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Iron Metabolism in African American Women in the Second and Third Trimesters of High-Risk Pregnancies

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Abstract

Objective: To examine iron metabolism during the second and third trimesters in African American women with high-risk pregnancies.

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Design: Longitudinal pilot study.

Setting: Large, university-based, urban Midwestern medical center.

Participants: Convenience sample of 32 African American women with high-risk pregnancies seeking care at an urban, maternal-fetal medicine clinic.

Methods: Non-fasting venous blood was collected in the second and third trimesters to assess iron status, hepcidin, and systemic inflammation. Anthropometric and survey data were obtained via self-report. Descriptive statistics were calculated from these data, and changes in the clinical parameters between the second and third trimester were evaluated via paired *t*-tests. Associations among demographic, reproductive, anthropometric, inflammatory and iron-related parameters were also assessed in each trimester.

Results: The mean age of participants was 28.3 (± 6.8) years, and pre-pregnancy body mass index was 31.9 (± 10.7) kg/m^2. In the longitudinal analysis, significant ($p < 0.05$) declines in serum iron, ferritin, transferrin saturation, and C-reactive protein were observed between the second and third trimester. There was no statistically significant change in hepcidin between trimesters. When using a ferritin cut-point of < 15 ng/ml and soluble transferrin receptor > 28.1 nmol/l, 48% of the participants (14 of 29) were classified with iron deficiency in the third trimester.

Conclusion.—In this pilot study, iron deficiency was prevalent among a small cohort of African American women with high-risk pregnancies. Hepcidin concentrations were higher than previously reported in healthy, pregnant, primarily White women, which suggests decreased iron bioavailability in this high-risk group.

Precis

In this sample, results indicated lower iron bioavailability in African American women with high-risk pregnancies.

Keywords

Pregnancy; iron metabolism; African American; high-risk

African American women are more likely to have high-risk pregnancies than women in other racial/ethnic groups (Centers for Disease Control and Prevention, 2013). This disparity may be due in part to higher rates of known risk factors, including pre-existing conditions such as obesity (Ogden, Carroll, & Flegal, 2014), hypertension (Go et al., 2013), type 2 diabetes (Chow, Foster, Gonzalez & McIver, 2012), and adverse pregnancy-related conditions such as gestational diabetes mellitus and gestational hypertension (Ferrara, 2007). Such disorders have also been reported to negatively influence iron metabolism in pregnant women of all racial/ethnic groups (Dao, Sen, Iyer, Klebenov, & Meydani, 2013; Garcia-Valdes et al., 2015; Phillips et al., 2014). Several research teams have linked obesity (Dao et al., 2013; Garcia-Valdes et al., 2015; Jones et al., 2016) and gestational diabetes mellitus (Phillips et al., 2014), conditions that would classify a pregnancy as high-risk, with iron deficiency (ID) and impaired iron metabolism in pregnancy.

Iron deficiency during pregnancy, defined as a measurable decrease in circulating and body iron stores (Suominen, Punnonen, Rajamäki, & Irjala, 1998), is associated with a multitude of well-documented negative maternal and fetal health events that include greater risk for maternal and infant morbidity (Allen, 2000), preterm birth (Siega-Riz et al., 2006), and transient and irreversible neurocognitive defects in infants (Beard, Murray-Kolb, Haas, & Lawrence, 2007). All pregnant women are at risk for ID because a substantial increase in iron is required to support the expansion of the woman's blood volume and the growth of the placenta and fetus (McArdle, Lang, Hayes, & Gambling, 2011). To fulfill this elevated iron demand, pregnant women are advised to increase their dietary iron intake from 16 to 27 milligrams daily (Institute of Medicine, Food and Nutrition Board, 2001). Further, women's bodies adapt to changing iron needs via compensatory mechanisms that allow for greater dietary iron absorption and enhanced placental iron uptake and flux to the developing fetus (Bothwell, 2000; Gambling, Lang, & McArdle, 2011; Rehu et al., 2010). Recent estimates suggest that approximately 30% to 40% of pregnant women in the United States are classified with ID during the third trimester of pregnancy, and racial/ethnic minorities, specifically African American women, are at even greater risk than their non-Hispanic White counterparts (Mei et al., 2011).

Systemic iron metabolism does not differ during gestation and is maintained by the hepatic-derived peptide hormone hepcidin (Ganz & Nemeth, 2012). Hepcidin promotes degradation of the body's only known iron exporter, ferroportin-1 (Fpn). Degradation of Fpn reduces the transport of iron into circulation from the diet and body storage sites (Ganz & Nemeth, 2012; Goodnough, Nemeth, & Ganz, 2010) (see Figure 1). In the non-pregnant and pregnant states, hepcidin is simultaneously regulated by body iron stores, erythropoiesis, and systemic inflammation (Darshan & Anderson, 2009) (see Figure 1). In the presence of systemic inflammation, hepcidin production and secretion from the liver is enhanced, which results in diminished Fpn expression and reduced iron export into circulation from stores and diet or in other words iron restriction (Goodnough et al., 2010) (see Figure 1).

In women experiencing uncomplicated pregnancies, serum hepcidin levels have been reported to be very low or undetectable in the third trimester (Rehu et al., 2010; van Santen et al., 2011; Young et al., 2010). It is believed that suppression of hepcidin is a compensatory mechanism that allows for enhanced dietary iron absorption and efflux of iron from a woman's body storage sites in pregnancy (Rehu et al., 2010). However, in conditions with underlying systemic inflammation, the flow of iron from the diet and body iron stores may be restricted due to elevated hepcidin (Dao et al., 2013). In fact, investigators in one study demonstrated that elevated hepcidin in women during the third trimester was associated with reduced dietary iron absorption (Young et al., 2010). Thus, when coupled with a high demand for iron, such a state could exacerbate ID given that iron from food and supplements may be less readily absorbed due to elevated hepcidin and suppressed Fpn. Therefore, the provision of additional oral iron may not resolve ID or improve iron status in pregnant women with inflammation-driven, hepcidin-mediated iron restriction.

Women with high-risk pregnancies may be a sub-set of pregnant women who are particularly vulnerable to adverse changes to iron metabolism and ID due to inflammation stemming from conditions of high-risk pregnancy (e.g., obesity, gestational diabetes, pre-

existing type 2 diabetes). The goal of this small pilot study was to explore systemic iron metabolism, including hepcidin and systemic inflammation, in African American women during the second and third trimesters of high-risk pregnancies.

Methods

Design and Recruitment

Women who sought care at an urban, maternal-fetal medicine (MFM) clinic affiliated with the University of Illinois Hospital and Health Sciences System were recruited for the pilot study. Eligibility criteria included singleton high-risk pregnancy, African American race, at least 15 years of age, born in the United States, living in the greater Chicago area, and able to read and write English. Exclusion criteria included major fetal anomaly, autoimmune disease (e.g., HIV, type 1 diabetes, rheumatoid arthritis, lupus, and Grave's disease), receiving steroid treatments (including inhalers for asthma), sickle cell disease, or placement of a cervical cerclage. An obstetrician classified women as having high-risk pregnancies based on their risk for prematurity or other pregnancy-related complications. At our MFM clinic, high-risk was defined as previous preterm birth; current preterm birth; younger (< 18 years old) or older age (> 35 years old); pregnancy-related conditions, including gestational diabetes mellitus and pre-eclampsia; and underlying health conditions, including pre-existing hypertension and obesity.

Potential research participants at greater than 19 weeks gestation were approached by research team (i.e., nursing students and student research assistants) in the MFM clinic and provided with a description of the research study. Those expressing interest in the study were invited to attend assessment visits in the second and third trimester of their pregnancies. Informed written consent was obtained at the first visit by research staff. All study procedures were approved by the University of Illinois at Chicago (UIC) Institutional Review Board.

Data Collection

Registered nurses from the UIC Clinical Research Center drew blood from the participants' antecubital veins; fasting was not required prior to the blood draw. At the first visit (second trimester), pregnant women completed a demographic and health questionnaire to gather information pertaining to relationship status, education level, smoking behavior, reproductive history, and past and current health status. The women also completed the Block brief food frequency questionnaire (FFQ) to report dietary intake and supplement use over the previous 3 months (Block et al., 1986). Development and validation of this widely used dietary assessment questionnaire have been reported previously (Block, Hartman, & Naughton, 1990). Participants with FFQ data that was deemed implausible (i.e., < 500 calories or > 5000 calories daily) were excluded ($n = 4$) from the dietary-related analyses. Pre-pregnancy body weight and height were obtained via self-report or abstracted from the UIC electronic health record (EHR) by the research team to calculate pre-pregnancy body-mass index (BMI).

Biochemical Analysis

Venous blood was processed for serum and stored at −80ºC until analysis. Table 1 includes a description of the biochemical indices assessed in this pilot study along with available, normal reference ranges in pregnancy. Serum ferritin, serum iron, transferrin, and transferrin saturation (TSAT) were assessed via immunoassay or spectrophotometry at a commercial lab. Circulating iron is delivered to cells via transferrin and its interaction with the cellular bound transferrin receptor (Beguin, 2003; Berlin, Meyer, Rotman-Pikielny, Natur, & Levy, 2011). Soluble transferrin receptor (sTfR), the inflammatory markers, high sensitivity C-reactive protein (CRP), and high sensitivity interleukin-6 (IL-6) were analyzed in house via immunoassay. Serum hepcidin was assessed by via competitive immunoassay (Ganz, Olbina, Girelli, Nemeth, & Westerman, 2008). The sample size for several of the analytes varied by trimester because of the limited availability of serum (samples were being utilized for several other investigations) and hemolysis of a few samples intended for the iron-related markers. The number of available samples by trimester and number of women included in the longitudinal biomarker analysis are indicated in Table 3.

Iron Status Classifications

We defined the prevalence of depleted iron stores, tissue ID, and ID in participants as follows. Depleted iron stores was defined as serum ferritin < 15 ng/ml (Centers for Disease Control and Prevention, 1998; Perry, Yip, & Zyrkowski, 1995). Tissue ID was defined as sTfR > 28.1 nmol/l (Duffy et al., 2010), with the understanding that sTfR mostly reflects ID of erythroid tissue. Similar to Duffy et al. (2010), a ferritin < 15 ng/ml combined with a sTfR > 28.1 nmol/l was used to define ID in the third trimester only.

Statistical Analyses

Data were managed using the Research Electronic Data Capture web application (Vanderbilt University, Nashville, TN). Statistical analysis was performed using SAS version 9.4 (Cary, NC). Variable distributions were assessed for normality. Log transformations were applied to ferritin, hepcidin, CRP, IL-6, and TSAT because of their skewed distributions. Continuous variables are presented as means [± standard deviations (SD)] or geometric means [95% confidence intervals (CI)], and categorical variables are presented as frequencies and percentages. Paired *t*-tests were used to compare changes in continuous variables between the second and third trimester of pregnancy for women with available data. Spearman correlation coefficients were used to assess associations among maternal age, gravida, pre-pregnancy BMI, inflammatory markers, and the iron-related parameters at each time-point. Statistical significance was set at $\alpha < 0.05$.

Results

Participant demographic, health, and dietary characteristics are presented in Table 2. Thirty-two women were recruited with a mean age of 28.3 ± 6.8 years, gravidity between 1–10, and mean self-report, pre-pregnancy BMI of 31.9 ± 10.7 kg/m^2; 47% of the women were classified as obese based on pre-pregnancy BMI. Due to missing responses for socio-economic data, implausible FFQ data, and limited availability of serum in each trimester, the sample size available for analysis varies and is indicated below and in Tables 2 and 3.

Seventy-seven percent (n = 24 of 31) of participants received benefits from the Special Supplemental Nutrition Program for Women, Infants and Children (WIC), and 71% (n = 22 of 31) received Illinois public aid. Thirteen percent of the participants (n = 4 of 32) reported that they smoked in the second trimester. Nine percent of women (n = 3 of 32) reported gestational diabetes mellitus, 6% (n = 2 of 32) gestational hypertension, and 6% (n = 2 of 32) pre-eclampsia in the second trimester. Mean dietary iron intake from food and supplements was 38.6 ± 26.9 milligrams daily as determined by the FFQ, and 57% of participants (n = 16 of 28) met the RDA for iron during pregnancy (≥ 27 mg/day). Twenty five percent of participants (n = 8 of 32) reported hypertension, and 13% (n = 4 of 32) reported type 2 diabetes mellitus as pre-existing medical conditions.

The biochemical data for the second and third trimesters are presented in Table 3. The mean values presented are for the total available sample for each gestational time point. The longitudinal paired analysis is based on the total available sample or the smaller of the two analytic samples available in the second or third trimester. A significant decline in serum iron, ferritin, TSAT, and a significant increase in transferrin and sTfR was observed between the second and third trimesters in women with available longitudinal data. No significant change in hepcidin was found between the trimesters. However, a significant reduction in CRP and no change in IL-6 from mid- to late gestation were observed.

In the second trimester, 25% of participants (n = 8 of 32) had depleted iron stores based on a ferritin cut point of < 15 ng/ml that increased in the third trimester to 53% (n = 17 of 32), respectively. In the second (n = 20 of 20) and third (n = 29 or 29) trimesters, 100% of participants with available serum were characterized with tissue ID based on sTfR levels > 28.1 nmol/l. In the third trimester, 48% of participants (14 of 29; sTfR missing for 3 women) were classified with ID based on serum ferritin < 15 ng/ml and sTfR > 28.1 nmol/l.

The relationship between demographic, reproductive health, anthropometric and biochemical parameters were examined in each trimester. In the second trimester, we observed a significant positive correlation between serum ferritin and hepcidin (r = 0.65; p < 0.001) and a modest inverse correlation between hepcidin and sTfR (r = − 0.40; p = 0.08), which suggests that hepcidin was being regulated to some degree by body iron stores. Pre-pregnancy BMI was significantly positively correlated with CRP (r = 0.77; p < 0.001) and IL-6 (r = 0.61; p = 0.005) at mid-gestation, which suggests that greater adiposity was associated with increased inflammation at mid-gestation.

In the third trimester, the relationship between hepcidin and ferritin (r = 0.30; p = 0.09) and sTfR (r = 0.30; p = 0.09) weakened (r = 0.30; p = 0.09), which suggests that that iron stores had less of an effect on regulating hepcidin concentrations later in gestation. Ferritin was significantly correlated with TSAT (r = 0.42; p = 0.02), serum iron (r = 0.42; p = 0.02), and sTfR (r = − 0.43; p = 0.02). Ferritin was also modestly correlated with IL-6 (r = 0.34; p = 0.07) providing some evidence that ferritin was influenced by inflammation. Third trimester hepcidin was significantly correlated with dietary iron intake (r = 0.42; p = 0.03), which is consistent with research results that indicate that higher intake of dietary iron can stimulate hepatic hepcidin production and secretion (Collins, Wessling-Resnick, & Knutson, 2008). Pre-pregnancy BMI remained significantly correlated with third trimester CRP (r = 0.69; p <

0.001) and IL-6 ($r = 0.57$; $p = 0.0001$). Age was significantly correlated with ferritin ($r = 0.47$; $p = 0.001$), TSAT ($r = 0.40$; $p = 0.03$) and serum iron ($r = 0.40$; $p = 0.03$), which suggests that greater age was associated with more favorable iron status. No other significant associations were observed in the second and third trimesters among the biochemical indices, pre-pregnancy BMI, age, gravida, and dietary iron intake.

Discussion

Findings from this pilot study of iron metabolism in African American women with high-risk pregnancies suggest a high prevalence of depleted iron stores in the third trimester. The rate of ID in our cohort was 48% in the third trimester, which is similar to the rate previously reported for African American adolescent girls in the third trimester (Iannotti et al., 2005). In an analysis of the National Health and Nutrition Examination Survey conducted by Mei and colleagues (2011), 39% of pregnant women from a nationally representative sample had ID during the third trimester based on low serum ferritin concentrations. Thus, given the importance of iron sufficiency for maternal and fetal health, women with high-risk pregnancies may require close monitoring and screening for ID during the third trimester.

We observed statistically significant reductions of serum ferritin, iron, and TSAT and marked increases in transferrin and sTfR between the second and third trimester, which suggests a decline in iron status during the later stages of pregnancy. This trend is comparable to results in previous reports of iron status during uncomplicated and high-risk pregnancy (Garcia-Valdes et al., 2015; Iannotti et al., 2005; Mei et al., 2011). The observed decrease in iron stores parallels the significant rise in iron transfer to the fetus in the third trimester (Bothwell, 2000).

We did not detect a significant decrease in hepcidin between trimesters. Further, the level of hepcidin in the third trimester observed in our small cohort was somewhat higher than what was reported by researchers in other studies of healthy, pregnant, non-minority adult women. Rehu et al. (2010) reported geometric mean hepcidin concentrations of 12.4 ng/ml (95% CI: 10.5 – 14.6 ng⁄ml) in women when measured at labor and birth, and Young et al. (2010) reported a third trimester median hepcidin concentration of < 5.0 ng/ml (range: < 5.0 – 207 ng/ml; the assay can only detect levels at or above 5 ng/ml). In a stable (i.e., not radioactive), iron isotope study (Young et al., 2010), women with third trimester serum hepcidin > 5.0 ng/ml had lower dietary iron absorption than pregnant women with low or undetectable hepcidin concentrations. Thus, the third trimester hepcidin level observed in our small cohort suggests that iron bioavailability may have been restricted (i.e., reduced dietary iron absorption due to elevated hepcidin). However, additional studies designed to objectively assess iron absorption via stable iron isotopes, including a control group with uncomplicated pregnancies, are needed to confirm this hypothesis.

Nonetheless, this finding is of concern given that hepcidin suppression late in pregnancy is believed to be the compensatory mechanism that facilitates enhanced dietary iron absorption and iron efflux from a woman's stores to be presented to and transferred across the placenta to the fetus (Rehu et al., 2010). Further, impaired iron bioavailability, particularly during the

third trimester, can be detrimental to fetal neurocognitive development (Lozoff et al., 2006; Piñero, Li, Connor, & Beard, 2000).

Hepcidin is simultaneously regulated by inflammation, body iron status, and erythropoiesis (Darshan & Anderson, 2009). Thus, hepcidin can be overexpressed because of underlying systemic inflammation even in the face of concurrent ID (Tussing-Humphreys et al., 2010a). This ID phenotype is considered a mixed ID in which the clinical hallmarks of frank ID (e.g., low serum ferritin, elevated sTfR) and the anemia of inflammation (e.g., elevated systemic inflammation, overexpressed hepcidin given the degree of ID) co-exist (Yanoff et al., 2007). In the third trimester, participants in our study had systemic inflammation higher than what has been reported in healthy pregnancy and comparable to what has been reported in high-risk pregnancy (Dao et al., 2013). Further, mean dietary iron intake was greater than the recommended daily allowance of 27 milligrams, and more than 50% of participants met this intake threshold based on data from the FFQ (Institute of Medicine, Food and Nutrition Board, 2001).

Thus, as suggested previously by our team and others, hepcidin-mediated changes to iron physiology and not inadequate dietary iron influence iron metabolism in persons with underlying inflammation (Cepeda-Lopez, Melse-Boonstra, Zimmerman, & Herter-Aeberli, 2015; Cepeda-Lopez et al., 2011; Menzie et al., 2008; Tussing-Humphreys, Liang, Nemeth, Freels, & Braunschweig, 2009). It is suggested that the degree of hepcidin observed (i.e., mildly overexpressed) in persons with mixed ID may allow for iron mobilization from body iron stores but may lead to restricted dietary iron absorption (Tussing-Humphreys et al., 2010a; Tussing-Humphreys et al., 2010b). Therefore, simply providing additional iron via food or oral supplements may not correct ID or improve iron status in those with inflammation-induced, hepcidin-mediated, mixed ID. Presumably, interventions designed to lower inflammation could positively influence the inflammation-hepcidin-iron axis.

Although we did not observe a significant relationship between inflammation and hepcidin at any point during gestation, it is well established that inflammatory cytokines, specifically IL-6, upregulate hepatic hepcidin production via the JAK/STAT3 pathway (Nemeth et al., 2003, 2004). In a study of pregnant obese and lean adolescents, Cao and colleagues (2015) reported no association between IL-6 and hepcidin at mid-gestation. In another study, inflammation was positively correlated with hepcidin, although the investigators cautioned readers not to overstate this finding given that ferritin, known to be similarly influenced by inflammation, decreased substantially during gestation and was only weakly correlated with inflammatory markers (Garcia-Valdes et al., 2015). Together these findings provide tentative evidence that other hepcidin-regulating signals, including body iron stores and erythropoiesis, may predominate in regulating hepcidin in pregnancy. This would account for why we observed only slightly overexpressed hepcidin in our cohort of women.

Strengths and Limitations

Our study had several strengths that include the focus on a largely understudied population of women with elevated risk for high-risk pregnancies and ID. We also used multiple markers to characterize iron status and concurrently examined systemic inflammation, hepcidin, and dietary iron intake, all of which contribute substantially to iron metabolism in

pregnant women. However, we acknowledge that this study is not without limitations. First, the cohort was a small convenience sample of pregnant African American women residing in Chicago. Because of the small sample size, our findings should be interpreted with caution. We were also not able to stratify the various high-risk conditions, pre-pregnancy BMI, or current smoking status because of the small sample size. Researchers recently suggested that a woman's hepcidin expression and iron metabolism can be affected differentially by pre-pregnancy BMI (Dao et al., 2013; Garcia-Valdes et al., 2015; Jones et al., 2016), gestational diabetes (Phillips et al., 2014), and smoking status (Chełchowska, Ambroszkiewicz, Jabłońska-Głąb, Maciejewski, & Ołtarzewski, 2016).

Also, the findings may not be generalizable to African American women living in other areas of the United States or to women of other race/ethnicities classified with high-risk pregnancies. Further, we did not have a healthy pregnant cohort for comparison. Pre-pregnancy body weight was typically obtained via self-report, which may have resulted in misclassification of pre-pregnancy BMI. However, classifying pre-pregnancy BMI based on self-reported weight is not uncommon in studies of pregnant women (Cao et al., 2015; Holland, Moore Simas, Doyle Curiale, Liao, & Waring, 2013). Many of the women in our study did not seek care at our institution before pregnancy, so often objectively measured weight could not be obtained from the EHR. The self-report nature of the surveys, including the FFQ, is not optimal. Self-reported data are subject to recall bias and socially desirable responses. Further, under-reporting of food intake is common, particularly among obese individuals (Muhlheim, Allison, Heshka, & Heymsfield, 1998). Lastly, we did not assess participants for erythropoiesis because of insufficient serum available for analysis. Erythropoiesis is known to influence iron metabolism and specifically hepcidin production (Nemeth & Ganz, 2009).

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Call outs

1. Pregnant women are prone to iron deficiency because additional iron is required to support the mother's expanding blood volume and growth of the placenta and fetus.
2. In conditions in which the mother's hepcidin is elevated, the flow of iron from mother to fetus may be impaired.
3. The provision of additional iron via food or oral supplements may not correct iron deficiency in pregnant women with inflammation-driven hepcidin-mediated iron restriction.

Clinical Considerations

Hepcidin is the master regulator of systemic iron metabolism (Ganz & Nemeth, 2012). Overexpressed hepcidin may impair a woman's dietary iron absorption and thus exacerbate ID and reduce the amount of iron presented to the placenta and ultimately transferred to the fetus (Koenig, Tussing-Humphreys, Day, Cadwell, & Nemeth, 2014). A mixed ID phenotype (Yanoff et al., 2007) triggered by inflammation-induced, hepcidin-mediated, iron restriction is vastly different than frank ID, which is largely precipitated by inadequate dietary iron intake. Nurses and clinicians alike must consider hepcidin-mediated iron restriction as a possible etiology for ID in some cases in which women have increased inflammation because of underlying medical conditions, including those associated with high-risk pregnancies. However, the use of hepcidin in routine clinical care remains limited although continuing analytical advances may soon allow for this biomarker to occupy a place in clinical practice (Miseta et al., 2015). In the future, hepcidin may be an important clinical biomarker used to determine adverse changes to iron metabolism in pregnancy and identify those for which iron supplementation would be most advantageous (i.e., patients with low hepcidin concentrations). Further, researchers suggest that hepcidin declines ahead of other iron status indicators (Rehu et al., 2010). Thus, hepcidin may help to more quickly identify women who are in need of iron supplementation during pregnancy (Rehu et al., 2010).

For now, in pregnant women with suspected hepcidin-mediated iron restriction or a mixed ID (i.e., those who are not responding to oral iron supplementation), examining inflammatory markers including CRP and erythrocyte sedimentation rate (ESR) in conjunction with iron-related parameters including hemoglobin, ferritin, and sTfR may help to distinguish the etiology of ID in pregnancy. Our group is focused on testing dietary approaches that have the potential to safely modulate systemic inflammation and hepcidin in an effort to improve iron bioavailability in high-risk pregnancy. Lastly, it remains to be determined if overexpressed hepcidin has pathologic effects on maternal, fetal, and long-term offspring health outcomes, which makes this a very ripe area for clinical investigation.

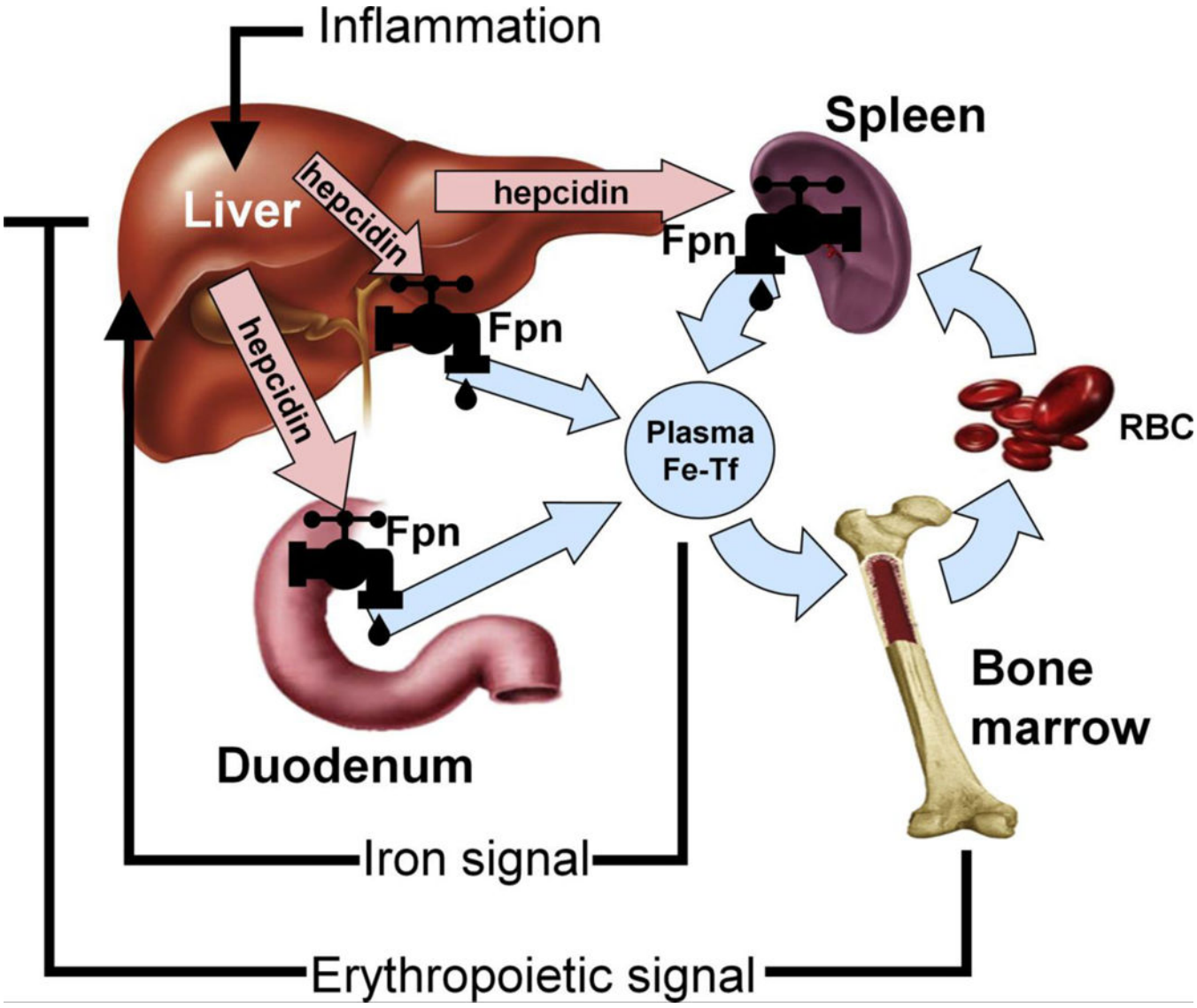

Figure 1.

The role of hepcidin in iron metabolism. Hepcidin-ferroportin interaction determines the flow of iron into plasma. Hepcidin concentration is in turn regulated by iron, erythropoietic activity, and inflammation. From Goodnough, L.T., Nemeth, E., & Ganz, T. (2010). Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood, 116*, 4754–4761. Retrieved from http://www.bloodjournal.org/content/116/23/4754?sso-checked=true.

Note. Fpn=ferroportin, RBC=red blood cells, Fe-TF=transferrin bound iron.

Table 1.

Description of The Biochemical Serum-Based Analytes Assessed In A Pilot Study Of Maternal Iron Metabolism In African American Women With A High Risk Pregnancy

Serum-based analyte	Description	Reported normal reference ranges in pregnancy
Iron status		
Iron (ug/dl)[a]	Measures the amount of iron bound to the iron transport protein transferrin.	2nd trimester: 44 – 178 μg/dl 3rd trimester: 30 – 193 μg/dl
Ferritin (ng/ml)[a]	An intracellular iron storage protein and acute phase protein (Koperdanova & Cullis, 2015). In non-inflammatory conditions, ferritin is a good marker of iron storage in the body. In inflammatory states, ferritin may be upregulated due to inflammation and thus a poor indicator of body iron stores (Baynes, Bezwoda, Bothwell, Khan, & Mansoor, 1986).	2nd trimester 2 – 230 ng/ml 3rd trimester 0 – 166 ng/ml
Transferrin (mg/dl)[a]	A protein that transports iron in circulation.	2nd trimester 220 – 441 mg/dl 3rd trimester 288– 530 mg/dl
Transferrin saturation (TSAT) (%)[a]	Indicates the percent of transferrin saturated with iron	2nd trimester 10 – 44% 3rd trimester 5 – 37%
Soluble transferrin receptor (sTfR) (nmol/l)[b]	A circulating marker that reflects the cellular expression of membrane bound transferrin receptor (Lee, Oh, Park, Lee, & Kim, 2002). Elevated sTfR is associated with increased cellular iron needs and ID. sTfR is not significantly influenced by chronic inflammation or the acute phase response and is thus useful in the differential diagnosis of frank ID versus inflammation-mediated ID (Ferguson, Skikne, Simpson, Baynes, & Cook, 1992)	2nd trimester 39.70 – 67.5 nmol/l 3rd trimester 47.7 – 80.5 nmol/l
Systemic iron regulation		
Serum hepcidin	A liver-derived peptide hormone that regulates systemic iron metabolism (Nemeth et al., 2004). Hepatic hepcidin production is simultaneously regulated by body iron stores, systemic inflammation, and erythropoiesis.	NR
Systemic inflammation		
C-reactive protein (CRP)[a]	An acute phase reactant produced by the liver in response to body inflammation. A non-specific global marker of systemic inflammation.	2nd trimester: 0.4 – 20.3 mg/l 3rd trimester 0.4 – 8.1 mg/l
Interleukin-6 (IL-6)	An inflammatory cytokine that has been shown to stimulate increased liver production of both CRP (Heikkilä, Ebrahim, Rumley, Lowe, & Lawlor, 2007) and hepcidin (Nemeth et al., 2004).	NR

Note. NR = not reported.

[a]Reported normal reference range from Abbassi-Ghanavati, Greer, & Cunningham (2009).

[b]Reported normal reference range from Choi, Im, & Pai (2000).

Table 2.

Second Trimester Characteristics of African American Women With High-Risk Pregnancies (N=32)

Characteristic	Mean ± SD or n (%)
Age (years)	28.3 ± 6.8
Self-reported pre-pregnancy BMI (kg/m^2)	31.9 ± 10.7
Gravida[a]	3.5 (1.0 – 10.0)
Gestational age (weeks)	21.2 ± 1.5
WIC (n = 31)	24 (77)
Public aid (n = 31)	22 (71)
Currently smoking	4 (13)
Obese (BMI ≥ 30.0 kg/m^2)	15(47)
Gestational hypertension	2(6)
Pre-eclampsia	2(6)
Gestational diabetes mellitus	3(9)
Placental abruption	1(3)
Mean daily calories (kcals) (n = 28)	2142.9 ± 715.8
Mean food iron (mg) (n = 28)	14.8 ± 5.2
Mean supplemental iron (mg) (n = 28)	23.8 ± 24.9
Mean total dietary iron (mg) (n = 28)	38.6 ± 26.9
Meeting the RDA for iron, ≥ 27 mg (n = 28)	16 (57)

Note: SD = standard deviation; WIC = Special Supplemental Nutrition Program for Women, Infants and Children, RDA = recommended dietary allowance.

[a]Gravida presented as median and (range).

Table 3.

Comparison Between Second and Third Trimester Anthropometric Iron-Related and Inflammatory Biomarkers For African American Women With High Risk Pregnancies

	2nd Trimester				3rd Trimester				
Measure	**n**	**Mean**	**95% CI**		**n**	**Mean**	**95% CI**		**P[a]**
Serum iron (ug/dl)	32	79.9	67.2	92.6	30	60.2	46.7	73.7	0.0177
Serum ferritin (ng/ml)[b]	32	24.5	18.7	29.9	32	13.5	11.6	16.9	<0.0001
Transferrin (mg/dl)	32	291.9	275.6	307.9	32	342.4	322.7	362.1	<0.0001
Transferrin saturation(%)[b]	32	20.1	14.1	20.8	32	10.5	8.7	12.7	<0.0001
sTfR(nmol/l)	20	43.8	39.7	47.9	29	49.0	44.8	53.3	0.0225
Hepcidin (ng/ml)[b]	32	20.8	16.4	24.5	31	16.6	14.3	19.3	0.0555
IL-6 (pg/ml)[b]	19	2.2	1.8	2.6	29	2.2	1.9	2.6	0.9409
CRP (ng/ml)[b]	19	4.9	3.9	6.2	28	3.9	3.2	4.9	0.0110

Note. CI = confidence interval, P = p-value for paired sample t test (change between trimesters), BMI = body mass index, sTfR = soluble transferrin receptor, IL-6 = interleukin-6, CRP = C-reactive protein.

[a] Sample size used for paired sample t test is the smaller of the two analytic samples sizes reported for the 2nd or 3rd trimester.

[b] Geometric mean and corresponding confidence intervals reported.