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Prevalence of anemia and associations between neonatal iron status, hepcidin, and maternal iron status among neonates born to pregnant adolescents

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BACKGROUND: Little is known about anemia and iron status in US newborns because screening for anemia is typically not undertaken until 1 y of age. This study was undertaken to characterize and identify determinants of iron status in newborns born to pregnant adolescents.

METHODS: Pregnant adolescents (≤ 18 y, n = 193) were followed from ≥ 12 wk gestation until delivery. Hemoglobin, ferritin, soluble transferrin receptor, serum iron, hepcidin, erythropoietin (EPO), IL-6, and C-reactive protein were assessed in maternal and cord blood.

RESULTS: At birth, 21% of the neonates were anemic (Hb < 13.0 g/dl) and 25% had low iron stores (ferritin < 76 μg/l). Cord serum ferritin concentrations were not significantly associated with gestational age (GA) at birth across the range of 37–42 wk. Neonates born to mothers with ferritin < 12 μg/l had significantly lower ferritin (P = 0.003) compared to their counterparts. Hepcidin and IL-6 were significantly (P < 0.05) higher in neonates born to mothers with longer durations of active labor.

CONCLUSION: Given the importance of the iron stores at birth on maintenance of iron homeostasis over early infancy, additional screening of iron status at birth is warranted among those born to this high risk obstetric population.

The iron endowment at birth helps support optimal growth and development throughout early infancy. Little attention has been focused on the assessment of iron status at birth because of the belief that sufficient iron will be accrued across gestation even in the face of mild-to-moderate maternal anemia (1). However, an increasing body of evidence indicates that iron stores may be compromised in newborns born to women with low iron stores (2). This is of concern given the growing literature on developmental programming that highlights the importance of iron acquisition *in utero* on long-term functional consequences (3), and animal data indicate that there are critical windows for the role of iron on fetal brain development (4). In spite of growing attention to the importance of optimal fetal iron accretion, US infants are not typically screened for anemia until 1 y of age, and normative data on comprehensive indicators of neonatal iron status at birth are limited.

The majority of fetal body iron is obtained during the last trimester of pregnancy (5); therefore, neonates born preterm or early-term may be at risk of insufficient iron endowment. The definition of "term", has recently been refined to classify neonates as early (37–39 wk gestation), full (39–41 wk gestation), late (41–42 wk gestation), or post-term (≥ 42 wk gestation) (6). Early-term neonates are at higher risk of nonoptimal developmental outcomes compared to infants born at 39 wk gestational age (GA) or later (7). At this time, little is known about iron status in healthy, term neonates born to pregnant adolescents and on relative changes in iron stores that can be accrued over the last 3–5 wk of gestation.

The fetus may be able to regulate placental iron trafficking via production of fetal hepcidin that is produced by the fetal liver early in gestation (8). Hepcidin is the master regulator of iron homeostasis, and the production of this hormone is known to be regulated by iron stores, inflammation, hypoxia, and erythropoietic activity (9). Despite its crucial role in regulation of iron homeostasis, currently, little is known about fetal hepcidin production and its regulatory function, and few normative hepcidin data are available in maternal–neonatal pairs at birth (10–12).

This study was undertaken to characterize iron status at birth in a group of healthy term neonates born to pregnant adolescents, a group at increased risk of maternal anemia and iron deficiency, and to explore the associations between neonatal iron status and hepcidin in relation to maternal iron status in a higher risk obstetric population.

RESULTS

The characteristics of the 193 term newborns are presented in **Table 1**. Although pregnant adolescents typically have higher risks of poor pregnancy outcomes when compared to adults (13), birth rates of early-term (21%), full-term (59%), and late/

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Table 1. Characteristics of the term newborns at birth ($n = 193$)

	Subjects with available data (n)	Mean ± SD or % (n)
Gestational age (weeks)	193	39.9 ± 1.2
Early term, % (n)[a]		21 (41)
Full term, % (n)[a]		59 (113)
Late term, % (n)[a]		19 (37)
Post-term, % (n)[a]		1 (2)
Mode of delivery	191	
Vaginal delivery, % (n)		92 (176)
Cesarean section, % (n)		8 (15)
Gender	193	
Male, % (n)		50 (96)
Female, % (n)		50 (97)
Birth weight (g)	193	3270.1 ± 439.1
Head circumference (cm)	153	33.5 ± 2.5
Birth length (cm)	182	51.1 ± 2.6
Apgar score (1 min)	189	7.8 ± 1.4
Race	193	
African-American, % (n)		70 (136)
Caucasian, % (n)		30 (57)
Ethnicity	193	
Hispanic, % (n)		26 (50)
Non-Hispanic, % (n)		74 (143)

[a]Early gestation (37-39 wk gestation), full (39–41 wk gestation), late (41–42 wk gestation), post-term (≥ 42 wk gestation) (6).

post-term neonates (20%) in this cohort were not significantly different ($P = 0.09$) from the national birth rates of early- (24.7%), full- (50.8%), or late/post-term neonates (14.6%), respectively (14). The mean birth weight of these term neonates was 3,270 ± 439 g, which was significantly lower ($P < 0.001$) than that reported for term US singleton births (3,389 ± 466 g) (15), but comparable to the mean birth weight of neonates born to adolescents (3.25 ± 0.02 kg) (16). Small-for-GA was observed in 18% ($n = 34$) of term newborns, which was significantly higher ($P = 0.001$) than the national rate (10–11%) (17).

Neonatal Anemia and Hemoglobin Concentrations

Cord blood Hb concentrations and iron status indicators are presented as a function of GA grouping in **Table 2**. Two neonates were born post-term (42.1 ± 0.1 wk gestation); data from these two neonates were combined into the late-term grouping. The mean Hb concentration in term neonates ($n = 113$) was 14.4 g/dl (95% confidence interval (CI): 13.9, 14.9) and anemia was evident in 21% ($n = 24$) of these neonates at birth. African-American neonates had significantly lower mean Hb concentrations compared to Caucasian neonates (14.1 g/dl, (95% CI: 13.6, 14.6), $n = 84$ vs. 15.4 g/dl, (95% CI: 14.3, 16.5), $n = 29$, $P = 0.03$); however, the prevalence of anemia did not differ significantly between the two racial groups (23% in African-American neonates vs. 17% in Caucasian neonates, $P = 0.54$). Neonatal anemia was observed among 16% of term neonates born to mothers who self-reported use of prenatal supplements ≥ 2–5 times/week compared to 33% of neonates born to mothers with who self-reported less frequent use of supplements ($P = 0.04$).

Neonatal Iron Status in Term Neonates at Birth

In term neonates ($n = 181$), the mean cord serum ferritin (SF) concentration was 114.4 μg/l (95% CI: 102.5, 127.7), with 25% ($n = 45$) having cord SF < 76 μg/l. SF concentrations were not significantly associated with GA at birth across the range of 37–42 wk ($P = 0.69$), nor were they associated with weight ($P = 0.99$), gender ($P = 0.97$), or race ($P = 0.93$). The percentage of term neonates with SF < 76 μg/l more than doubled in neonates born to mothers with dietary iron intakes less than the estimated average requirement (23 mg/d) (18), compared to those with iron intakes ≥ 23 mg/d (27 vs. 11%, $P = 0.04$).

The mean concentration of cord soluble transferrin receptor (sTfR) was 7.6 mg/l (95% CI: 7.2, 8.1), $n = 183$, and cord sTfR was positively correlated with GA ($r = 0.32$, $P < 0.0001$, $n = 183$). A significantly ($P < 0.05$) higher concentration of cord sTfR (9.0 mg/dl) was observed in late/post-term newborns (95% CI: 7.8, 10.4), $n = 38$, when compared to early-term (6.6 mg/l, (95% CI: 6.0, 7.2), $n = 39$) or full-term neonates (7.6 mg/l, (95% CI: 7.1, 8.1), $n = 106$). This finding was not significantly different if the sTfR data from the one post-term newborn in whom sTfR data was available was excluded from this analysis.

The mean concentration of cord erythropoietin (EPO) was 32.1 mIU/ml (95% CI: 28.2, 36.2), $n = 163$. Cord EPO was positively correlated with GA ($r = 0.32$, $P < 0.0001$, $n = 163$) and early-term newborns had a significantly ($P < 0.05$) lower EPO concentration (21.8 mIU/ml, (95% CI: 17.3, 27.1), $n = 32$) compared to full-term (32.8 mIU/ml, (95% CI: 28.2, 38.5), $n = 97$) and late/post-term neonates (43.4 mIU/ml, (95% CI: 31.5, 59.7), $n = 34$).

The mean concentration of cord hepcidin was 92.8 ng/ml (95% CI: 82.3, 105.6), $n = 179$. Cord hepcidin was significantly positively associated with the total duration of labor ($R^2 = 0.07$, $P < 0.001$, $n = 152$), and with the duration of Stage 1 ($R^2 = 0.05$, $P = 0.005$, $n = 152$) and Stage 2 labor ($R^2 = 0.08$, $P < 0.001$, $n = 153$). The mean concentrations of IL-6 was 9.4 pg/ml (95% CI: 7.2, 12.2), $n = 156$, and similar to the hepcidin findings, cord IL-6 was significantly positively associated with the total duration of labor ($R^2 = 0.04$, $P = 0.02$, $n = 131$), and with the duration of Stage 2 labor ($R^2 = 0.06$, $P = 0.007$, $n = 132$).

Correlations Between Neonatal Iron Status Indicators

Table 3 presents correlations between cord Hb concentrations and indicators of iron status. Among term-neonates, cord Hb was significantly negatively associated with cord SF ($R^2 = 0.07$, $P = 0.006$, $n = 109$), and cord sTfR was significantly positively associated with cord EPO ($R^2 = 0.14$, $P < 0.001$, $n = 163$). Cord hepcidin concentrations were significantly positively associated with cord SF ($R^2 = 0.18$, $P < 0.001$, $n = 178$) and cord IL-6 ($R^2 = 0.09$, $P < 0.001$, $n = 155$), and significantly negatively associated with cord EPO ($R^2 = 0.05$, $P = 0.004$, $n = 162$) and cord sTfR ($R^2 = 0.03$, $P = 0.02$, $n = 179$).

Table 2. Neonatal hemoglobin concentrations and indicators of iron status by gestational age groups[a]

	n	Early term (37–39 wk gestation) (6)	*n*	Full term (39–41 wk gestation) (6)	*n*	Late and post-term (≥ 41 wk gestation) (6)
Gestational age (weeks)[b]	41	38.1 ± 0.5[e]	113	40.0 ± 0.6[f]	39	41.4 ± 0.3[g]
Birth weight (g)[b]	41	2,940.1 ± 358.1[e]	113	3,261.9 ± 368.4[f]	39	3,640.8 ± 425.0[g]
Neonatal gender	41		113		39	
Male, % (*n*)[c]		66 (27)		42 (48)		54 (21)
Female, % (*n*)[c]		34 (14)		58 (65)		46 (18)
Hemoglobin (g/dl)	26	14.6 ± 2.4 (8.2–18.7)	62	14.2 ± 2.8 (7.0–20.8)	25	14.7 ± 2.1 (10.4–17.3)
Anemia, % (*n*)[c]		19 (5)		24 (15)		16 (4)
Serum Ferritin (μg/l)	39	122 (91.8, 161)	104	114 (100, 130)	38	108 (80.6, 144)
< 76 μg/l, % (*n*)[c]		21 (8)		26 (27)		26 (10)
Serum sTfR (mg/l)[b]	39	6.6 (6.0, 7.2)[e]	106	7.6 (7.1, 8.1)[e]	38	9.0 (7.8, 10.4)[f]
Total body iron (mg/kg)	39	9.0 ± 3.4 (1.1–17.2)	104	8.3 ± 2.8 (2.2–15.4)	38	7.4 ± 3.4 (−0.6–13.1)
Serum EPO (mIU/ml)[b]	32	21.8 (17.3, 27.1)[e]	97	32.8 (28.2, 38.5)[f]	34	43.4 (31.5, 59.7)[f]
Serum Iron (μg/dl)	22	192 (144, 257)	82	198 (179, 219)	23	202 (172, 237)
Serum Hepcidin (ng/ml)	39	104 (78.3, 138)	104	87.4 (74.4, 103)	36	99.5 (72.2, 137)
Serum IL-6 (pg/ml)[d]	29	9.5 (6.0, 14.9)	95	8.8 (6.2, 12.7)	32	11.1 (5.9, 20.9)
< 0.13 pg/ml, % (*n*)[c]		3 (1)		0		0
Serum CRP (mg/l)[d]	5	3.5 (0.23, 51.4)	11	0.8 (0.32, 2.0)	5	0.6 (0.2, 2.0)
< 0.2 mg/l[c], % (*n*)[c]		76 (16)		81 (48)		77 (17)

CI, confidence interval; CRP, C-reactive protein; EPO, erythropoietin; IL-6, Interleukin-6; sTfR, soluble transferrin receptor.
[a]Data are presented as the mean ± SD (range) for hemoglobin and total body iron; geometric mean (95% CI) for CRP, EPO, ferritin, hepcidin, IL-6, and serum iron. [b]Values within a row that do not share a common superscript statistically differ. Gestational age, $P < 0.0001$; birth weight, $P < 0.0001$; serum sTfR, $P < 0.05$; serum EPO, $P < 0.05$. [c]No significant differences were observed in the number or percentage between gestational age groups. [d]Undetectable values were excluded from calculations. [e,f,g]Group means without a common letter in a row differ, $P < 0.05$.

Table 3. Correlations between gestational age, birth weight, and iron status indicators of term neonates

	Birth weight (g)	Cord hemoglobin	Log cord ferritin	Log cord sTfR	Cord total body iron	Log cord EPO	Log cord serum Fe	Log cord hepcidin	Log cord IL-6
Gestational age at birth	$r = 0.51^*$; $n = 193$	$r = 0.05$; $n = 113$	$r = -0.03$; $n = 181$	$r = 0.35^*$; $n = 183$	$r = -0.18^\dagger$; $n = 181$	$r = 0.36^*$; $n = 163$	$r = 0.02$; $n = 127$	$r = -0.02$; $n = 179$	$r = 0.09$; $n = 157$
Birth weight (g)		$r = -0.05$; $n = 113$	$r = 0.0006$; $n = 181$	$r = 0.04$; $n = 183$	$r = -0.02$; $n = 181$	$r = 0.07$; $n = 163$	$r = 0.06$; $n = 127$	$r = 0.11$; $n = 179$	$r = 0.16$; $n = 157$
Cord hemoglobin			$r = -0.26^\ddagger$; $n = 109$	$r = 0.15$; $n = 109$	$r = -0.30^\ddagger$; $n = 109$	$r = -0.01$; $n = 98$	$r = 0.08$; $n = 76$	$r = 0.0001$; $n = 108$	$r = -0.007$; $n = 98$
Log cord ferritin				$r = -0.04$; $n = 181$	$r = 0.90^*$; $n = 181$	$r = -0.09$; $n = 162$	$r = 0.22^\dagger$; $n = 125$	$r = 0.43^*$; $n = 178$	$r = 0.03$; $n = 156$
Log cord sTfR					$r = -0.47^*$; $n = 181$	$r = 0.38^\dagger$; $n = 163$	$r = 0.06$; $n = 125$	$r = -0.17^\dagger$; $n = 179$	$r = -0.08$; $n = 156$
Cord total body iron						$r = -0.25^\ddagger$; $n = 162$	$r = 0.16$; $n = 125$	$r = 0.45^*$; $n = 178$	$r = 0.06$; $n = 156$
Log cord EPO							$r = 0.02$; $n = 116$	$r = -0.22^\ddagger$; $n = 162$	$r = -0.05$; $n = 149$
Log cord serum Fe								$r = 0.05$; $n = 125$	$r = 0.08$; $n = 114$
Log cord hepcidin									$r = 0.30^*$; $n = 155$

EPO, erythropoietin; Fe, iron; IL-6, interleukin-6; sTfR, soluble transferrin receptor.
$^*P < 0.001$; $^\dagger P < 0.05$; $^\ddagger P < 0.01$.

Associations Between Neonatal Iron Status and Maternal Iron Status

Table 4 presents the correlations between maternal and neonatal iron status indicators obtained at mid-gestation and at delivery. Cord SF concentrations were significantly positively associated with maternal SF at mid-gestation ($R^2 = 0.11$, $P < 0.001$, $n = 106$). Neonates born to mothers with SF < 12.0 μg/l at mid-gestation (27.1 ± 3.5 wk gestation) had on average 34%

Table 4. Correlations between maternal and neonatal iron status indicators at mid-gestation (26.1 ± 3.5 wk gestation) and at delivery (39.9 ± 1.2 wk gestation)[a]

	N	Maternal mid-gestation	Cord blood	*r* (*P*)
Hemoglobin (g/dl)	61	11.2 ± 0.9 (8.7–13.3)	14.2 ± 2.7 (8.2–20.8)	−0.06 (0.63)
Ferritin (μg/l)	106	16.8 (14.4, 19.7)	122 (108, 137)	0.34 (< 0.001)
sTfR (mg/l)	108	4.7 (4.3, 5.1)	7.9 (7.4, 8.4)	0.21 (0.03)
Total body iron (mg/kg)	106	3.2 ± 3.8 (−10.3–12.4)	8.4 ± 2.6 (1.2–13.1)	0.25 (0.01)
Hepcidin (ng/ml)	104	21.8 (18.4, 25.8)	98.5 (83.1, 116)	0.31 (0.001)
EPO (mIU/ml)	93	29.7 (27.1, 32.8)	34.8 (29.1, 41.3)	0.13 (0.22)
	n	Maternal delivery	Cord blood	*r* (*P*)
Hemoglobin (g/dl)	112	11.5 ± 1.6 (6.5–17.2)	14.4 ± 2.6 (7.0–20.8)	−0.08 (0.40)
Ferritin (μg/l)	175	21.8 (19.3, 24.8)	117 (104, 130)	0.08 (0.30)
sTfR (mg/l)	177	5.0 (4.6, 5.4)	7.6 (7.2, 8.1)	0.18 (0.02)
Total body iron (mg/kg)	175	4.0 ± 4.1 (−9.1–19.9)	8.3 ± 3.1 (−0.6–17.2)	0.05 (0.52)
Hepcidin (ng/ml)	171	25.8 (22.2, 30.3)	95.6 (84, 109)	0.13 (0.10)
EPO (mIU/ml)	153	27.4 (24.5, 30.3)	32.8 (28.8, 37.3)	0.21 (0.01)

EPO, erythropoietin; sTfR, soluble transferrin receptor.
[a]Data are presented as the mean ± SD (range) for hemoglobin and total body iron; geometric mean (95% CI) for EPO, ferritin, hepcidin, and sTfR.

lower cord SF (88.2 μg/l, (95% CI: 69.4, 113.3), $n = 28$) compared to neonates born to mothers with SF ≥ 12.0 μg/l at mid-gestation (26.1 ± 3.5 wk gestation) (135.6 μg/l, (95% CI: 119.1, 154.5), $n = 78$, $P = 0.003$). Furthermore, a significantly higher percentage of neonates with cord SF < 76 μg/l were born to mothers with SF < 12.0 μg/l compared to neonates born to mothers with SF ≥ 12.0 μg/l (29 vs. 12%, $P = 0.04$). Cord hepcidin was significantly positively associated with maternal hepcidin at mid-gestation ($R^2 = 0.10$, $P = 0.001$, $n = 104$).

At delivery, cord EPO was also significantly negatively correlated with maternal hemoglobin concentrations at delivery ($r = -0.19$, $P = 0.01$, $n = 156$). In contrast to the relationships observed at mid-gestation, no significant associations were observed between cord SF and maternal SF at delivery ($P = 0.30$) or between cord hepcidin and maternal hepcidin at delivery ($P = 0.10$). This may be explained by labor-induced inflammation as we have previously found that ferritin and hepcidin concentrations were affected by the increased maternal inflammation observed at delivery (19).

DISCUSSION

In this study, anemia was evident in nearly one-quarter (21%) of healthy term newborns born to pregnant adolescents. At this time, there are no national data to directly compare this prevalence to, as 1- to 2-y olds are the youngest age group monitored for anemia prevalence by the NHANES (20). Neonatal Hb concentrations have recently been compiled in archived records of nonanemic US newborns with minimal hematology-related pathologies ($n = 24{,}416$) (21). In relation to our data, nonanemic term neonates in our cohort exhibited a 2.7 g/dl lower Hb concentration on average than the national reference data. Direct comparison of these values is challenging because the US reference data were obtained from a mixture of capillary, venous, and arterial blood, and Hb concentrations are known to vary between these sources (22). To better control for variability due to site of sampling, our data were compared to weighted mean cord Hb concentrations published in a review of nine studies (15.9 g/dl, $n = 1{,}538$) (23). Using this reference value, mean cord Hb concentrations of neonates in our study remained on average 1.8 g/dl lower than expected. African-American neonates in this study exhibited significantly lower average Hb concentrations (1.3 g/dl, $P = 0.03$) in comparison to the mean Hb observed in the Caucasian neonates studied. The lower Hb observed among the African-American neonates is consistent with the 0.8 g/dl lower Hb concentration reported in adult African-American women compared to Caucasian women (ages 18–44 y, $n = 2{,}974$) (24).

Deficits in Hb are a late sign of iron insufficiency and the reductions in Hb concentrations observed in these neonates may be indicative of insufficient iron reserves at birth. In this cohort, fully 25% of neonates were born with cord SF < 76 μg/l, a percentage that was 2.6- and 2.3-times higher than observed among Chinese ($n = 3{,}699$) (2) and Norwegian neonates ($n = 363$) (25), respectively ($P < 0.001$ for both). The median cord SF in our study (124.1 μg/l) was approximately 27% lower than median values reported from both China (170 μg/l) (2) and Norway (169 μg/l) (25), and approximately 7.4% lower than US data reported in term newborns (134 μg/l, $n = 308$) (26). In addition, the mean cord SF of neonates born to mothers with low iron stores at delivery (SF < 12 μg/l) in our study (91 μg/l) was approximately 43% lower than the mean value reported in recent neonatal data from Spain (160 μg/l) (12). The combined Hb and ferritin data in this group of neonates are indicative of insufficient iron stores at birth when compared to other neonatal populations.

In this study, an unexpected inverse relationship was observed between cord Hb concentrations and SF, which was opposite to the positive association observed among the

pregnant adolescents that gave birth to these newborns (19), and to that reported among pregnant women (27). An inverse correlation between neonatal Hb and storage iron has previously been reported in term newborns born to diabetic mothers (28) in support of the premise that iron is preferentially utilized for fetal red cell mass over storage iron but few normative data are available regarding these correlations in otherwise healthy term neonates. Low ferritin concentrations in neonates with high Hb concentrations may reflect a preferential utilization of iron in support of erythropoiesis. However, the mechanisms responsible for higher ferritin concentrations in neonates with low Hb concentrations are unknown and do not appear to be due to inflammation given the lack of an association between cord SF and markers of inflammation (IL-6 or hepcidin) in this group as a whole. At this time, the physiological mechanism behind this relationship has not been well established and more studies are needed to address this association.

Neonatal iron stores in umbilical cord serum at birth were best predicted by maternal SF concentrations at mid-gestation. A large study (n = 3,247) in maternal-neonatal pairs from China found maternal SF at delivery was related to cord blood SF only among women with SF < 13.6 μg/l; no associations between maternal and neonatal SF were evident in women with SF > 13.6 μg/l at delivery (2). We did not identify an inflection point between adolescent SF at delivery and cord blood SF, and no significant differences were noted in mean cord blood SF of neonates as a function of maternal SF (< 12 or ≥ 12 g/l at delivery). Maternal SF concentrations at delivery are affected by acute delivery-associated inflammatory events. In a previous publication of maternal data from this same study cohort (19), concentrations of SF, hepcidin, and IL-6 were significantly higher at delivery compared to values obtained at mid-gestation. Thus, inflammation may confound the utility of ferritin and hepcidin as iron status indicators at delivery.

Early chemical analysis studies found that fetal total body iron content increased rapidly over the last 4 wk of gestation (29). However, of all the iron status indicators measured in our study, only sTfR and EPO exhibited an increase as a function of the GA at birth across 37–42 wk of gestation. Ferritin concentrations did not significantly increase across the final 3–5 wk of gestation, but remained constant in the face of increased neonatal birth weight and length. This pattern is different from that reported by Siddappa *et al.* (26) where cord SF increased with GA across a wider range of GA (23–41 wk gestation). Because we are only measuring circulating iron biomarkers at birth, we cannot evaluate possible changes in iron stores in other body tissues and compartments. Currently, there are insufficient data to tell if the patterns observed are unique to neonates born to pregnant adolescents, or if similar patterns are evident across the same time window in newborns born to biologically mature or nonanemic women.

At this time, little is known regarding the magnitude and determinants of the expression of fetal hepcidin. All term neonates in this study had detectable hepcidin at birth and mean concentrations observed were similar to those reported among term neonates using the same hepcidin assay (30). Hepcidin concentrations were higher in newborns born to mothers with longer durations of labor. This is likely due to inflammation as maternal hepcidin was significantly elevated at delivery when compared to mid-gestation (19), and hepcidin was significantly associated with IL-6 in both the adolescent at delivery and in neonatal cord blood samples. This should be taken into account when considering use of either hepcidin or ferritin as an indicator of iron status in neonates and their mothers at delivery. Similar findings on the impact of iron stores and inflammation on hepcidin concentrations have been recently reported in healthy infants and toddlers ages 0.5–3 y (31).

The iron endowment at birth is critical in ensuring that the neonate maintains sufficient iron stores over early infancy (4–6 mo), a time when the regulation of intestinal iron absorption in response to infant iron stores may not be fully developed (32,33). In this group of neonates, maternal ferritin at mid-gestation was the most significant predictor of neonatal iron stores at birth. This finding has implications for a more rigorous screening for maternal iron status at a time when there is a window of opportunity to augment maternal and possibly fetal iron stores by increasing maternal iron intake through diet or prenatal supplementation.

To our knowledge, our study cohort is the largest of its kind with data on a panel of cord iron and inflammation biomarkers in neonates born to pregnant adolescents. Given the logistical difficulties and challenges in recruiting teen mothers and obtaining longitudinal data across gestation, our study provides unique and important data from this high-risk obstetric population. We acknowledge that there were several limitations in this study. First, the time of cord clamping was unknown; however delayed cord clamping is standard of practice at the clinic. Second, the clinical significance of the findings remains unclear given the small R^2 values. Third, generalizability of these findings are limited due to the small sample size and because the study cohort was predominantly African-American reflecting the disproportionately higher adolescent birth rates among non-Hispanic black (43.9/1,000) compared to non-Hispanic white teens (20.5/1,000) (14).

Ensuring optimal iron stores at birth and across gestation is essential. Moreover, intervening at key windows of gestation may have clinical implications for the growing fetus as animal models have found that the timing of maternal ID plays a key role in fetal brain development (4). Additional studies are needed to identify optimal concentrations of iron status biomarkers at birth that best support subsequent health and development.

METHODS

Pregnant adolescents (n = 255) were recruited from the Rochester Adolescent Maternity Program at The University of Rochester Medical Center in Rochester, NY (2006–2012). Adolescents were: ≤ 18 y; ≥ 12 wk gestation; carrying a singleton, and had no pre-existing medical complications (HIV infection, diabetes, eating disorders, or malabsorption diseases). Informed written consent was obtained from all adolescents. In teens ≤ 14 y of age, both the teen's assent and parental consent were obtained. The study was approved by the Institutional Review Boards of the University of Rochester and Cornell University.

GA was determined based on self-reported maternal menstrual history. If the maternal self-report and sonogram data differed by more than 10 d, GA was based on the ultrasound data. Infant medical records were reviewed to abstract birth data and to screen for the following adverse birth events or conditions: admission to the Neonatal Intensive Care Unit, sepsis, respiratory distress, hyperbilirubinemia (> 12 mg/dl), antibiotic treatment, and TORCH (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex, and HIV) infections. At birth, neonates were classified as small-for-GA if the birth weight was < 10th percentile of the growth curves based on 80,000 deliveries in the Finger Lakes Region of NY State (34). Duration of each stage of labor was defined as Stage 1 (time from onset of contractions to full cervical dilation), Stage 2 (time from full dilation to delivery of the baby), and Stage 3 (time elapsed between delivery of the baby to delivery of the placenta) (35).

Umbilical cord blood (15 ml) was obtained at delivery. Cord blood was not obtained in 50 newborns due to study staffing availability at delivery (n = 9); delivery at a different hospital (n = 8); fetal death *in utero* (n = 5); maternal refusal to allow blood sampling (n = 3); and other miscellaneous reasons associated with adverse birth outcomes (n = 25). Cord blood hemoglobin (Hb) concentrations were measured at the Strong Memorial Hospital clinical laboratory (Cell-Dyn 4000 hematology analyzer, Abbott diagnostics, Santa Clara, CA) using Clinical Laboratory Improvement Amendments certified methodology. Neonatal anemia was defined using standard criteria as a cord blood Hb concentration < 13.0 g/dl (22). Of the 205 cord blood samples collected, 12 neonates (Neonatal Intensive Care Unit admission, n = 6; cystic fibrosis, n = 1; preterm birth, n = 5) were excluded from the final analyses to focus the analyses on iron status of heathy, term neonates. Hemoglobin concentrations were not measured in 65 newborns because the cord blood samples clotted prior to analysis, or staff was not available at the time of blood collection. Thus late in the study additional Hb data were obtained in the delivery room using a HemoCue (n = 46). Of the 128 Hb values, 64% (n = 82) were analyzed at the Strong Memorial Hospital laboratory only, 12% (n = 15) were assessed by HemoCue only, and 24% (n = 31) had Hb measures analyzed in both the laboratory and by HemoCue. When Hb data were available by both methods, the values were significantly correlated (r = 0.70, P < 0.001), yet to a lower degree than the correlations reported by others (36). Therefore, only the laboratory values were utilized in the statistical analyses presented.

Nonfasted maternal blood (15 ml) was collected at mid-gestation (26.1 ± 3.5 wk gestation, n = 120) and at admission into the hospital for delivery (39.9 ± 1.2 wk gestation, n = 193). SF (ELISA, Ramco Laboratories, Stafford, TX), sTfR (ELISA, Ramco Laboratories), hepcidin (Intrinsic Life Sciences LLC, LaJolla, CA), serum iron (Perkin Elmer AAnalyst 800, Waltham, MA), interleukin-6 (IL-6) (Millipore Magnetic Multiplex, Temecula, CA), C-reactive protein, and EPO (Siemens Immulite 2000 immunoassay system, Erlangen, Germany) were measured in cord and maternal blood. Maternal dietary iron intake was assessed by 24-h dietary recall, and frequency of prenatal supplement use was self-reported via questionnaire. Details of the methodology used for each assay, maternal iron status, and maternal dietary iron intake have been previously reported (19). At present, there is no definitive cut-off that is indicative of suboptimal cord SF concentrations. In accordance with several studies that related cord SF to neurodevelopmental outcomes (37–39), for data analysis purposes, we used the same criteria of a cord SF concentration < 76 μg/l to define suboptimal SF concentrations at birth.

Statistical Analysis

The distributions of variables were examined and geometric means (95% CI) were calculated for the variables with skewed distributions. Differences in group means of normally distributed variables were compared using independent t-tests or ANOVA with Tukey HSD for multiple comparisons. Bivariate correlations between indicators were examined with scatter plots, and correlation coefficients were calculated. Linear regression analyses were performed to identify the determinants of each neonatal iron status indicator. Non-normally distributed variables were log-transformed to achieve normality of the residuals. Statistical analyses were conducted using JMP 10.0 (SAS Institute Cary, NC). Statistical significance was determined at P < 0.05 in all analyses.

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