.222	0.01
EPO	37	0.404	0.01	122	−0.146	0.11
Neonatal						
Hemoglobin	87	−0.154	0.15	82	0.093	0.40
SF	84	−0.125	0.26	123	−0.016	0.86
sTfR	84	−0.126	0.25	124	−0.114	0.21
TBI[2]	84	−0.032	0.77	123	0.030	0.74
Serum Fe	82	−0.051	0.65	111	−0.209	0.03
Hepcidin	84	−0.048	0.66	120	0.065	0.48
EPO	82	−0.094	0.40	109	−0.051	0.59

[1] To account for nonindependent observations in the multiples cohort, average placental iron concentration of placentas from the same mother was used to calculate the correlation coefficients for maternal variables. For mothers with both fused and discrete placentas, the average iron concentration of the fused placentas was averaged with the iron concentration of the discrete placenta. Log transformed values of placental iron concentration in micrograms per gram wet weight and other nonnormal data were used. Correlations were considered significant if $P < 0.05$. EPO, erythropoietin; SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron.
[2] Total body iron was calculated with the following equation: .TBI(mg/kg) = −[log 10(sTfR/SF) − 2.8229/0.1207]

cohorts (32, 33). When cutoffs for anemia were not adjusted downwards for black mothers, the difference in rates of anemia at delivery became nonsignificant ($P = 0.09$). The prevalence of mothers with depleted iron stores (SF <12 μg/L) did not differ between cohorts and was similar to previous reports from our laboratory (11, 12). Data on maternal and neonatal iron status are presented in **Supplemental Tables 4** and **5**.

Determinants of placental iron content

Correlation analyses.

Correlations between maternal and neonatal iron status indicators and hormones and p[Fe] are shown in Table 2. For both the multiples and teen cohorts, maternal iron status variables at delivery showed stronger correlations with p[Fe] compared with the same variables at mid-gestation, but the strength and direction of correlations differed between cohorts. In women carrying multiples, low maternal iron status (lower SF and lower TBI) was associated with higher p[Fe], whereas in teens, better iron status (lower sTfR and higher TBI) was associated with higher p[Fe]. These divergent observations were supported by associations between p[Fe] and hormonal markers at delivery. In women carrying multiples, EPO was positively correlated with p[Fe], whereas in teens, hepcidin was positively correlated with p[Fe]. With respect to neonatal variables, p[Fe] was negatively associated with umbilical cord serum iron in the teen cohort only.

In the multiples cohort, 3 women with the lowest hemoglobin concentrations (<8 g/dL) had significantly lower p[Fe] compared to other mothers with hemoglobin concentrations between 8.8 and 9.9 g/dL ($n = 10$; $P = 0.01$). When these 3 outliers were excluded, the correlation between delivery hemoglobin and p[Fe] became highly significant (Figure 2). No teens had hemoglobin concentrations <8 g/dL.

Correlations between maternal and neonatal characteristics and p[Fe] are shown in **Supplemental Table 6**. p[Fe] was not correlated with maternal characteristics. Among the neonatal and placental characteristics evaluated, only placental area was significantly and positively correlated with p[Fe] in the teens.

Mixed-effects interaction and additive model analyses.

Multivariate mixed-effects models were constructed to identify determinants of p[Fe] (Table 3). Maternal iron status parameters at mid-gestation were not associated with p[Fe]. Observed associations between p[Fe] and maternal anemia at delivery, iron status parameters at delivery (SF and TBI), and frequency of prenatal supplement use differed significantly by cohort (Figure 3). These moderating effects of study cohort remained significant after controlling for characteristics that significantly differed between the 2 cohorts (PW:BW, maternal age at delivery, and ppBMI) (**Supplemental Table 7**).

To further understand the moderating effects of study population, observed associations were explored separately by cohort. p[Fe] was higher in anemic (175 μg/g; 95% CI: 120, 254 μg/g) compared with nonanemic mothers (46 μg/g; 95% CI: 26, 82 μg/g) carrying multiples ($\beta = 1.3$; $P = 0.009$), but did not differ in anemic (62 μg/g; 95% CI: 40, 102

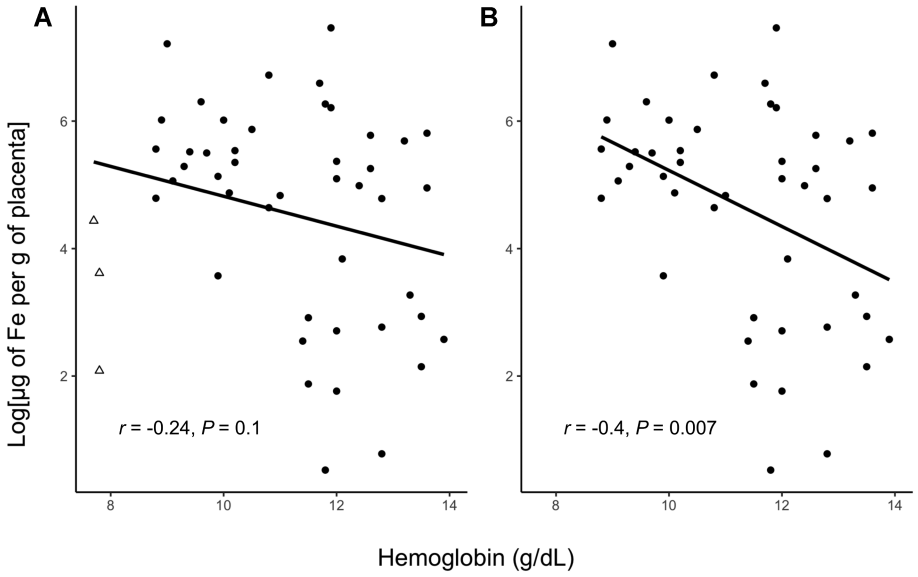

FIGURE 2 Correlation between maternal hemoglobin concentration at delivery and placental iron concentration in women carrying multiple fetuses including or excluding mothers with hemoglobin concentrations <8 g/dL at delivery. Bivariate correlation between hemoglobin concentration at delivery and log(µg of Fe per g of placenta), (A) including 3 women with hemoglobin concentrations <8.0 g/dL (represented by empty triangles) or (B) excluding 3 women with hemoglobin concentrations <8.0 g/dL.

µg/g) compared with nonanemic (73 µg/g; 95% CI: 56, 97 µg/g) teens ($\beta = -0.1$; $P = 0.6$). When these models were analyzed using the ethnicity-adjusted values for anemia (18, 19), the difference in p[Fe] between anemic and nonanemic mothers in the multiples cohort remained significant ($\beta = 1.2$; $P = 0.03$), and the difference between anemic (41 µg/g; 95% CI: 23, 74 µg/g) and nonanemic (79 µg/g; 95% CI: 61, 101 µg/g) teens became significant ($\beta = -0.6$; $P = 0.04$). In the multiples cohort, mothers with SF <12 µg/L had higher p[Fe] (288 µg/g; 95% CI: 159, 522 µg/g) compared with mothers with normal SF concentrations (73 µg/g; 95% CI: 44, 120 µg/g) ($\beta = 1.5$; $P = 0.04$). Consistent with the correlation analyses, p[Fe] did not change as a function of SF status in the teens. Despite the significant moderating effect of study population on the relation between TBI and p[Fe], there were no significant differences in p[Fe] in mothers with delivery TBI <0 mg/kg compared with mothers with TBI >0 mg/kg in either cohort. However, the correlation analyses showed significant, yet opposing, correlations between TBI at delivery and p[Fe] in both cohorts. This suggests that the lack of significance when TBI was analyzed as a binary variable could be attributed to lack of power when groups were subdivided by TBI status.

Differences in prenatal supplement use were also evident. Women carrying multiples who reported taking prenatal supplements daily had significantly lower p[Fe] than those who took prenatal supplements less frequently (≤2–5 times/wk; $\beta = -2.1$; $P = 0.008$). In contrast, teens who reported taking prenatal supplements daily had significantly higher p[Fe] than those who took prenatal supplements less frequently (≤2–5 times/wk; $\beta = 0.9$; $P = 0.0002$). Women who took prenatal supplements less frequently likely had suboptimal concentrations of other relevant nutrients, potentially driving the observed differences in p[Fe] between groups. In the multiples cohort, women who took prenatal supplements daily had significantly higher delivery hemoglobin concentrations than those who took prenatal supplements less frequently ($P < 0.0001$). However, the difference in p[Fe] remained significant after adjustment for hemoglobin concentrations ($\beta = -1.8$; $P = 0.02$). In the teen cohort, women who took prenatal supplements daily had significantly higher delivery SF concentrations than those who took prenatal supplements less frequently ($P = 0.002$). Similarly, the difference in p[Fe] remained significant after adjustment for SF concentrations ($\beta = 0.8$; $P = 0.0009$).

Among all variables evaluated, only the association between p[Fe] and parity was consistent between the 2 cohorts, with p[Fe] being significantly higher in multiparas (≥1 birth) compared with primiparas in both the multiples and teen cohorts ($\beta = 1.0$, $P = 0.03$ and $\beta = 0.6$, $P = 0.02$, respectively). In the multiples cohort, multiparas had lower hemoglobin concentrations at delivery compared with primiparas ($P = 0.02$). In the teens, the difference in hemoglobin between multiparas and primiparas was nonsignificant ($P = 0.09$). Due to the known effect of increasing parity on hemoglobin concentrations (34–36), models evaluating parity were adjusted for hemoglobin concentration. In the multiples cohort, the significant difference in p[Fe] between multiparas and primiparas disappeared after adjustment for delivery hemoglobin concentrations ($\beta = 0.9$; $P = 0.07$), whereas in the teens, the difference became more pronounced ($\beta = 0.9$; $P = 0.002$).

Literature search on the iron content of the human placenta.

Twenty-eight papers published between 1961 and 2020 met the inclusion criteria for the review (Supplemental Table 1). Mean p[Fe] was 83 ± 26 µg Fe/g wet tissue, and total milligrams iron was 41 ± 13 for a 496-g placenta when assuming a placental water content of 84% (28). Total milligrams iron did not differ as a function of analytical technique; but varied by sampling location even after controlling for analytical technique. Total milligrams iron was significantly higher in samples collected around the umbilical cord insertion compared with those collected from both central and peripheral regions or peripheral regions only ($\beta = 26.8$; $P = 0.009$) (Figure 1).

TABLE 3 Unadjusted 2-factor mixed-effects interaction models of maternal and neonatal determinants of placental iron concentration in women carrying multiple fetuses and their neonates and pregnant teens and their neonates[1]

	n	$\beta_{interaction}$	*P* value
Maternal characteristics			
Ethnicity[2]	233	−0.20	0.14
Parity	229	0.11	0.35
Cigarette use	233	−0.09	0.53
Supplement use	227	0.73	<0.0001
Mid-gestation			
Anemia[3]	151	−0.20	0.19
Mild anemia[4]	135	−0.22	0.22
Moderate anemia[4]	121	−0.25	0.37
SF	168	−0.09	0.54
sTfR	168	−0.05	0.81
TBI[5]	168	−0.09	0.63
Delivery			
Anemia[3]	227	−0.37	0.003
Mild anemia[4]	188	−0.40	0.02
Moderate anemia[4]	184	−0.47	0.008
SF	207	−0.38	0.02
sTfR	207	−0.29	0.07
TBI[5]	206	−0.36	0.03
Neonates			
Anemia[6]	169	−0.31	0.04

[1] Nonindependence of observations in the multiples cohorts is accounted for by adding a mom identification variable as a random effect. Log transformed values of iron content in placental tissue in micrograms per gram wet weight used. Significant interaction terms defined as $P < 0.05$. SF, serum ferritin, sTfR, soluble transferrin receptor; TBI, total body iron.
[2] Ethnicity was self-reported by each woman as black or nonblack.
[3] Maternal anemia was defined as hemoglobin concentrations <11.0 g/dL in the first and third trimesters and <10.5 g/dL in the second trimester.
[4] Mild anemia was defined as hemoglobin concentrations between 10.0 and 10.9 g/dL and moderate anemia was defined as hemoglobin concentrations between 7.0 and 9.9 g/dL.
[5] TBI was calculated with the following equation:
TBI(mg/kg) = −[log 10(sTfR/SF) − 2.8229/0.1207].
[6] Neonatal anemia was defined as umbilical cord hemoglobin <13.0 g/dL.

Discussion

In these women at increased risk of iron insufficiency, placentas contained an average of 83 μg Fe/g wet tissue and p[Fe] did not significantly differ between the 2 cohorts. Total milligrams iron per placenta was significantly lower in the multiples compared with the teens given the significantly lower placental weight per fetus between the 2 cohorts (23 mg compared with 40 mg Fe per placenta). Mean total p[Fe] in these 2 cohorts was considerably lower than the value used by the National Academy of Medicine when setting maternal iron requirements during pregnancy (90 mg) (37), but was consistent with more recent values reported in healthy and high-risk pregnancies using a range of analytical methods. Additionally, there were marked differences between study cohorts with respect to determinants of p[Fe]. In the multiples cohort, p[Fe] was higher in anemic compared with nonanemic mothers, whereas in the teens, p[Fe] was not significantly different in anemic compared with nonanemic mothers when using non–ethnicity-adjusted cutoffs for anemia. These contrasting observations were supported by differing associations between p[Fe] and maternal iron status indicators and regulatory hormones between cohorts. In both cohorts, maternal iron status and hormonal markers at delivery were stronger determinants of p[Fe] compared with the same variables at mid-gestation.

In women carrying multiples, mean p[Fe] was higher in anemic compared with nonanemic women. Poor maternal iron status (lower SF and lower TBI) was associated with higher p[Fe], and p[Fe] was higher in women with SF <12 μg/L compared with women with SF ≥12 μg/L. In humans and animals, expression of placental TfR1 is increased in response to maternal iron deficiency (9, 10), which might explain the higher concentrations of placental iron observed in anemic and iron-deficient mothers carrying multiples. Altogether, these observations support the concept of the "selfish placenta," whereby the placenta continues to sequester iron to support its own demands even at the expense of the mother. Interestingly, although p[Fe] was higher in anemic women with hemoglobin concentrations between 8.8 and 11 g/dL, 3 mothers with hemoglobin concentrations <8 g/dL had lower p[Fe]. This could suggest that at a hemoglobin concentration <8.8 g/dL, the ability of the placenta to maintain its own iron reserve is compromised. Consistent with this idea, it has been shown that in women with hemoglobin concentrations <9 g/dL, TfR1 expression is not different from that of nonanemic women but is significantly higher in anemic women with hemoglobin concentrations of 9–11 g/dL (38). Finally, in contrast to the significant differences in p[Fe] between mothers with low and normal SF, a recent study in healthy women carrying singletons found no difference in p[Fe] between mothers with SF <10 μg/L ($n = 18$) and mothers with SF ≥10 μg/L ($n = 25$) (10). These divergent results could be attributed to inherent differences between singleton and multiple pregnancies, smaller sample size of the study population, or methodological differences. Nonetheless, p[Fe] was maintained in adult women with depleted iron stores, also supporting the concept of the selfish placenta.

Different associations between p[Fe] and maternal iron status were observed in the teen cohort. Mean total p[Fe] did not differ between anemic and nonanemic teens, but improved iron status (lower sTfR and higher TBI) was associated with higher p[Fe]. These observations are perhaps explained by competition for nutrients in a pregnancy that occurs while adolescent growth is still occurring (39). Our group previously reported that, in pregnant adolescents, maternal iron status was inversely associated with placental TfR1 protein abundance (7, 8), consistent with reports in adults (38). However, the fact that poor maternal iron status was associated with lower p[Fe] and that there was a higher proportion of newborns with anemia in the teen cohort indicates that placental adaptation in teens might not be sufficient to fully supply the neonate with iron. Consistent with this idea, we have previously shown that expression of the heme transporter feline leukemia virus subgroup C receptor–related protein is positively correlated with p[Fe] and is lower in anemic mothers (27). Therefore, teens may utilize additional adaptive mechanisms to prioritize iron for maternal use, which might not be favorable to the placenta or fetus.

Conflicting associations between p[Fe] and frequency of prenatal supplement use were observed. In the multiples cohort, women who took prenatal supplements daily had lower p[Fe] than those who took prenatal supplements less frequently, whereas in the teens, p[Fe] was higher in mothers who took prenatal supplements daily compared with those who took prenatal supplements less frequently. In both cohorts, significant differences in p[Fe] between these groups remained after controlling for differences in hemoglobin concentration or iron

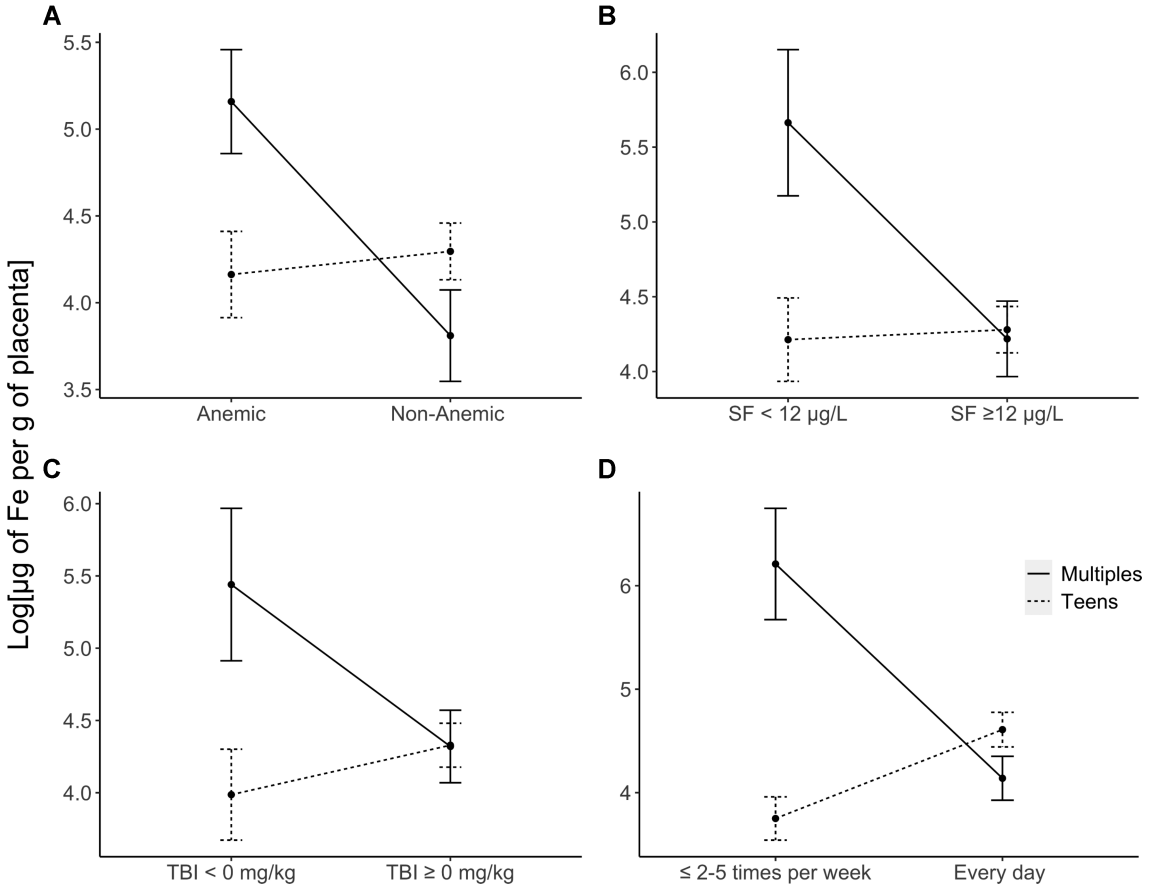

FIGURE 3 Least square means plots of placental iron concentration for maternal variables with a significant moderating effect of study population in women carrying multiple fetuses and pregnant teens. Presented as least square means of log transformed placental iron concentration ± SE for (A) maternal anemia at delivery, (B) maternal serum ferritin at delivery, (C) maternal total body iron at delivery, and (D) prenatal supplement use frequency. All interaction terms were significant, $P < 0.05$. Cutoffs for anemia were not adjusted for ethnicity. SF, serum ferritin; TBI, total body iron.

status, likely indicating that the supplements are providing additional nutrients that influence p[Fe]. Few studies have investigated prenatal supplement use and p[Fe]. A recent study in 62 women carrying singletons (aged 31–40 y) found p[Fe] to be lower in women who took iron supplements compared with those who did not (40). In contrast, a study conducted in 61 pregnant women with and without gestational diabetes (mean age 34 y), found no relation between supplement use and p[Fe]. However, no information was provided on the type of dietary supplement (41).

The relation between parity and p[Fe] was the only observation that was consistent in both cohorts. Overall, multiparas had higher p[Fe] compared with primiparas. Increasing parity has been associated with lower maternal hemoglobin and iron status (34–36). The relation between parity and p[Fe] in the multiples cohort was partially mediated by maternal hemoglobin concentrations and was consistent with the observation that anemic women had higher p[Fe] than nonanemic women. The relation between parity and p[Fe] in the teens was unexpected because p[Fe] was lower in anemic compared with nonanemic teens. However, unlike in the multiples cohort, the relation between parity and p[Fe] in the teens was not mediated by hemoglobin. Primiparous teens have the highest odds of adverse birth outcomes (42), suggesting that parity in adolescents is associated with p[Fe] independently of hemoglobin concentrations (35), and that increasing parity can provide a protective effect in younger women possibly mediated by higher placental efficiency (43).

To the best of our knowledge, this is the first study to explore maternal and neonatal iron status and hormonal determinants of p[Fe] in higher-risk obstetric populations. Moreover, our literature review of p[Fe] is the first comprehensive review on this topic in humans in >2 decades (29). Pregnant teens and women carrying multiple fetuses are at increased risk of iron insufficiency (34), and therefore are good candidates to explore possible variations in p[Fe] in relation to maternal and neonatal iron insufficiency. However, use of only higher-risk obstetric populations did not allow for comparison with normative values. All samples were run in the same laboratory using the same procedures, thus minimizing this potential source of variation between cohorts. Although we had relatively large cohorts, our sample sizes were reduced when analyses were stratified based on maternal or neonatal characteristics, possibly lowering our power to detect significant associations. Moreover, the data on supplement use were self-reported and we were not able to control for the additional iron supplementation. Finally, the ethnic composition differed between cohorts, with more minorities among the teens, limiting the generalizability of these findings. The clinical implications and rationale for using ethnicity-adjusted anemia cutoffs for black mothers should be further evaluated because differences in the results from the teen cohort were observed when using adjusted and nonadjusted values.

To date, little is known about determinants of p[Fe]. Our findings support the idea that the human placenta responds to changes in maternal iron status by adjusting how much iron it retains or transfers to the fetus. We also show that specific determinants of p[Fe] and placental adaptations in response to these factors differ between study populations, thus highlighting the importance of studying placental homeostatic mechanisms as a function of the study population. Based on our current values and the literature review of published values, we propose that iron retention in the human placenta is actually 50% lower than the estimated value of 90 mg. Finally, our results highlight the challenges inherent in the interpretation of studies evaluating iron partitioning between the mother, placenta, and fetus, and the need to further understand placental adaptations to the highly dynamic gestational environment.

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Data Availability

Data described in the article, code book, and analytic code will not be made available because of the composition of the patient population and the confidential nature of the data collected.

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