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Journal

Journal of Nutrition, 149(3)

ISSN

0022-3166

Authors

Delaney, Katherine M
Guillet, Ronnie
Fleming, Robert E
et al.

Publication Date

2019-03-01

DOI

10.1093/jn/nxy286

Peer reviewed

The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Umbilical Cord Serum Ferritin Concentration is Inversely Associated with Umbilical Cord Hemoglobin in Neonates Born to Adolescents Carrying Singletons and Women Carrying Multiples

Katherine M Delaney,[1] Ronnie Guillet,[3] Robert E Fleming,[5] Yuan Ru,[6] Eva K Pressman,[4] Francoise Vermeylen,[2] Elizabeta Nemeth,[7] and Kimberly O O'Brien[1]

[1]Division of Nutritional Sciences; [2]Cornell Statistical Consulting Unit, Cornell University, Ithaca, NY; [3]Department of Pediatrics Division of Neonatology; [4]Department of Obstetrics and Gynecology, The University of Rochester School of Medicine, Rochester, NY; [5]Division of Neonatal-Perinatal Medicine, Saint Louis School of Medicine, St. Louis, MO; [6]School of Public Health, Zhejiang University, Hangzhou, China; and [7]Department of Medicine, University of California Los Angeles, Los Angeles, CA

ABSTRACT

Background: It has been proposed that the fetus prioritizes iron for hemoglobin production over delivery to tissues. However, few studies have evaluated the interrelations between hemoglobin and multiple iron status biomarkers in umbilical cord blood. A full understanding is needed of how these parameters influence each other within cord blood to fully interpret iron and hematologic status at birth.

Objectives: We evaluated the determinants of neonatal hemoglobin and assessed the interrelations between hemoglobin, serum iron status indicators, and serum iron regulatory hormones in healthy neonates.

Methods: This was an observational study that assessed umbilical cord hemoglobin (Hb), serum ferritin (SF), erythropoietin (EPO), soluble transferrin receptor (sTfR), serum iron, hepcidin, vitamin B-12, folate, IL-6, and CRP measured in 234 neonates born to adolescents or to women carrying multiples. Correlations between these indicators were evaluated and mediation models consistent with the observed significant determinants of cord Hb concentrations were developed.

Results: A highly significant inverse association was found between cord SF and Hb concentrations that was not attributable to neonatal or maternal inflammation (as measured by IL-6 and CRP). The inverse association was present in the combined cohort, as well as in the adolescent and multiples cohorts independently. Mediation analyses found that EPO and hepcidin had significant indirect effects on cord Hb, associations that are explicable by mediation through SF and sTfR.

Conclusion: In contrast to observations made in older infants, a highly significant inverse association between Hb and SF, as well positive associations between Hb and both sTfR and EPO, were observed in umbilical cord blood from neonates born to adolescents or women carrying multiples. These findings, combined with review of the published literature, indicate a need for analysis of the relations between multiple parameters to assess iron and hematologic status at birth. These clinical trials were registered at clinicaltrials.gov as NCT01582802 (https://clinicaltrials.gov/ct2/show/NCT01582802) and NCT01019902 (https://clinicaltrials.gov/ct2/show/NCT01019902) *J Nutr* 2019;149:406–415.

Keywords: neonates, anemia, hepcidin, erythropoietin, transferrin receptor, multiple births, iron

Introduction

Iron (Fe) is an essential nutrient involved in numerous metabolic processes such as oxygen transport, mitochondrial function, as well as growth and development (1). Iron deficiency during fetal and early life developmental periods has been associated with multiple adverse neurodevelopmental and cognitive outcomes (2–6). The fetus relies on maternal Fe stores during pregnancy, and by late gestation 5–8 mg Fe/d are transported across the placenta to support fetal demands. When Fe availability in utero is limited, the human fetus has been shown to prioritize the Fe demands of erythropoiesis over other tissue demands, including the brain (7, 8). In utero, hemoglobin (Hb) concentrations are elevated compared to values any time thereafter in response to a relatively hypoxemic environment (9). After birth, Fe that was

Manuscript received August 7, 2018. Initial review completed September 12, 2018. Revision accepted October 18, 2018.
First published online February 15, 2019; doi: https://doi.org/10.1093/jn/nxy286.

once in erythrocyte heme is redistributed and used by other tissues or stored as ferritin. As such, there are dynamic changes in biomarkers of Fe status across early infancy (10).

Normative data on Hb concentrations across a range of gestational ages at birth and over the first few weeks of life have been reported in large cohorts of neonates (9, 11). However, data evaluating Fe status indicators in umbilical cord blood have been compiled from much smaller study populations and many have evaluated outcomes assuming that associations between Hb and Fe status indicators in cord blood mirror those found in older infant, pediatric, and adult populations. Few studies, however, have tested these assumptions or assessed relations between Fe status indicators and Hb or erythropoietic regulatory hormones in cord blood. In particular, serum ferritin (SF) is commonly used as a biomarker of body Fe stores but the vast majority (>80%) of identified studies that published both Hb and SF data in cord blood did not report any data on possible associations between these 2 biomarkers (**Supplemental Table 1**). Recent studies have also raised concerns with the interpretation of SF in older pediatric and pregnant cohorts and have highlighted the need to evaluate this indicator in relation to markers of inflammation and to established iron status biomarkers (12, 13).

We recently completed 2 studies evaluating neonatal Fe status indicators in umbilical cord blood in a group of 234 neonates born to 2 obstetric groups known to be at higher risk for maternal Fe deficiency and anemia (14, 15). In each of these neonatal cohorts, Hb concentrations, Fe status indicators, Fe regulatory hormones, and inflammatory markers were evaluated. In both cohorts, an unexpected and highly significant inverse association between SF and Hb concentrations was evident (14, 16). The main goal of this analysis was to investigate the interrelations between Hb concentrations in umbilical cord blood and a more comprehensive panel of Fe status indicators and Fe regulatory hormones across late gestation. A secondary goal was to compare our data to other published findings on Hb and SF in umbilical cord blood to determine whether our SF and Hb findings were unique to our particular neonatal cohorts, or whether similar findings have been reported in other neonatal study populations.

Materials and methods

Participants

Pregnant women were recruited from Strong Memorial Hospital and Highland Hospital in Rochester, NY as previously reported (15, 17). Informed written consent was obtained from all participants aged >14 y, and parental consent and adolescent assent were obtained from adolescents aged ≤14 y. The total study population consisted of 374 neonates born to 2 cohorts of higher-risk, but otherwise healthy gravida. Neonates had Fe status indicators measured within cord blood at birth and were excluded if they did not have both Hb and SF measurements within cord blood, resulting in a study population of 234 neonates. Therefore, the final study population comprised 108 neonates born to 64 women carrying multiples (aged ≥ 21 y) and 126 singleton neonates born to pregnant adolescents (aged 13–18 y) (**Figure 1**). All studies were approved by the institutional review boards of the University of Rochester and Cornell University. Gestational age for both populations was determined using standard criteria or using the known date of in vitro fertilization in the multiples cohort. Preterm delivery was defined as birth occurring before 37 weeks of gestation, while early term delivery was defined as birth between 37 and 38 weeks of gestation. Low birth weight (LBW) was defined as birth weight < 2500 g. In all women, a baseline health history was obtained and pertinent maternal and neonatal data at birth were abstracted from medical records. Maternal Fe status data from the adolescent (14, 17, 18), and multiples cohorts (15), and descriptive data on Fe status indicators in the neonates (15, 16), have been published.

Serum collection and biochemical analyses

Umbilical cord blood (~15 mL) was obtained at delivery. In both cohorts, whole blood was sent to the University of Rochester core laboratory for assessment of Hb concentrations using a Cell-Dyn 4000 hematology analyzer (Abbott Diagnostics). The remaining blood samples were centrifuged, separated, and stored at −80°C until analysis. Cord blood was not obtained from 50 newborns in the adolescent cohort because of lack of study personnel at delivery ($n = 9$), delivery at a different hospital ($n = 8$), fetal death in utero ($n = 5$), maternal refusal ($n = 3$), and other miscellaneous reasons ($n = 25$). Neonatal mortality rate in the multiples cohort was 1.6% (3 deaths per 186 live births). Neonatal anemia was defined using standard definitions as a cord Hb concentration <130 g/L (19). SF, soluble transferrin receptor (sTfR), and hepcidin were measured by ELISA (Ramco Laboratories) as previously described (14, 15). The SF CV of control samples provided in the kit fell within specifications for all kits and averaged 8% for the multiples cohort and 4% for the adolescent cohort. Cord serum Fe was measured by atomic absorption spectrophotometry (Perkin Elmer AAnalyst 800). Cord erythropoietin (EPO) values were measured by immunoassay (Siemens Immulite 2000). Hepcidin, C-reactive protein (CRP), and IL-6 were measured with 2 different assays between cohorts. Within the multiples cohort, hepcidin, CRP, and IL-6 were measured with ELISA as previously described (15). Within the adolescent cohort, hepcidin was measured with an ELISA and IL-6 and CRP were measured using a commercial immunoassay as previously described (14). Sample sizes vary per indicator because of insufficient sample volume to analyze all indicators.

Statistical analysis

Subject characteristics and Fe status indicators were compared between cohorts using 2-tailed t-tests or chi-squared test. Indicators that were analyzed with different assays between cohorts (CRP, IL-6, and hepcidin) were standardized by dividing each measurement by its respective cohort's mean and dividing this number by the standard deviation of the cohort. Pairwise and partial Pearson correlations were assessed between Fe status indicators, inflammatory markers, and Hb. Neonates were then divided into tertiles based on their Hb status. Iron

Supported by the Gerber Foundation, USDA grants 2005-35200-15218 and 2009-35200-05171 and The U.S. National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant T32-DK007158.
Author disclosures: KMD, RG, REF, YR, EKP, FV, and KOO, no conflicts of interest. EN is a stockholder and consultant for Intrinsic LifeSciences, LLC. The Gerber Foundation had no conflict of interest in the study and was not involved in the study design or interpretation of the data.
Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
Address correspondence to KOO (e-mail: koo4@cornell.edu).
Abbreviations used: CRP, C-reactive protein; EPO, erythropoietin; Hb, hemoglobin; LBW, low birth weight; SF, serum ferritin; sTfR, soluble transferrin receptor.

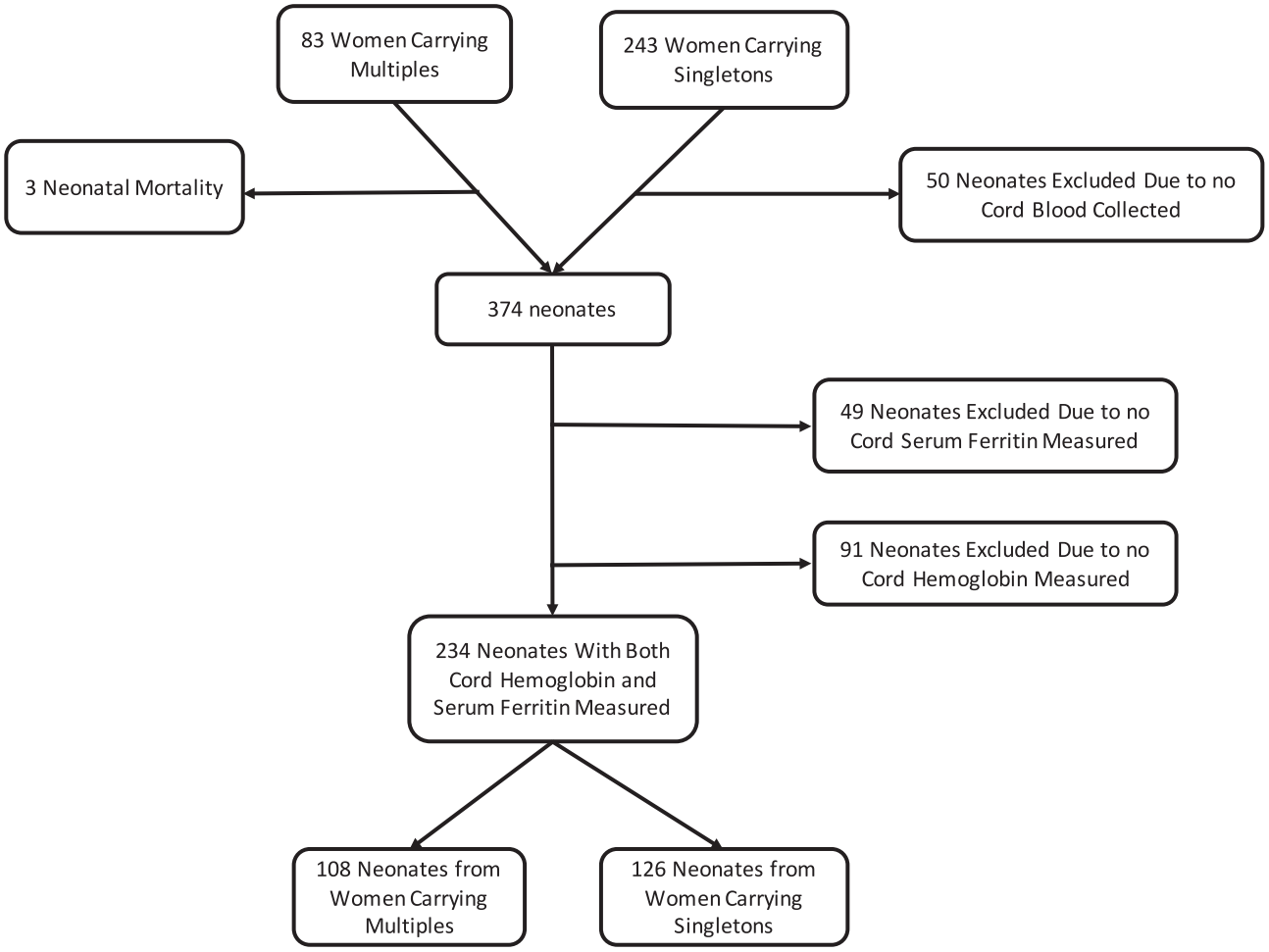

FIGURE 1 Participant flowchart for the pregnant women in the multiples or adolescent cohorts. Participants were recruited to Strong Memorial Hospital or Highland Hospital and included in the study according to umbilical cord iron status indicator measurements.

status indicators and regulatory hormones were evaluated by tertile. Differences in iron status indicators by Hb tertiles were compared using a general linear model controlling for birth weight. This was followed by Tukey honest significant difference analyses for multiple comparisons. Multiple regression was used to determine predictors of Hb status. Variables were tested simultaneously and eliminated by backward selection until only statistically significant predictors remained. All analyses were performed using the multiple mothers ID number as a random coefficient to control for lack of independence in the multiples cohort. The previous statistical analyses were performed using JMP 12.0 (SAS Institute Inc). To assess interrelations between Fe status indicators and Hb, a mediation model was developed using STATA 15 (StataCorp LLC). To determine the amount of variance captured by the model, an R^2 for linear mixed models was calculated following published methods (20). Results of statistical tests were considered significant at P values <0.05.

Literature search

A PUBMED and Web of Science search for articles written in English was used to identify studies that measured both SF and Hb in cord blood. The Web of Science search was as follows: [(Hemoglobin or Anemia) AND ("serum ferritin" or ferritins) AND ("cord blood" or "fetal blood")]. The PUBMED search was as follows: (Hemoglobins[Mesh] OR Hematocrit[Mesh] OR Anemia, Iron Deficiency[Mesh] OR Hemoglobin*[tiab] OR haemoglobin*[tiab] OR hematocrit*[tiab] OR iron deficiency[tiab]) AND (Ferritins[Mesh] OR ferritin*[tiab]) AND (Fetal blood[Mesh] OR fetal blood[tiab] OR cord blood[tiab]). Figure 2 demonstrates the results from this literature search and a list of main findings from the 106 identified studies can be found in Supplemental Table 1.

Results

Neonatal characteristics

Neonatal characteristics are summarized in Table 1. Maternal characteristics, background data, recruitment procedures, and loss to follow-up have been described in detail elsewhere (15, 17, 21). The mean number of offspring within the multiples cohort was 2 newborns. Consistent with national data in women carrying multiples (22–24), 65% of neonates born to women carrying multiple fetuses were born preterm. One-quarter of these preterm births were caused by pregnancy complications that have been previously reported (16). As expected (24), the majority (71%) of multiple birth neonates were born LBW and 92% of these LBW neonates were those born prematurely. Although pregnant adolescents typically have a higher risk of poor pregnancy outcomes (25), only 2% of babies born to this adolescent population were LBW and none were

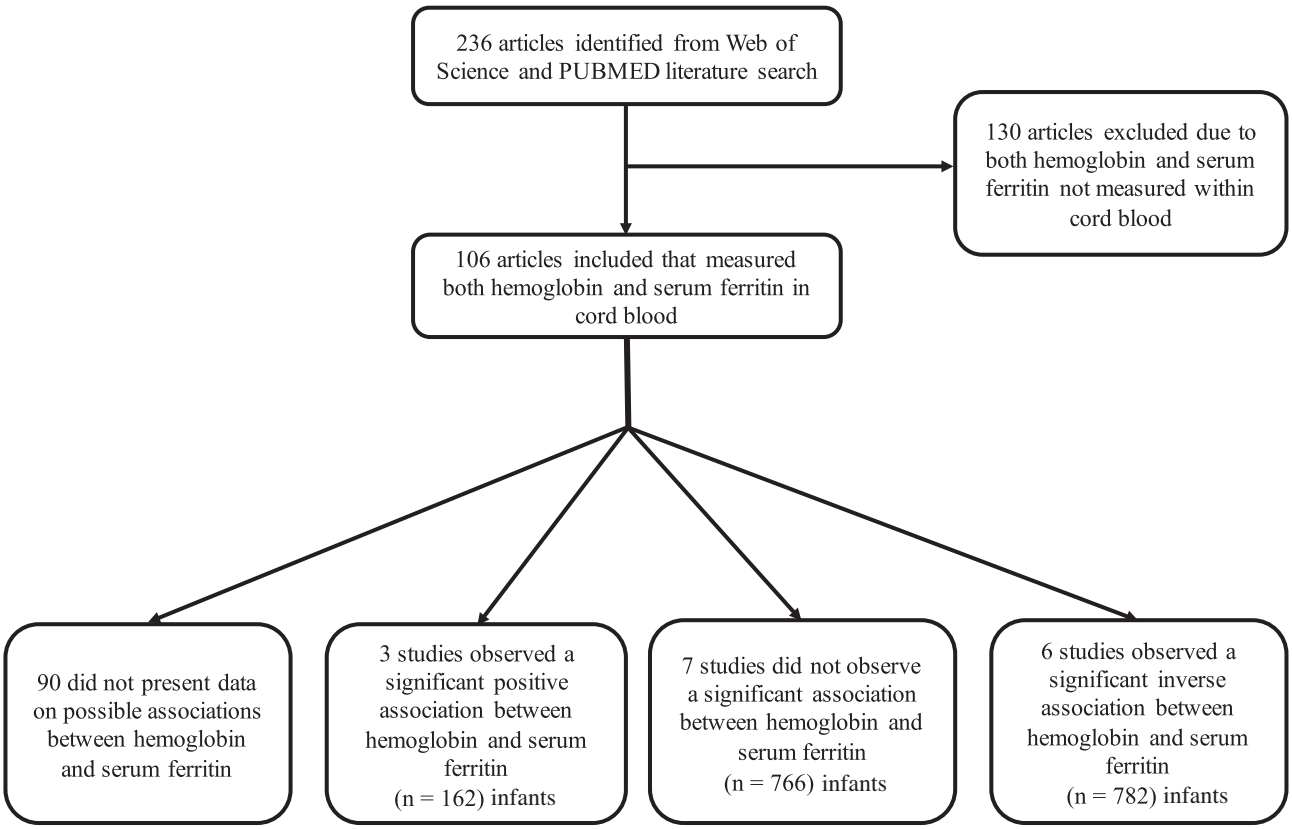

FIGURE 2 Literature search on hemoglobin and serum ferritin in umbilical cord blood. Flowchart of all identified articles from both PUBMED and Web of Science and those included when both serum ferritin and hemoglobin were measured in umbilical cord blood.

born prematurely. There was a higher prevalence of African-American females within the adolescent cohort, consistent with national data demonstrating that adolescent pregnancy disproportionately impacts minorities (24). Within the multiples cohort the majority of neonates (74%) were delivered via C-section, consistent with national data (26). However, only 10% of neonates within the adolescent cohort were delivered via C-section. There was a higher prevalence of female neonates within the multiples cohort, a finding that was consistent with published data documenting an increased prevalence of female births in women carrying multiples (27–30).

Neonatal iron status indicators

Data on Hb, prevalence of anemia, neonatal Fe status indicators, and inflammatory markers are presented in Table 2. There was a significant difference in cord Hb concentrations between the multiples and adolescent cohorts [153.5 g/L (n = 126) versus 143.2 g/L (n = 108), P = 0.03] and within the entire cohort

TABLE 1 Characteristics of neonates born to adolescents or women carrying multiples at birth[1]

Variable	Whole population (n = 234)	Multiples cohort (n = 126)	Adolescent cohort (n = 108)
Gestational age, wk	37.07 ± 3.36	34.70 ± 2.73	39.84 ± 1.22*
Preterm, < 37 wk, %	35	65	0*
Early term, 37–38 wks, %	30	35	24*
Birth weight, g	2709.21 ± 726.32	2219.71 ± 539.80	3280.29 ± 443.87*
Low birth weight, < 2500g, %	39	71	2*
Very low birth weight, < 1500g, %	04	07	00*
Race			
African American, %	46	21	75*
Ethnicity			
Hispanic, %	17	09	26*
Mode of delivery			
Cesarean section, %	45	74	10*
M/F ratio	0.97	0.88	1.08

[1] Data are presented as arithmetic mean ± SD, or percentage.
*Indicates statistical difference from multiples cohort (P < 0.05).

TABLE 2 Umbilical cord red blood cell indices, serum iron status indicators, and iron regulatory hormones in neonates born to adolescents or women carrying multiples[1]

Variable	*n*	Whole population	*n*	Multiples cohort	*n*	Adolescent cohort
Hemoglobin, g/L	234	147.6 ± 1.80	126	153.4 ± 2.30	108	143.2 ± 2.50*
Anemia, %	39	17	15	12	24	22
Serum ferritin, μg/L	234	110.82 (1.06)	126	103.28 (1.09)	108	120.31 (1.07)
Serum iron, mg/L	224	2.64 (1.04)	123	2.97 (1.06)	101	2.28 (1.05)*
Serum EPO, mIU/mL	218	22.40 (1.07)	121	16.64 (1.10)	97	32.45 (1.09)*
sTfR, mg/L	234	6.51 (1.03)	126	5.86 (1.05)	108	7.38 (1.04)*
Standardized serum hepcidin	—	—	126	0.38 (0.99)	107	−0.03 (0.09)
Serum hepcidin, ng/mL			126	13.78 (1.10)	107	92.13 ± 1.09
Standardized serum IL-6	—	—	121	0.50 (1.11)	96	0.67 ± 1.06
Serum IL-6, pg/mL			121	3.81 (1.18)	96	10.22 (1.15)
Standardized serum CRP	—	—	118	0.05 (1.08)	54	0.002 (1.33)
Serum CRP, mg/L			118	0.10 (1.10)	54	0.28 (1.13)
Undetectable CRP, %			80	68	43	80
MCV	191	105.96 ± 0.58	123	107.76 ± 0.74	68	102.72 ± 0.81*
MCH	183	35.76 ± 0.21	117	36.44 ± 0.26	66	34.55 ± 0.28*
MCHC	183	33.75 ± 0.08	116	33.82 ± 0.09	67	33.66 ± 0.14
Serum vitamin B-12, pg/mL	150	717.5 (1.05)	101	699.79 (1.07)	49	755.44 (1.07)*
Serum folate, nmol/L	150	35.25 ± 1.11	112	38.45 ± 1.50	46	31.51 ± 1.48*

[1]Values are arithmetic means ± SE and geometric mean (SE). CRP, C-reactive protein; EPO, erythropoietin; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; sTfR, soluble transferrin receptor.
*Indicates significant difference from multiples cohort ($P < 0.05$).

Hb was negatively associated with gestational age ($\beta = -0.12$, $P = 0.04$, $n = 234$). Other biomarkers that were significantly negatively associated with gestational age were serum Fe ($\beta = -0.04$, $P = 0.001$, $n = 224$), CRP ($\beta = -0.38$, $P < 0.001$, $n = 172$), and folate ($\beta = -1.09$, $P = 0.002$, $n = 158$). Biomarkers that were significantly positively associated with gestational age were sTfR ($\beta = 0.05$, $P < 0.001$, $n = 234$) and EPO ($\beta = 0.13$, $P < 0.001$, $n = 218$). The biomarkers that were not associated with gestational age included: SF ($\beta = 0.024$, $P = 0.16$, $n = 234$), hepcidin ($\beta = 0.02$, $P = 0.32$, $n = 233$), vitamin B-12 ($\beta = 0.01$, $P = 0.37$, $n = 150$), and IL-6 ($\beta = 0.03$, $P = 0.11$, $n = 217$).

Associations between Fe status indicators and regulatory hormones

Pairwise correlations for all Fe status indicators and regulatory hormones are listed in **Table 3**. Within each individual cohort (multiples: $P < 0.0001$, $n = 108$; and adolescent: $P = 0.004$, $n = 126$) and in the combined population ($P < 0.0001$, $n = 234$), a highly significant inverse association between SF and Hb was observed (**Figure 3**). This inverse association remained significant after controlling for maternal and neonatal inflammation using cord IL-6 ($P < 0.01$, $n = 217$), cord CRP ($P < 0.01$, $n = 172$), or both ($P < 0.01$, $n = 165$). In addition, CRP and IL-6 concentrations did not significantly differ in neonates within the highest and lowest Hb tertiles (**Table 4**). The inverse association did not appear to be driven by the high proportion of anemic neonates within this study population, as the inverse association remained significant when only the non-anemic neonates were evaluated ($\beta = -0.63$, $P < 0.001$, $n = 195$). Moreover, the negative association between cord SF and Hb was independent of neonatal race, ethnicity, mode of delivery, gestational age, maternal inflammation, or maternal pre-pregnancy BMI.

Impact of Fe status and regulatory hormones on Hb status

Multiple regression analysis revealed that neonatal determinants of Hb status in the combined cohort were birth weight, SF, sTfR, and race. Together these 4 variables accounted for 22% of the variance in cord Hb (**Table 5**). To further explore interrelations between study variables and their impact on Hb status, a mediation model was developed (**Figure 4**). Using standardized variables within this approach, the strongest associations were evident between cord Hb and sTfR concentrations ($\beta = 0.45$; $P < 0.001$, $n = 221$), and between cord Hb and SF concentrations ($\beta = -0.21$; $P < 0.01$, $n = 221$). Hepcidin exhibited significant direct associations with SF, and EPO exhibited significant direct associations with SF and sTfR. Both regulatory hormones exhibited indirect associations with Hb (**Table 6**). The mediation model explained 23% of the variance found in Hb status.

Literature search on hemoglobin and ferritin in cord blood

The literature search identified 106 studies that reported data on both Hb and SF in umbilical cord blood (Supplemental Table 1). In this relatively large body of literature, only 16 studies reported any information on possible associations between these 2 indicators. The results of these findings can be seen in Figure 2 and Supplemental Table 1. Six of these 16 studies (38%) reported a significant inverse association between Hb and SF. Five of the 6 studies that found an inverse association were undertaken in healthy term neonates born to both anemic and non-anemic mothers (14, 31–34), and the other was a study of neonates born to diabetic mothers (35). Two of the 16 studies (13%) reported a positive association between Hb and SF; these studies were undertaken in 135 total neonates born to women with Fe deficiency anemia ($n = 100$) and non-anemic women ($n = 35$) (36, 37). One additional study also reported

TABLE 3 Pairwise correlations between serum iron status indicators, serum iron regulatory hormones and hemoglobin in umbilical cord blood from neonates born to adolescents or women carrying multiples[1]

	Hb, g/L ($n = 233$–234)	SF, μg/L ($n = 234$)	sTfR, mg/L ($n = 49$–234)	Serum Fe, mg/L ($n = 48$–224)	EPO, mIU/mL ($n = 49$–218)	Std Hep ($n = 217$–234)	Std IL-6 ($n = 213$–217)	Vitamin B-12, pg/mL ($n = 149$–150)	Folate, nmol/L ($n = 145$–150)	Std CRP ($n = 37$–47)
Hb, g/L	1.0000	−0.2610*	0.1724*	0.1452*	−0.0291	−0.1401*	0.0378	0.0833	0.2026*	−0.2543
SF, μg/L		1.0000	−0.1318*	0.1328*	−0.1512*	0.4288*	0.0344	0.1714*	0.0869	−0.0804
sTfR, mg/L			1.0000	−0.0464	0.4457*	−0.2265*	0.1282	0.1174	−0.1376	−0.0962
Serum Fe, mg/L	0.0987			1.0000	−0.0225	−0.0078	0.0586	0.0369	0.3282*	−0.3817*
Serum EPO, mIU/mL					1.0000	−0.0877	0.2666*	0.1307	−0.1776*	0.4240*
Std serum Hep	−0.1201				−0.1367*	1.0000	0.1773*	0.2014*	0.0512	0.3680*
Std serum IL-6							1.0000	0.0000	0.0842	0.4511*
Serum vitamin B-12, pg/mL								1.0000	0.1209	−0.1684
Serum folate, nmol/L									1.0000	−0.3007
Std CRP			−0.4572*	−0.1552	−0.0044					1.0000

[1]Values are Pearson pairwise correlation coefficients (r) for relations between serum iron status indicators, iron regulatory hormones, and hemoglobin in umbilical cord blood from neonates born to pregnant adolescents and women carrying multiples and are shown above the diagonal. Partial correlation coefficients (partial r) for relations between serum iron status indicators, iron regulatory hormones, and hemoglobin in umbilical cord blood from neonates born to pregnant adolescents and women carrying multiples, while controlling for birth weight and using each individual mothers ID number as a random effect, are shown below the diagonal. CRP, c-reactive protein; EPO, erythropoietin; HB, hemoglobin; Hep, hepcidin; SF, serum ferritin; sTfR, soluble transferrin receptor; std, standardized. Serum ferritin, sTfR, Hep, serum Fe, EPO, Hep, IL6, CRP, and vitamin B-12 are logarithmically transformed because of non-normality and serum Hep, serum IL-6 and serum CRP are standardized.
*Indicates $P < 0.05$.

a positive association, but this study evaluated percutaneous umbilical cord sampling over a wide gestational range during pregnancy, which might not reflect status within cord blood at birth (38). The remaining 7 of the 15 identified studies assessed the possible relation between Hb and SF but found that it was not significant. All 7 of these study cohorts comprised healthy term neonates (sample sizes ranging from 44 to 193 neonates) (39–45). In addition, although only 15 of the studies identified in the literature review reported data on the correlation between Hb and SF, 1 additional study (Supplemental Table 1) in a group of 300 neonates born to anemic mothers noted that neonates with anemia (assessed within cord blood) exhibited higher SF concentrations (46).

Discussion

The present study uniquely evaluated Hb, serum Fe status indicators, serum Fe regulatory hormones, and inflammatory markers in umbilical cord blood obtained from a large cohort of neonates. Neonates studied were born to women at higher risk of Fe deficiency, providing opportunities to evaluate interactions between these biomarkers when iron availability across gestation may have been constrained. Similarly, the increased risk of preterm birth in women carrying multiples provided an opportunity to evaluate the possible impact of gestational age on observed associations. A highly significant inverse association between cord Hb and cord SF was evident in these neonates, a finding that was not driven by maternal or neonatal inflammation (CRP or IL-6), or influenced by the gestational age of the neonate at birth. Moreover, a comprehensive literature search on cord Hb and SF lends support to our findings. Our results highlight the challenges inherent in interpretation of Fe status biomarkers in umbilical cord blood and the need to obtain normative data on Fe status at birth in larger cohorts of neonates.

Nearly 20% of neonates studied were anemic and 40–50% of the women who gave birth to these neonates were anemic at delivery (14, 15). The prevalence of anemia was significantly higher among African-American neonates and among their African-American mothers. Normative data on possible racial differences in neonatal Hb concentrations are lacking. Currently, the Institute of Medicine recommends lowering the Hb cutoff for African-American women by 8.0 g/L and for African-American children aged <5 y by 4.0 g/L, although the Centers for Disease Control and Prevention does not recommend race-specific cutoffs for children aged <5 y (47, 48). National Hb data from newborns are not available as The National Health and Nutrition Examination Survey does not include individuals aged <1 y (49). Currently neonatal Hb concentrations are not routinely monitored at birth unless the newborn is identified as "at-risk." The American Academy of Pediatrics has defined "at-risk" neonates as those born prematurely, at low birth weight, or born to women of low socioeconomic status (50).

The majority of Fe found in the human neonate at birth (70%) is present within the RBC compartment (51). Newborn autopsy studies have estimated that every 1 μg/L of SF represents 2.7 mg/kg of storage Fe (31), and that in the presence of fetal hypoxia, Fe use is prioritized for erythropoietic demands at the expense of tissue and storage Fe (8, 52, 53). The exact amount of Fe stored within SF in umbilical cord blood is unknown (54). Within our cohort we observed a highly significant, inverse association between Hb and SF

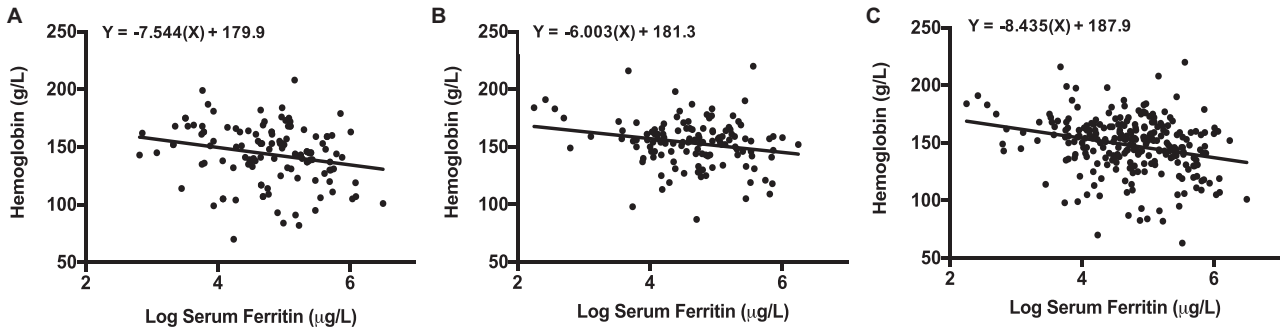

FIGURE 3 Inverse association between umbilical cord hemoglobin and serum ferritin in neonates born to adolescents or women carrying multiples. (A) Neonates in the multiples cohort. (B) Neonates in the adolescent cohort. (C) All neonates as a combined cohort.

concentrations, such that neonates with the lowest Hb status exhibited the highest SF concentrations. Other studies with data on these biomarkers in cord blood support this inverse association between Hb and SF and have attributed this finding to a preferential use of Fe in support of erythropoietic demands (14, 31–35). This interpretation, however, does not explain the presence of elevated umbilical cord ferritin concentrations in neonates with the lowest Hb concentrations.

The unexpected association between low cord Hb and elevated cord SF concentrations highlights what appears to be an inability of some neonates within this cohort to use iron stored within SF for RBC production, a finding that did not appear to be driven by inflammation as detailed above. In addition to Fe, folate and vitamin B-12 deficiencies have also been associated with anemia, but this cohort of neonates were not deficient in either vitamin using standard cutoffs (55, 56). Red blood cell production is also influenced by other nutrients that were not evaluated in this cohort, including vitamin D, zinc, selenium, copper, and vitamin A (57–59). When compared to adult erythrocytes, newborn erythrocytes exhibit markedly different metabolic characteristics, morphologies, and membrane composition that may alter the ability to use storage Fe for Hb production in utero (9).

Hepcidin, EPO, and erythroferrone are regulatory hormones that play a role in Fe metabolism and RBC production (60). Hepcidin limits Fe export from the enterocyte and decreases release from body stores during conditions of Fe sufficiency or inflammation. Hepcidin's role in regulating fetal Fe accretion is increasingly recognized (16, 61), but findings relating hepcidin to Hb concentrations in cord blood are mixed. In our cohort, significant negative direct and indirect associations were observed between Hb and hepcidin. This observation differs from 2 recent studies in 291 term (62, 63) and 121 preterm neonates (63), both of which found no significant correlation between these variables. Additionally, a small study of 45 newborns born to anemic ($n = 30$) or non-anemic ($n = 15$) mothers found a positive association between Hb and hepcidin (36). However, none of these studies included concurrent measures of a full panel of Fe regulatory hormones or inflammatory markers that may also be influencing study outcomes.

Erythropoietinis an erythropoietic hormone that increases Fe use in support of RBC production under conditions of hypoxia. Studies assessing cord blood EPO concentrations in neonates under hypoxic in utero conditions (including pre-eclampsia, placental dysfunction, maternal smokers, or intrauterine growth restriction) found that cord EPO concentrations were elevated compared to neonates under non-hypoxic conditions (64–67). Only 1 of these studies concurrently measured Hb concentrations and found that neonates born to mothers who smoked during pregnancy had higher EPO and Hb concentrations

TABLE 4 Umbilical cord serum iron status indicator means by hemoglobin tertiles from neonates born to adolescents or women carrying multiples[1]

	n	Tertile 1	*n*	Tertile 2	*n*	Tertile 3	*P* value
Hb, g/L	60	11.87 (11.57, 12.17)[a]	125	15.22 (15.01, 15.43)[b]	49	17.69 (17.39, 18.02)[c]	<0.01
SF, μg/L	60	148.90 [123.5, 179.50][a]	125	108.50 [94.77, 124.23][b]	49	78.44 [64.44, 95.48] [c]	<0.01
sTfR, mg/L	60	5.93 [5.34, 6.59] [a]	125	6.43 [5.96, 6.93][a]	49	8.17 [7.31, 9.13][b]	<0.01
BW, g	60	3034.40 (2885.77, 3183.02)[a]	125	2876.18 (2757.51, 2994.86)[ab]	49	2715.05 (2603.97, 2898.13)[b]	<0.01
African American, %	37	62	56	45	15	31	<0.01
Serum EPO, mIU/mL	55	23.45 [18.76, 29.31][ab]	118	20.77 [17.70, 24.36][b]	45	30.5 [24.01, 38.87][a]	0.01
GA, wk	60	37.19 (36.78, 37.60)	125	37.33 (37.03, 37.64)	49	37.77 (37.37, 38.17)	0.10
Serum Fe, mg/L	57	2.59 [2.26, 2.99]	121	2.56 [2.32, 2.83]	46	2.96 [2.53, 3.45]	0.15
Std serum IL-6	53	0.05 [-0.25, 0.34]	118	−0.08 [−0.28, 0.13]	46	0.21 [−0.10, 0.52]	0.33
Serum folate, nmol/L	36	34.19 (30.49, 37.90)	94	36.10 (33.53, 38.68)	28	35.84 (32.09, 39.58)	0.33
Male, %	32	53	55	45	28	57	0.37
Serum vitamin B-12, pg/mL	33	714.23 [599.07, 851.59]	90	695.25 [616.82, 783.66]	27	826.05 [689.00, 990.36]	0.40
Std serum Hep	60	0.20 [−0.05, 0.45]	124	−0.08 [−0.26, 0.10]	49	−0.15 [−0.42, 0.13]	0.44

[1]Data are presented as arithmetic mean (95% CI) or geometric mean [95% CI]. All means were determined controlling for birth weight. Values within a row that do not share a superscript demonstrate statistical differences between serum ferritin tertiles ($P < 0.05$). Undetectable values were excluded from calculations. BW, birth weight; CRP, c-reactive protein; EPO, erythropoietin; GA, gestational age; Hb, hemoglobin; Hep, hepcidin; SF, serum ferritin; sTfR, soluble transferrin receptor; std, standardized.

TABLE 5 Multiple regression analysis to capture determinants of umbilical cord hemoglobin from neonates born to adolescents or women carrying multiples[1]

Hemoglobin	Coefficient	Standard error	*P* value
Intercept	16.931	1.330	< 0.01
Log serum ferritin, μg/L	− 0.764	0.189	< 0.01
Log serum transferrin receptor, mg/L	1.582	0.332	< 0.01
Race	0.597	0.178	< 0.01
Birth weight, g	− 0.001	0.0002	0.03

[1] Multivariate analysis with hemoglobin as the response.

TABLE 6 Indirect effects of umbilical cord iron status indicators and iron regulatory hormones on hemoglobin status from neonates born to adolescents or women carrying multiples[1]

Pathway	Coefficient	Std error	*P* value
EPO-sTfR-Hb	0.1578	0.051	0.002
Hep-SF-Hb	− 0.0843	0.029	0.004
EPO-SF-Hb	0.0475	0.020	0.018
SF-sTfR-Hb	− 0.1389	0.073	0.057
Hep-SF-sTfR-Hb	− 0.0562	0.030	0.063
EPO-Hep-SF-Hb	0.0152	0.010	0.064
EPO-SF-sTfR-Hb	0.03164	0.018	0.075
Hep-sTfR-Hb	− 0.0735	0.048	0.123
EPO-Hep-SF-sTfR-Hb	0.0101	0.010	0.145
EPO-Hep-sTfR-Hb	0.0132	0.010	0.177

[1] Indirect effects on hemoglobin from the mediation model in Figure 4. EPO, erythropoietin; Hb, hemoglobin; Hep, hepcidin; SF, serum ferritin; sTfR, soluble transferrin receptor.

compared to non-smokers (67). Within our cohort no direct association was observed between EPO and Hb, but a significant positive indirect association was mediated through EPO's association with SF, sTfR, and hepcidin. sTfR concentration can be used as a marker of iron restrictive erythropoiesis, but it is important to note that within newborns and infants this marker is also reflective of increased erythropoietic activity. The positive association between EPO and Hb was unexpected, because low Hb concentrations would be expected to stimulate EPO production as seen in adults (68) and neonates with severe anemia (hemolytic anemia and Rh immunization) (69, 70). However, other studies in healthy term neonates also reported a lack of significant direct association between Hb and EPO, although these study cohorts included only non-anemic neonates (71, 72).

Erythroferrone is a newly identified Fe regulatory hormone, which is stimulated by EPO to reduce hepcidin expression (60). A limitation of the current study is that no erythroferrone measurements were obtained because of lack of an available validated human assay when these studies were undertaken. Of note, our current mediation analysis found a direct negative relation between umbilical cord EPO and hepcidin concentrations, an association found to be mediated by erythroferrone in other studies (60, 73–75). More data are needed on neonatal erythroferrone concentrations to determine whether this marker has additional diagnostic utility at this life stage.

The present observations in a group of newborns at increased risk for anemia demonstrated unexpected relations between common Fe status biomarkers and regulators of Fe homeostasis and erythropoiesis. Until normative umbilical cord blood data on these indicators are available, care should be taken when applying assumptions based on observations made in older infants or adults. Our findings highlight concerns with the use of SF as an index of neonatal Fe sufficiency. Elevated concentrations of SF in cord blood were more common among anemic newborns, which may indicate that other factors (e.g., inflammation, EPO insensitivity) may limit use of this pool of iron for erythropoiesis. Improvements in interpretation of these biomarkers at birth is needed to identify neonates with insufficient Fe stores given the increasing links between suboptimal Fe status and subsequent adverse neurodevelopmental outcomes.

Acknowledgments

The authors' responsibilities were as follows—KMD: analyzed and interpreted the data and wrote the manuscript; KOO: designed the research, performed experiments, analyzed and interpreted the data, and wrote the manuscript; YR: performed experiments and assisted with data analysis; FV: assisted with statistical analysis and interpretation of data; RG, EKP, RF, and EN: assisted with data interpretation; and all authors: reviewed and critiqued the manuscript, and read and approved the final paper.

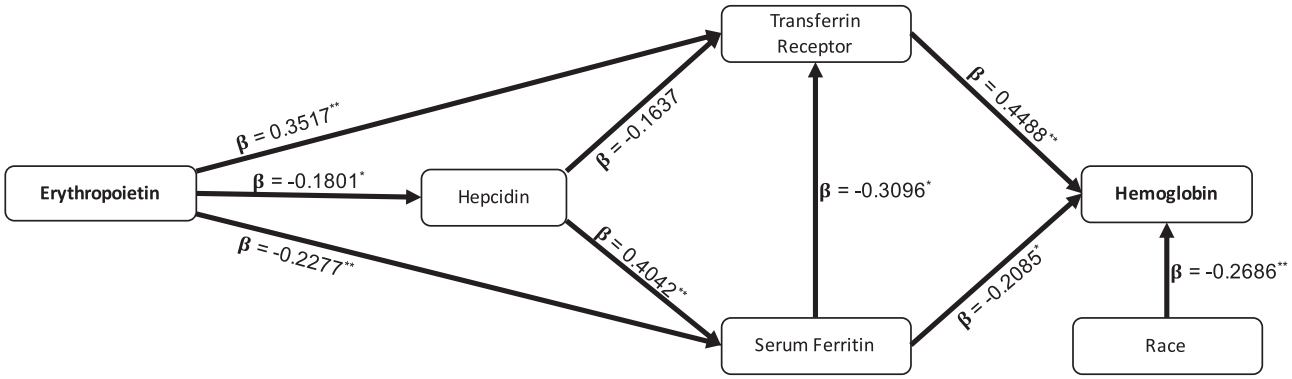

FIGURE 4 Direct effects of umbilical cord serum iron status indicators and serum iron regulatory hormones on hemoglobin status in neonates born to adolescents or women carrying multiples. A mediation model was developed to evaluate the direct and indirect effects of these iron status indicators and regulatory hormones on hemoglobin status in 234 neonates. Each indicator was standardized and the model is controlled for birth weight. * ($P < 0.05$) and ** ($P < 0.001$). Direct pathways are shown with a solid line. Indirect pathways are shown in Table 6.

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