Maternal Obesity Affects Inflammatory and Iron Indices in Umbilical Cord Blood

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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/m^2 or above or below 35 kg/m^2. 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/m^2 and above, predicted higher C-reactive protein and TNF-α in umbilical cord plasma ($P < .05$ for both), and also proinflammatory cytokines (IL-1β, 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/m^2 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/m^2 ($P < .03$). 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. *(J Pediatr 2016;172:20-8)*.

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/m^2 and GWG increase.[4] C-reactive protein (CRP) levels rise after rapid weight gain in nongravid 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)-6[7] 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-1β and IL-6,[10] and, in one study, also upregulated maternal hepcidin levels,[8] decreasing both maternal and fetal iron bioavailability.[8] Fetal hepcidin levels are likely regulated in a similar fashion to that in adults, with lower hepcidin reported in rat pups with gestational iron deficiency[11] 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

BMI	Body mass index	RBC	Red blood cell
CRP	C-reactive protein	RDW	RBC distribution width
GWG	Gestational weight gain	Th	T-helper
Hb	Hemoglobin	TNF	Tumor necrosis factor
IFN	Interferon	WBC	White blood cell
IL	Interleukin	ZnPP:H	Zinc protoporphyrin to heme
MNC	Mononuclear cell		

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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 kg[13] 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/m^2), 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,[24] 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, Mulelein, 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.[26] 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 × g for 22 minutes followed by washing of the pellet by centrifugation at 1250 × 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 μg/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-1β, IL-6, IL-8, IL-12, and TNF-α using a Human Proinflammatory 7-Plex array (Meso Scale Discovery, Rockville, Maryland).