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Maternal Obesity Affects Inflammatory and Iron Indices in Umbilical Cord Blood

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Abstract

Objective—To determine the impact of maternal obesity and gestational weight gain across pregnancy on fetal indices of inflammation and iron status.

Study design—Eighty-five healthy term newborns delivered via elective cesarean were categorized by 2 maternal body mass index (BMI) thresholds; above or below 30 kg/m² or above or below 35 kg/m². Umbilical cord plasma levels of C-reactive protein, interleukin (IL)-6, tumor necrosis factor (TNF)- α , ferritin, and hepcidin were assayed. Cytokines released by phytohemagglutinin-stimulated umbilical cord mononuclear cells (MNCs) were assayed.

Results—Maternal class II obesity, defined as BMI of 35 kg/m² and above, predicted higher C-reactive protein and TNF- α in umbilical cord plasma ($P < .05$ for both), and also proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) from stimulated MNC ($P < .05$ for all). The rise in plasma TNF- α and MNC TNF- α was not linear but occurred when the threshold of BMI 35 kg/m² was reached ($P < .005$, $P < .06$). Poorer umbilical cord iron indices were associated with maternal obesity. When ferritin was low, IL-6 was higher ($P < .04$), but this relationship was present primarily when maternal BMI exceeded 35 kg/m² ($P < .03$). Ferritin was correlated with hepcidin ($P < .0001$), but hepcidin was unrelated to either maternal BMI or inflammatory indices.

Conclusions—Class II obesity and above during pregnancy is associated with fetal inflammation in a threshold fashion. Although maternal BMI negatively impacted fetal iron status, hepcidin, related to obesity in adults, was related to iron status and not obesity in fetuses. Pediatricians should be aware of these relationships.

Children born to women with prepregnancy obesity are more likely to develop allergic asthma, obesity, and adult hypertension.¹ Excess gestational weight gain (GWG) may pose similar risks.² Women who are obese prepregnancy are twice as likely to exceed GWG guidelines set by the Institute of Medicine.³ Inflammation may rise as pregnancy body mass index (BMI) in kg/m² and GWG increase.⁴ C-reactive protein (CRP) levels rise after rapid

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weight gain in nonpregnant adults.^{5,6} Excessive GWG has been linked to higher umbilical cord tumor necrosis factor (TNF)- α ,² and prepregnancy obesity has been linked to higher umbilical cord plasma interleukin (IL)-6⁷ and other inflammatory indicators.^{8,9} Data specifically defining the threshold when maternal obesity, and GWG impact newborn inflammatory pathways are limited.

Iron-related and inflammatory pathways are linked by the master iron-regulatory peptide, hepcidin. Normal pregnancy lowers maternal and fetal hepcidin, which works to increase bioavailable iron to the fetus. In adults, hepcidin levels fall in anemia, iron deficiency, and hypoxia, but conversely, hepcidin rises in response to inflammation to reduce iron supply and increase iron sequestration. Obesity stimulates the iron pathway cytokines, including IL-1 β and IL-6,¹⁰ and, in one study, also upregulated maternal hepcidin levels,⁸ decreasing both maternal and fetal iron bioavailability. Fetal hepcidin levels⁸ are likely regulated in a similar fashion to that in adults, with lower hepcidin reported in rat pups with gestational iron deficiency¹¹ and higher umbilical cord levels in human newborns after intrauterine infection.¹² We previously showed that maternal obesity and excess GWG interfered with fetal iron endowment,¹³ but it is not known whether fetal hepcidin levels will mount an appropriate response and decline under low fetal iron conditions. Because iron status impacts fetal neurodevelopment and health,¹⁴ it is important to understand the physiological regulation of fetal iron in maternal obesity and/or excess GWG.

Maternal obesity and large GWG were previously shown to influence innate immune status of newborn infants,² but it is unclear what role iron-related pathways played in this outcome. Iron is essential for normal immune development. Iron deficiency lowers circulating T lymphocyte numbers and can increase infection risks.^{15,16} Maternal iron deficiency during pregnancy has also been linked to a higher likelihood of childhood wheezing.^{17,18} An additional study found that lower umbilical tissue iron content was linked to childhood wheezing,¹⁹ but did not measure immune indices at birth. We previously found poorer umbilical cord blood iron status in the presence of maternal obesity or with GWG exceeding 18 kg¹³ and also demonstrated that either poor umbilical cord iron status or GWG exceeding 18 kg predicted the development of eosinophilia during the first year of life.²⁰ Eosinophilia is used to predict the development of allergic asthma.^{21,22} Cytokine responses from umbilical cord blood mononuclear cell (MNC) cultures are also predictive of future allergic disease.^{2,23} Therefore, it is important for clinicians to understand the roles of maternal obesity, excess GWG, and iron biology in regulating newborn immune function. Our study objective was to measure umbilical cord plasma and MNC cytokines, as well as iron status and hepcidin levels in the same newborns. We hypothesized that obesity at delivery and excess GWG would be related to iron and inflammatory pathways in umbilical cord blood.

Methods

Between June 2012 and July 2014, umbilical cord blood was collected from healthy term newborns with gestational age ≥ 37 weeks. All infants were born by scheduled cesarean delivery at a single center (Meriter Hospital, Madison, Wisconsin), either as repeat cesarean delivery or because of unfavorable fetal lie, with none because of fetal complications or anomaly. Cesarean deliveries were selected to limit the possibility of subclinical

chorioamnionitis and allow for more consistency in the duration of time before sample processing, and because previous work showed that umbilical cord cytokine responses does not differ based on mode of delivery.²⁴ Based on evidence of seasonal fluctuation in cytokine responses,²⁴ enrollment was limited to the summer months. Institutional Review Boards approved this study as exempt from maternal consent. Exclusion criteria included maternal HIV infection, cancer, fetal hemolytic disease, neonatal intensive care unit admission, <37 weeks gestation, major fetal anomaly (cardiac, vascular, renal, or central nervous system), or estimated fetal weight below the first percentile. Markedly abnormal inflammatory indices may reflect sample clotting or other activation during processing, thus, samples were excluded for umbilical cord white cell count or MNC levels of interferon (IFN)- γ or TNF- α either less than the first percentile (1 sample) or greater than the 99th percentile (2 samples). In a prospective fashion, demographic and anthropometric data were extracted from the electronic medical records, including maternal prepregnancy and delivery BMI, race/ethnicity, presence of maternal diabetes, other medical conditions, healthcare payment method as a surrogate of socioeconomic status, newborn sex, and whether the newborn was small, large, or appropriate size for gestational age. Smoking was not recorded, but women delivering at Meriter Hospital have a 9.5% smoking rate. Currently, known risks for developing iron deficiency as an infant were tallied: maternal obesity (BMI ≥ 30 kg/m²), maternal diabetes, minority status, Medicaid insurance, and fetal growth disturbance (small or large for gestational age).^{13,25} Subjects were selected after electronic medical record evaluation for inclusion and exclusion criteria. All subject data were de-identified.

Laboratory Procedures

After delivery, umbilical cord blood was collected in sodium heparin and processed within 18 hours. Because previous work showed subtle differences in cytokine responses in samples held 12 hours before processing,⁴ time before processing was minimized, with median time 2.25 hours before processing, and only 4 samples held more than 12 hours. No maternal blood samples were collected. Complete blood counts were assayed via a Sysmex Poch-100i hematology analyzer (Sysmex, Mulelelin, Illinois) to yield white blood cells (WBCs), WBC differentials, hemoglobin (Hb), hematocrit, mean cell volume, and red blood cell (RBC) distribution width (RDW). A zinc protoporphyrin to heme ratio (ZnPP:H) measured iron-deficient erythropoiesis using a hematofluorometer (Aviv Biomedical, Lakewood, New Jersey), with reticulocyte-enriched ZnPP:H improving ZnPP:H sensitivity. A blinded reviewer determined manual reticulocytes (Reticulocyte Stain, Sigma-Aldrich, St. Louis, Missouri) and nucleated RBC and eosinophil counts on Wright-Giemsa stain smears (Astral Diagnostics, West Deptford, New Jersey).

WBCs and lymphocytes counts were corrected if nucleated RBC were $>5\%$ of the WBCs. Additional plasma assays included ferritin (Genway Biotech, San Diego, California), transferrin (ICL, Portland, Oregon), unsaturated iron binding capacity and iron (Pointe Scientific, Canton, Michigan), hepcidin (DRG International, Springfield, New Jersey), CRP (Genway Biotech), IL-6 (R&D Systems, Minneapolis, Minnesota), and TNF- α (R&D Systems).

After plasma removal, the blood cellular fraction underwent processing for MNC using lymphocyte separation medium (Lonza, Walkersville, Maryland) with centrifugation at $1200 \times g$ for 22 minutes followed by washing of the pellet by centrifugation at $1250 \times g$ for 12 minutes. The PocH-100i quantified the MNC yield. Cells were cultured at 0.5 to 1×10^6 cells/mL in Roswell Park Memorial Institute-1640 with 10% fetal bovine serum, L-glutamine (2 mM), Pen Strep (100 U/mL), and Hepes (25 mM), because preliminary data showed that cytokine responses at this range of cell concentrations did not differ. Cell suspensions with and without phytohemagglutinin stimulant ($200 \mu\text{g/mL}$ final concentration) were added to a 24-well plate incubated at 37°C for 24 hours. After 24 hours incubation, well contents were harvested and centrifuged. Supernatants were snap-frozen with liquid nitrogen and stored at -80°C , and later assayed for IFN- γ , IL-1 β , IL-6, IL-8, IL-12, and TNF- α using a Human Proinflammatory 7-Plex array (Meso Scale Discovery, Rockville, Maryland). Supernatant IL-5 concentrations were assayed separately via a high-sensitivity enzyme-linked immunosorbent assay (R&D Systems). To estimate MNC viability, a thiazolyl blue tetrazolium bromide 0.9 mg/mL solution (Alfa Aesar, Ward Hill, Massachusetts) was incubated at 37°C for at least 4 hours. After incubation, dimethyl sulfide at 200% of the total volume solubilized the cells before spectrophotometer analysis at 570 nm, reference at 630 nm.

Statistical Analyses

Data were initially categorized into 2 groups by the nonpregnant definition of obesity; either maternal BMI $<30 \text{ kg/m}^2$ or by BMI $\geq 30 \text{ kg/m}^2$ at time of delivery. To further distinguish the effects of more extreme obesity (class II obesity and above, defined as BMI $\geq 35 \text{ kg/m}^2$), the women were then subdivided into two groups, either with a BMI $<35 \text{ kg/m}^2$ or by BMI $\geq 35 \text{ kg/m}^2$ at time of delivery. Relative maternal GWG was also determined by the difference between prepregnancy and delivery BMIs (ie, ΔBMI). Once assays were performed, plasma ferritin data were also characterized as high, $>25\text{th}$ percentile ($>84 \text{ mg/L}$), or low ferritin ($\leq 25\text{th}$ percentile).²⁷ Ratios of MNC cytokines were also calculated to assess the relative expression of the targeted cytokines. The Shapiro-Wilk normality test, which uses the null hypothesis principle to determine if the samples originate from a normally distributed population, was used to assess data distribution. If data were normally distributed or if data were normalized after natural log-conversion, a Student t test or 1-way ANOVA with Fisher post hoc test was used. If the distribution was skewed, data were analyzed by Wilcoxon rank-sum test and natural log-converted for linear regression analysis. Simple and stepwise regression analyses examined relationships between variables. BMI categories, presence of diabetes, hepcidin values, ferritin values, and grouping by high or low ferritin were modeled as independent variables.

CRP, plasma cytokines, or MNC cytokine responses were modeled as dependent variables. A P value of $<.05$ was considered significant.

Result

A total of 85 umbilical cord blood samples from healthy babies were studied. Table I presents demographic data, although these were not available for all subjects. Most mothers

identified as Caucasian, and most carried insurance. Fetal sex nearly matched, and 78% were deemed appropriate for gestational age. At delivery, maternal BMI was less than 30 kg/m² in 34 women, and BMI was greater than or equal to 30 kg/m² in 51 women. Diabetes was not present in women with a BMI less than 30 kg/m². Fifty-five percent of neonates had the lowest quartile of umbilical cord plasma ferritin for a healthy newborn population.^{25,27}

Maternal BMI and Umbilical Cord Blood Erythropoietic and Iron Indices

The distribution of ferritin values was lower than that of a normal population,²⁷ with over one-half exhibiting umbilical cord ferritin in the lowest quartile and only 3.5% in the highest quartile. BMI before pregnancy did not impact the umbilical cord erythrocyte iron or other iron indices. However, Figure 1 shows significant relationships between BMI at delivery and iron indices. As maternal BMI at delivery increased, umbilical cord plasma ferritin levels decreased ($R^2 = -0.07$; $P < .04$). BMI, an indicator of GWG, was associated with ZnPP:H ($R^2 = 0.21$; $P < .008$). A direct relationship was also found between DBMI and umbilical cord nucleated RBC ($R^2 = 0.15$; $P < .02$). Nucleated RBCs, median (IQR), were higher in the lowest quartile of umbilical cord ferritin; 193/ μ L (47-496/ μ L) vs 100/ μ L (0-205/ μ L) for newborns in the normal ferritin group, ($P < .05$). When delivery BMI ≥ 35 kg/m² was considered, the umbilical cord RDW was higher ($P < .02$), and plasma ferritin trended lower ($P < .06$) than for neonates with maternal delivery BMI < 35 kg/m² (Table II).

Maternal Delivery BMI and Umbilical Cord Plasma Inflammatory and Iron Status

No significant relationships between pregravid BMI and any umbilical cord inflammatory measurements were found. However, at delivery, maternal BMI was associated with umbilical cord plasma CRP ($R^2 = 0.15$, $P < .002$), percentage of lymphocytes ($R^2 = 0.17$; $P < .0007$), and total lymphocyte number ($R^2 = 0.12$; $P < .007$). When categorized by delivery BMI < 30 kg/m² or ≥ 30 kg/m² groups, inflammatory measurements did not differ between groups. However, when considering delivery BMI ≥ 35 kg/m², then umbilical cord plasma CRP ($P < .02$), IL-6 ($P < .03$), and TNF- α ($P < .007$), lymphocyte percent ($P < .004$), and lymphocyte number ($P < .03$) were all higher than for those with a delivery BMI < 35 kg/m² (Table II).

Most of the iron measures were unrelated to inflammatory measures. However, umbilical cord lymphocyte number was directly related to umbilical cord plasma ferritin ($R^2 = 0.06$, $P < .03$). Umbilical cord plasma IL-6 levels, median (IQR), were higher, 1.51 pg/mL (1.08-2.13 pg/mL), if umbilical cord ferritin levels were in the lowest quartile compared with 1.12 pg/mL (0.79-1.99 pg/mL), with normal umbilical cord ferritin ($P < .04$). When umbilical cord plasma IL-6 levels were examined in a multivariate analysis, having a delivery BMI ≥ 35 kg/m² was predictive of IL-6 ($R^2 = 0.075$, $P < .035$), but the relationship with ferritin approached but did not attain significance ($R^2 = 0.049$, $P < .085$).

Influence of Maternal Delivery BMI and Umbilical Cord Iron Status on Stimulated Cytokine Release

Cell viability was assessed; the MNC mitochondrial activity (thiazolyl blue tetrazolium bromide) postincubation did not differ if grouped by prepregnancy BMI, delivery BMI, or BMI, or by high and low ferritin.

The level of the cytokines, IL-1 β , IL-6, IL-8, IL-12, and TNF- α , released by umbilical cord MNC following phytohemagglutinin stimulation, was higher ($P < .05$ for all) in new-borns with a delivery BMI ≥ 35 kg/m² than in the BMI < 35 kg/m² group (Table II). Cytokine responses did not differ by group when the delivery BMI cut-off was set at ≥ 30 kg/m² or < 30 kg/m². To determine the incremental influence of maternal adiposity on TNF- α , BMI was categorized into 3 groups; BMI < 30 kg/m², BMI ≥ 30 but < 35 kg/m², or ≥ 35 kg/m² (Figure 2), which illustrates a threshold effect of maternal BMI with the shift in TNF- α evident as BMIs exceeded ≥ 35 kg/m². Because lower umbilical cord MNC IFN- γ responses predict asthma,²³ we examined ratios of several cytokines to IFN- γ . The TNF- α :IFN- γ was directly related to delivery BMI ($R^2 = 0.11$, $P < .008$). When categorized by normal or lowest quartile of plasma ferritin, TNF- α :IFN- γ did not differ, but the ratio of Th2 cytokine IL-5 to IFN- γ , 0.045 (0.014-0.90), trended higher in the low ferritin group ($P < .1$), 0.028 (0.18-0.051).

Maternal Delivery BMI and Umbilical Cord Plasma Haptoglobin

Maternal BMI was not directly associated with umbilical cord plasma haptoglobin. However, haptoglobin was associated with other indicators of fetal iron status. Umbilical cord haptoglobin was lower ($P < .0001$) in the lowest quartile of umbilical cord plasma ferritin levels and was related to plasma ferritin ($R^2 = 0.25$; $P < .0001$) (Figure 3). Umbilical cord haptoglobin was also inversely related to Hb ($R^2 = -0.05$; $P < .05$) and to RDW ($R^2 = -0.13$; $P < .001$). Analyzing multiple factors associated with umbilical cord haptoglobin confirmed the relationships with umbilical cord plasma ferritin ($R^2 = 0.15$, $P < .0001$) and Hb ($R^2 = -0.12$, $P < .05$), but showed that maternal BMI was only indirectly linked to haptoglobin via its influence on fetal iron status.

Influence of Maternal Diabetes

Maternal diabetes was present only in those with a delivery BMI ≥ 30 kg/m², including 37.5% of the BMI ≥ 35 group, but only 6.7% of the BMI < 35 kg/m² ($P < .008$). Maternal diabetes increased umbilical cord inflammatory indicators including plasma CRP; 5.00 mg/L (2.7-14.5 mg/L) with diabetes and 4.30 mg/L (0.69-4.41 mg/L) without diabetes ($P < .009$). Multivariate analysis showed that experiencing both maternal diabetes and a BMI ≥ 35 kg/m² was a stronger predictor of plasma CRP ($P < .003$) than was BMI alone ($P < .015$), supporting an interaction between diabetes and BMI. However in multivariate examination of the dual impact of BMI ≥ 35 kg/m² and diabetes on cytokine responses, only BMI, and not diabetes, predicted umbilical cord plasma levels of IL-6 ($P = .051$) or TNF- α ($P < .017$) and MNC levels of IL-6 ($P < .025$) or TNF- α ($P < .045$).

Discussion

Expert panels have not yet defined the threshold at which pregnancy BMI or GWG negatively impacts the fetus. The standard definition of obesity in nonpregnant adults, BMI ≥ 30 kg/m², does not account for pregnancy-related increases in body mass. However, over one-half of women in the US begin pregnancy overweight or obese.²⁸ Since the Institute of Medicine established GWG guidelines in 2009, nearly one-half of women still gain more than is recommended for their prepregnancy weight.²⁹ Overweight and obese women are the

most likely to exceed these recommendations.³⁰ We previously found that obesity adversely impacted fetal iron status¹³ and that fetal iron or GWG predicted altered infant inflammatory pathways.²⁰

Our study shows that an array of fetal inflammatory responses can be predicted by maternal BMI exceeding a threshold of 35 kg/m² at the time of cesarean delivery.

The standard definition of obesity either prepregnancy or at delivery (BMI ≥ 30 kg/m²) did not predict fetal inflammation, but class II obesity or greater at delivery (maternal BMI ≥ 35 kg/m²) impacted iron and increased inflammatory markers, including both plasma and MNC TNF- α . The relationship between delivery BMI and inflammatory markers was not linear but occurred when the threshold of 35 kg/m² was reached. However, the relationship between obesity and umbilical cord iron was linear, with an incremental decrease in iron stores as BMI rose. Increased GWG also appeared to stimulate erythropoiesis, but there was evidence that iron bioavailability did not keep pace with exaggerated erythropoiesis. Umbilical cord plasma levels of hepcidin, the iron-regulatory peptide that is normally an intermediary between iron and inflammation, solely reflected fetal iron status, but not maternal BMI or plasma indices of newborn inflammation (IL-6, TNF- α , or CRP). This finding supports an appropriate fetal adaptation in an attempt to increase iron availability and not upregulation of fetal hepcidin because of inflammatory processes.

We compared umbilical cord plasma and umbilical cord MNC inflammatory indices based on varying definitions of maternal obesity. Fetal inflammatory measurements did not differ at BMI ≥ 30 kg/m² either prepregnancy or at delivery but were higher when categorizing by class II obesity, delivery BMI ≥ 35 kg/m². These data are supported by previous published data showing that umbilical cord plasma CRP levels¹³ and plasma cytokines^{8,9} were unchanged with delivery at BMI ≥ 30 kg/m². However, our data contrast with a study reporting higher umbilical cord plasma IL-6 with prepregnancy BMI ≥ 30 kg/m²,⁷ and a study reporting higher levels of inflammatory mediators in both maternal and umbilical cord plasma with presence of obesity mid-pregnancy.³¹ The other published studies^{7-9,31} did not report delivery BMI, so our previous and current work may not be in disagreement with these other works, if excessive GWG contributed to a BMI at delivery higher than 35 kg/m². The current study included a limited number of measured cytokines, but other cytokines responses, including those in the transforming growth factor family, may be altered by maternal obesity during pregnancy and should be studied.

Expression of cytokines, IL-6 and TNF- α can be upregulated in iron deficiency.³² Obesity in nonpregnant adults is characterized by chronic inflammation that is further propagated by altered adipocyte function, including increased expression of pro-inflammatory TNF- α and IL-6.^{33,34} We found higher fetal plasma IL-6 in those with poorer fetal iron status, but the relationship was primarily driven by BMI once it exceeded 35 kg/m². Because diabetes was common in our higher BMI cohort, our data also support work describing that maternal diabetes upregulated umbilical cord inflammatory markers and plasma TNF- α .^{35,36} Our data in the higher BMI group are consistent with findings that greater maternal GWG predicted higher umbilical cord MNC production of TNF- α .² This agreement is important because if higher umbilical cord MNC TNF- α persisted into infancy, it predicted asthma.²

Iron is essential for proper function and maintenance of the immune system, affecting both innate and adaptive cell function.³⁷ This mechanistic study was undertaken because we previously found that fetal iron endowment was the link between excessive maternal GWG¹³ and eosinophilia at 6-12 months of age.²⁰ Umbilical cord MNC and plasma TNF- α and IL-6 were investigated in the current study because previous work found that GWG was linked to high umbilical cord levels of MNC TNF- α ,² and that gestational iron deficiency upregulated placental expression of IL-6 and TNF- α .³⁸ Published work in children also showed that iron deficiency stimulated T-cell production of IL-6 and TNF- α .¹⁸ However, in the current study, no relationship between umbilical cord plasma TNF- α and iron was found, although iron status independently predicted IL-6 until BMI exceeded 35 kg/m². It is possible that our sample size limited our findings or that diabetes,^{39,40} rather than obesity, controlled the interaction between iron and inflammation.

However, when this question was tested specifically in multivariate analysis, BMI, and not diabetes, predicted umbilical cord plasma and MNC levels of IL-6 or TNF- α .

Previously, greater levels of plasma or MNC IL-6 and TNF- α were found in plasma of macrosomic offspring of mothers with diabetes,³⁶ a population known to be at great risk for tissue iron depletion.^{39,41} Despite the current study and others reporting low fetal iron status after diabetic gestation, maternal iron status in diabetic pregnancy has been reported as high, not low, consistent with tissue sequestering and diabetic placental dysfunction that results in inadequate transfer of iron to the fetus.⁴² Because excessive body iron may accompany diabetes in nonpregnant adults,⁴³ further investigation of both maternal and fetal iron status, as well as the regulation of iron transfer to the fetus during diabetic pregnancy is warranted.

Balance between T-lymphocyte cytokine responses may be important, particularly with excessive T-helper (Th) responses in allergy and asthma.⁴⁴ We studied the Th2 cytokine IL-5⁴⁵ and Th1 cytokine IFN- γ to determine the MNC Th2:Th1, and saw a trend for a greater ratio in the low ferritin group. However, imbalanced Th2:Th1 cytokine responses may not be seen in umbilical cord MNC of children ultimately developing asthma, and relatively higher umbilical cord MNC levels of either IL-13, a Th2 cytokine, or of IFN- γ , a Th1 cytokine,²³ may protect against wheezing in childhood. With recent advent of more sensitive cytokine assays, further work is needed to understand MNC cytokine balance.

Iron status at birth is critical because one-half of the iron needed for infant growth is obtained before birth and impaired iron status may persist into early childhood.¹⁴ Maternal obesity is an emerging risk factor for newborn iron deficiency.^{8,13} The current study reaffirms our prior work showing a direct relationship between maternal obesity and fetal iron status in newborns with risk factors for developing iron deficiency in infancy.¹³ Fetal iron status in this cohort of elective cesarean deliveries may be less impacted at BMI 30 kg/m² than those with risk factors for developing iron deficiency,¹³ but iron status was clearly impacted at BMI 35 kg/m². All 3 compartments of iron in fetal blood (storage, erythrocyte, and transport) appeared to adapt to the block in iron delivery, as evidenced by the relationship between iron transporter levels and worsening erythrocyte iron indices. It is unlikely that excess iron contributed to inflammation, as one-half of the fetuses exhibited values in the lowest quartile for umbilical cord ferritin, but none were above the 90th

percentile.²⁷ Our cohort consisted entirely of elective scheduled cesarean deliveries and contained a large percentage of women with obesity. Our observations may be important to pediatricians because iron status in infancy can be made poorer by cesarean delivery because of a lack of normal umbilical cord transfusion of iron-rich erythrocytes.⁴⁶

The current study analyzed inflammatory indices and iron indices with hepcidin levels in umbilical cord blood of neonates born to mothers with obesity. The peptide hepcidin is the master regulator of iron homeostasis, with higher levels inhibiting the iron export channel in enterocytes, trophoblasts or hepatic macrophages.^{47,48} Elevated hepcidin is both an adaptive response to infection and a propagator of inflammation in older adults.^{8,48,49} In normal pregnancy, maternal hepcidin falls in response to lower fetal hepcidin in order to maximize maternal-fetal iron transfer.⁸ However, proinflammatory cytokines in inflammation or obesity upregulate hepcidin,⁴⁷ an acute phase reactant.⁴⁹ A small study found that maternal, but not fetal, hepcidin levels were higher with maternal obesity⁸ and that maternal hepcidin was unrelated to fetal hepcidin.⁸ The current study similarly found that the fetal hepcidin-iron axis was unrelated to maternal BMI, but also found that fetal hepcidin was related to iron stores, appropriately falling in response to depleted fetal tissue iron, but not affected by the proinflammatory fetal milieu. These results pinpoint maternal iron delivery in class II obesity as the problem and not impaired fetal signaling of iron needs to the placenta. This is an important distinction because the fetus may be amenable to interventions to improve fetal or neonatal iron status.

Study limitations include the relatively small sample size and the lack of prepregnancy and delivery BMI data for some samples. However, an advantage was the highly sensitive new generation multiplex cytokine analysis, including very few cytokines reading below detectable limits. Current findings may be limited to children delivered without labor, although one study reported that umbilical cord MNC release of IFN- γ , IL-5, IL-13, and IL-10 cytokine responses did not differ between those with vaginal and cesarean delivery.²⁴ Because epidemiologic analyses have suggested cesarean delivery may be associated with greater risk of allergy and asthma,⁵⁰ additional data about cytokine activity in umbilical cord blood may set forth putative immune pathways for future investigation.

In summary, obesity decreased fetal iron stores in a linear fashion. If maternal BMI reached 35 kg/m² by the end of pregnancy, cytokine responses were driven predominantly by BMI and not by iron. At delivery, once the threshold of BMI at 35 kg/m² was reached, either as a consequence of excessive GWG and/or higher baseline BMI, an inflammatory fetal milieu was observed. Class II obesity at delivery predicted fetal plasma CRP, IL-6, and TNF- α , as well as several MNC cytokines including TNF- α , findings that were not appreciated below this BMI threshold. We speculate that this cascade established a pattern that may predispose a child to an allergic or inflammatory phenotype later in life. Mechanisms behind the epigenetic programming of allergic airway disease are not well established. These altered inflammatory responses may inform potential pathways programmed during fetal life that lead to allergic airway disease. Although the predictions described above do not confirm causality, maternal BMI at delivery might play a role in altering childhood inflammatory patterns and chronic health risks for children.

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Glossary

BMI	Body mass index
CRP	C-reactive protein
GWG	Gestational weight gain
Hb	Hemoglobin
IFN	Interferon

IL	Interleukin
MNC	Mononuclear cell
RBC	Red blood cell
RDW	RBC distribution width
Th	T-helper
TNF	Tumor necrosis factor
WBC	White blood cell
ZnPP:H	Zinc protoporphyrin to heme

Figure 1.

Relationships between iron status and BMI. Umbilical cord plasma ferritin and ZnPP:H are expressed on $\log_{10}$ scale. **A**, Umbilical cord plasma ferritin levels ($\mu\text{g/L}$), a measure of storage iron, were indirectly related to maternal delivery BMI (kg/m^2). **B**, Umbilical cord ZnPP:H ($\mu\text{mol/mol}$) rise as erythrocyte iron falls and were directly related to BMI (kg/m^2), a surrogate of GWG.

Figure 2.

Relationship between obesity and TNF- α by grouping delivery BMI into 3 groups, BMI <30 kg/m², n = 33; BMI $\geq$ 30 and <35 kg/m², n = 21; and BMI $\geq$ 35 kg/m², n= 16. Bar graph indicates mean (SD), *P* values listed. **A**, Umbilical cord plasma TNF- α (pg/mL) is higher in group with BMI $\geq$ 35 kg/m² than other 2 groups. **B**, PHA-stimulated umbilical cord MNC TNF- α (pg/mL) trends higher in group with BMI $\geq$ 35 kg/m² than other 2 groups. *NS*, not significant.

Figure 3.

Relationships between umbilical cord levels of the master iron regulator, plasma hepcidin (ng/mL), and umbilical cord plasma ferritin (mg/L). Data were categorized by high (normal) ferritin (≥ 84 mg/L) vs lowest quartile of umbilical cord plasma ferritin (< 84 mg/L) showing that **A**, umbilical cord plasma hepcidin levels were lower in the low ferritin group. **B**, A direct relationship between umbilical cord plasma levels of hepcidin (ng/mL) and ferritin (mg/L) was determined.

Table I
Maternal and fetal demographic data

Ethnic and racial distribution	
Caucasian	73%
African American	5%
Latina	11%
Asian/Southeast Asian	11%
Insurance coverage	
Medicaid or self-pay	27%
HMO insurance	73%
Sex	
Male	48%
Female	52%
Fetal growth measurements	
AGA	78%
SGA	11%
LGA	11%
Maternal gestational diabetes	
If BMI <30 kg/m ²	0%
If BMI 30 kg/m ²	25%
If BMI 35 kg/m ²	37.5%
Umbilical cord ferritin *	
Lowest quartile	55%
Highest 3 quartiles	45%

AGA, appropriate for gestational age; HMO, health maintenance organization; LGA, large for gestational age; SGA, small for gestational age.

* Lowest quartile 84 µg/L; highest quartile >84 µg/L.^{25,27}

Table II
Umbilical cord iron and inflammatory indices by BMI <35 or ≥ 35 kg/m²

	BMI <35 kg/m ² , n = 47	BMI ≥ 35 kg/m ² , n = 16	P value
Iron measurements			
Hb (g/dL)	14.6 (1.4)	14.5 (1.7)	.8
HCT (%)	44.7 (4.1)	44.2 (5.7)	.8
RDW (%)	16.9 (1.2)	17.8 (1.5)	<.012
MCV (fL)	107.3 (4.8)	108.0 (5.3)	.6
Nucleated RBC (cells/μL)			
ZnPP* (μmol/mol)	102 (90-125)	101 (86-144)	.6
Plasma iron* (μg/dL)	239 (147-260)	192 (76-213)	.11
TSAT* (%)	58.5 (40.3-79.2)	64.3 (43.5-76.9)	.8
Plasma Tf† (g/L)	1.78 (1.58-2.24)	2.09 (1.73-2.29)	.18
Plasma ferritin* (mg/L)	76.4 (55.3-128.6)	64.7 (43.0-80.3)	<.06
Plasma hepcidin (ng/mL)	13.4 (5.8)	12.5 (5.4)	.6
Inflammatory measurements			
WBC (cells × 10 ³ /μL)	12.7 (3.4)	11.9 (2.8)	.4
Eosinophils† (cells/μL)	378 (207-527)	301 (165-563)	.4
Lymphocyte percent† (%)	38.4 (33.5-44.2)	49.8 (38.4-54.0)	<.004
Lymphocytes† (cells/μL)	1543 (1476-1610)	1681 (1531-1831)	<.03
Plasma CRP† (mg/L)	4.0 (2.1-5.9)	5.6 (2.9-12.2)	.02
Plasma IL-6† (pg/mL)	1.37 (1.01-1.99)	2.00 (1.44-2.96)	<.03
Plasma TNF-α (pg/mL)	1.56 (0.38)	1.87 (0.35)	<.007
Cytokines (pg/mL)			
IFN-γ†	241 (192-419)	342 (225-450)	.3
IL-1β*	1268 (419-2724)	2111 (1149-5058)	<.045
IL-6*	3330 (3044-2635)	5110 (3174-6847)	<.015
IL-8*	3288 (3174-3653)	4790 (3351-6691)	<.005
IL-12†	95.6 (82.4-122.6)	104.1 (85.3-178.4)	<.042
TNF-α*	2761 (2165-3046)	3565 (2570-5174)	<.047
IL-10	255 (150-444)	394 (237-529)	.14
IL-5†	8.44 (4.47-15.3)	18.13 (3.79-32.93)	.6
Cytokine ratios			
TNF-α:IFN-γ†	10.3 (6.8-13.8)	12.2 (8.5-15.9)	.16
IL-5:IFN-γ†	0.039 (0.022-0.067)	0.060 (0.013-0.098)	.8

HCT, hematocrit; MCV, mean cell volume; TSAT, transferrin saturation; Tf, transferrin.

All others = mean (SD), *t* test.

Significant differences are indicated by bolded data.

* Median (IQR), log-converted, nonparametric test.

[†] Median (IQR), log-converted, *t* test.

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