Maternal Perceived Stress during Pregnancy Increases Risk for Low Neonatal Iron at Delivery and Depletion of Storage Iron at One Year

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Objective To investigate the impact of maternal stress during pregnancy on newborn iron and stage 1 iron deficiency at 1 year of age.

Study design In total, 245 mothers and their newborn infants (52% male; 72% white) were recruited at the Meriter Hospital Birthing Center on the basis of known risk factors for iron deficiency. Umbilical cord blood hemoglobin and zinc protoporphyrin/heme (ZnPP/H) were determined to evaluate erythrocyte iron and plasma ferritin was determined to reflect storage iron. Mothers retrospectively reported stress experienced previously during pregnancy on a 25-item questionnaire. Blood was also was collected from 79 infants who were breastfed at 1 year of age.

Results Maternal recall of distress and health concerns during pregnancy correlated with cord blood ZnPP/H indices ($r = 0.21$, $P < .01$), even in the absence of major traumatic events. When concurrent with other known risks for iron deficiency, including maternal adiposity, socioeconomic status, and race, maternal stress had a summative effect, lowering cord blood iron. At 1 year, 24% of infants who were breastfed had moderate iron deficiency (plasma ferritin <12 μg/L). Higher cord blood ZnPP/H was predictive of this moderate iron deficiency (95% CI 0.26-1.47, $P = .007$). When coincident with maternal reports of gestational stress, the likelihood of low plasma ferritin at 1 year increased 36-fold in breastfed infants as compared with low-stress pregnancies (95% CI 1.33-6.83, $P = .007$).

Conclusions Maternal recall of stress during pregnancy was associated with lower iron stores at birth. High cord blood ZnPP/H, reflecting low erythrocyte iron, was correlated with the likelihood of stage 1 iron deficiency at 1 year, when rapid growth can deplete storage iron in breastfed infants. *(J Pediatr 2018;■■:■■-■■).*

Iron deficiency is prevalent worldwide,[1] more common in women,[2] and of clinical concern for children.[3-6] Several gestational conditions can reduce iron levels in newborn infants, including maternal obesity, gestational diabetes, hypertension, and fetal overgrowth, increasing the subsequent risk for infantile iron deficiency.[7-9] Despite a reduction in clinical anemia, moderate (stage 1) iron deficiency in the US has remained stable at >7%, especially in infants of younger and poorer mothers.[2,10-12] The American Academy of Pediatrics Committee on Nutrition and the World Health Organization now recommend universal screening for anemic or preanemic iron deficiency at 1 year of age, in addition to earlier screening of high-risk infants.[1,13] Prevention programs would benefit from a more comprehensive identification of maternal and prenatal risks that contribute to iron deficiency.

Iron sequestration is an evolutionarily conserved response to infection, trauma, and stress, mediated in part by a hepcidin-induced reduction of iron absorption. Although adaptive acutely, sequestration may inhibit placental iron transfer.[14-17] Consistent with this view, stress can decrease iron absorption, inhibiting erythropoiesis and hemoglobin (Hb) synthesis.[18,19] Daily maternal stress in pregnant monkeys increased the likelihood that infants became anemic.[20] Furthermore, Israeli women who were living in a war zone during their first trimester of pregnancy delivered infants with lower cord blood ferritin (cord blood plasma ferritin), a primary iron-storage protein.[21] Our study evaluated whether these conclusions generalize to subjective experiences of a stressful pregnancy and included a follow-up determination of the incidence of stage 1 iron deficiency in breastfed infants. We focused on enduring effects in infants who were breastfed because they would not have access to the extra iron in fortified formula.[6] Maternal stress also may synergize with other factors known to affect the placental transfer of iron,[9] such as obesity, poverty, and minority background, and thus participants with several of these known risk factors were recruited at delivery.[10,22,23]

BMI	Body mass index
Δ-ZnPP/H	Delta zinc protoporphyrin/heme
Hb	Hemoglobin
LGA	Large for gestational age
RE	Reticulocyte-enriched
SES	Socioeconomic status
ZnPP/H	Zinc protoporphyrin/heme

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Methods

This prospective, observational cohort study enrolled women and their healthy newborn infants with risk factors for infantile iron deficiency. Known risk factors for insufficient fetal iron allotment included maternal anemia diagnosed at the initiation of prenatal care, maternal obesity, gestational diabetes, late preterm birth, fetal undergrowth or overgrowth, and maternal ethnic minorities or low socioeconomic status (SES).[7,9,24] To evaluate obesity, maternal morphometric measures were used to calculate prepregnancy and delivery body mass index (BMI). Small for gestational age (birth weight <10th percentile), large for gestational age (LGA; birth weight >90th percentile), and maternal diabetes were verified from the digital health records. Assistance from the supplemental program for Women, Infants, and Children or Medicaid was used as a proxy for designating lower SES. Because male infants often are more vulnerable to gestational stress, and their tendency for faster growth places greater demands on iron,[25,26] we considered the additional influence of infant sex.

After informed written consent was obtained, women who were >18 years old, fluent in English or Spanish, and with a singleton pregnancy participated. Exclusion criteria included fetal congenital anomaly, chromosomal abnormality, neonatal intensive care unit admission or other complications, and birth at <35 weeks of gestation (given that placental iron transfer is greatest in late pregnancy).[27] Women were not selected for having experienced extreme stress or major stressful life events. To maximize generalizability, we included infants born at the Meriter Hospital Birthing Center via vaginal or cesarean delivery. A subset of the vaginal births, through provider preference, had cord clamping delayed until pulsations had stopped. This study was approved by the institutional review boards of the University of Wisconsin-Madison and Meriter Hospital, and recruitment took place between June 2008 and August 2010.

Blood Collection at 1 Year of Age in Infants Who Were Breastfed

Follow-up blood collection evaluated infant iron status at 1 year, with a focus on the subset who breastfed for >6 months to preclude the influence of iron-fortified formulas. Of the 245 participants who met inclusion criteria, completed the stress questionnaire, and had cord blood iron indices at delivery, 136 returned for the later blood tests. Seventy-nine of the 136 infants (32% of original sample) were breastfed until at least 6 months of age and were included in this follow-up analysis. Stage 1 iron deficiency at 1 year was defined as plasma ferritin <12 μg/L.[28] Three children had mildly elevated white blood cell counts (<16.5 10^9/L) at 1 year of age. We verified that plasma ferritin did not differ in these infants and found no relationship between cell count and iron indices. Dietary histories also were obtained, including duration of breastfeeding, formula use, and age when solid foods were introduced.

Sample Collection and Laboratory Tests

Umbilical cord blood was collected at delivery, stored at 4°C in EDTA anticoagulant tubes, and assayed within 8 days. After being washed to remove interfering pigments, zinc protoporphyrin/heme (ZnPP/H) was measured with a FrontFace Hematofluorometer (Aviv Biomedical Co, Lakewood, New Jersey).[29] ZnPP/H is a measure of zinc substitution for unavailable iron in red blood cells and known to be sensitive to moderate iron deficiency. The top fraction or reticulocyte-enriched (RE) ZnPP/H was measured in the immature, lightest 6.25% of cells, in a fashion analogous to reticulocyte Hb. Δ-ZnPP/H was calculated as the difference between the washed and RE-ZnPP/H and is especially sensitive for identifying impaired erythrocyte iron delivery in neonates.[29] Plasma ferritin was quantified with a commercial human enzyme-linked immunosorbent assay kit by Bio-Quant (San Diego, California) in duplicate, with intra-assay coefficient of variation <7.5%

Perceived Maternal Stress

Women completed the Perceived Stress During Pregnancy questionnaire within 48 hours after delivery. Because pregnancy-specific anxiety rather than general anxiety has been more related to birth outcomes and neuroendocrine activation,[30,31] our questionnaire focused on perceived stress during gestation. It was adapted from the Prenatal Social Environment Inventory[32] used by others.[33,34] The adapted questionnaire probed stressors specific to pregnancy health and wellbeing concerns and stress associated with finances, parenting, familial and partner relationships, housing, and employment (see **Appendix** for final questionnaire and validation [available at www.jpeds.com]). All items were scored on a 0-10 Likert scale, ranging from 0 (did not happen) to 10 (extremely disturbing), with the lowest total score being 0 and 250 the highest score. Total scores were used, and a stress score greater ≥32 (mean score) was considered to be indicative of having experienced some stress during pregnancy.

Statistical Analyses

Statistical tests were conducted via SPSS, Version 23.0 (IBM Corp, Armonk, New York) and R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2015). For the questionnaire evaluation, internal-consistency reliability was examined with the Cronbach α and item-total correlation. Factor analysis with principal axis factoring and varimax rotation was conducted to explore factor structure and to determine construct validity. The normality of the distribution for each continuous study measure was tested with the Shapiro–Wilk test and log-transformed as appropriate. To limit the influence of extreme outliers, 1 RE-ZnPP/H and Δ-ZnPP/H value was winsorized and set at the upper 3 SD point.[35] ANCOVA and linear regression were used to test the association between maternal stress and the infants' iron status. Post hoc testing was based on planned orthogonal contrasts. Logistic regression was used to assess the likelihood of iron deficiency at 12 months of age. Given our aim to examine the association between maternal stress in the context of established risk factors, covariates known to affect perinatal iron status were identified.[7]

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