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Is maternal obesity associated with sustained inflammation in extremely low gestational age newborns?☆

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ABSTRACT

Background: The offspring of obese women are at increased risk for systemic inflammation. Blood concentrations of inflammatory proteins in preterm newborns of obese women have not been reported.
Aim: To compare blood concentrations in the highest quartile for gestational age of inflammatory proteins and day of blood specimen collection on two days at least one week apart of newborns of overweight (i.e., BMI 25–29) and obese women (i.e., BMI ≥ 30) with newborns of women with lower BMIs. Because deliveries for spontaneous indications are more likely than those for other indications to be associated with inflammation, we evaluated spontaneous indication deliveries separately from maternal or fetal indications.
Study design: Prospective cohort study.
Subjects and outcome measures: We measured from 939 children born before the 28th week of gestation 25 inflammation-related proteins in blood obtained on postnatal day 1 (range 1–3), day 7 (range 5–8) and day 14 (range 12–15).
Results: Among infants delivered for spontaneous indications, maternal BMI was not related to elevated concentrations of any protein. Among infants delivered for maternal (i.e., preeclampsia) or fetal indications, those whose mother was overweight or obese were more likely than others to have elevated concentrations of inflammation proteins.
Conclusions: Maternal pre-pregnancy overweight and obesity appear to contribute to a pro-inflammatory state in very preterm newborns delivered for maternal or fetal indications. Our failure to see a similar pattern among newborns delivered for spontaneous indications, which often have inflammatory characteristics, might reflect competing risks.

1. Introduction

The prevalence of maternal overweight and obesity is rapidly increasing in the United States, Europe, and Asia [1]. Women who are overweight or obese before pregnancy appear to be at increased risk for gestational diabetes, preeclampsia, labor complications, and hypertension [2,3]. Obesity is a chronic inflammatory state [4–10]. Compared to their leaner peers, obese women tend to have higher blood concentrations of pro-inflammatory proteins when not pregnant [11–14] and when pregnant [11–15].

In addition to these potential risks to the mother, maternal obesity appears to have adverse effects on the offspring [16,17]. For example, children of obese mothers are prone to obesity [18], systemic inflammation [19–21], and metabolic syndrome [22], as well as cognitive problems [23–28]. While selected inflammatory markers were assessed in adolescents and adults born to obese mothers and/or fathers, respectively [19,20], we did not find any study that measured blood concentrations of inflammatory proteins in newborns of obese mothers. In this report, we compare the tendency of extremely preterm newborns of

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overweight (i.e., BMI 25–29) and obese (i.e., BMI ≥ 30) women to have elevated blood concentrations of inflammatory proteins to that of infants born to women with lower BMIs.

A large prospective cohort of extremely low gestation age newborns (ELGAN) showed that ELGANs who had elevated concentrations of inflammation-related proteins measurements made at least one week apart (interpretable as intermittent or sustained systemic inflammation abbreviated here as ISSI) were at increased risk of numerous brain disorders such as cerebral white matter damage evident on ultrasound scans [29], later severe cognitive limitations [30], and microcephaly two years after birth [31]. These findings prompted us to evaluate in the same ELGAN cohort if the infants of overweight and obese mothers had ISSI within the first month of birth when compared to infants of women with lower BMIs.

2. Methods

2.1. Sample

The ELGAN Study, a prospective cohort study designed to identify characteristics and exposures that increase the risk of structural and functional neurologic disorders, enrolled 1506 extremely low gestational age newborns (ELGANs) (birth between 23 and 27 6/7 weeks of gestation) at 14 participating institutions between 2002 and 2004 [32]. The consent and enrollment procedures were approved by the individual institutional review boards.

Inflammation-related proteins were measured in the blood of the 939 children who had a developmental assessment at age 2 years. These children are the subjects of this report.

2.2. Maternal body mass index (BMI)

Each mother was asked to provide her height and her pre-pregnancy weight. These were used to calculate her BMI. The United States government classifies BMIs as follows: <18.5 is underweight, 18.5–24.9 is normal, 25.0–29.9 is overweight, 30.0–34.9 is obese, 35.0–39.9 is very obese, and ≥40 is extreme obesity [33]. We collapsed these groups into <25, 25–29.9, and ≥30.

2.3. Pregnancy disorders

The clinical circumstances that led to each maternal admission and ultimately to each preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the medical record [34]. We were interested in the potential preconditioning/sensitization by antenatal inflammation. Therefore, we divided our sample into two groups defined by spontaneous indications for delivery (preterm labor, preterm premature rupture of membranes (pPROM), abruption or cervical insufficiency) vs. maternal (preeclampsia) or fetal indications. The rationale for this was that spontaneous indications are often associated with inflammation, while fetal and maternal indications are mostly not [35,36].

2.4. Placenta characteristics

Placentas were collected, and their histological characteristics analyzed, according to the procedures previously described [37]. In this paper, we used only the variable for "histologic chorioamnionitis", defined as the presence of any neutrophils in the fetal membranes [37].

2.5. Infant characteristics

Gestational age estimates were based on a hierarchy of the quality of available information with estimates based on the dates of embryo retrieval or intrauterine insemination or fetal ultrasound before the 14th week of gestation (62%) as the most desirable. Next most desirable in sequential order were estimates based on a fetal ultrasound at 14 or more weeks of gestation (29%), last menstrual period without fetal ultrasound (7%), and recorded in the log of the neonatal intensive care unit.

The birth weight Z-score represents the number of standard deviations the infant's birth weight was above or below the median weight of infants at the same gestational age in referent samples not delivered for preeclampsia or fetal indications [38,39]. We evaluated 2 groups of growth-restricted infants. The more severely growth-restricted infants had a birth weight Z-score < −2 (i.e., more than 2 standard deviations below the median of the referent group). Infants in the less severely growth-restricted group had a birth weight Z-score ≥ −2 and <−1 (i.e., between 1 and 2 standard deviations below the median of the referent group).

2.6. Blood spot collection

Drops of blood from the newborns were collected on filter paper on postnatal days 1 (range: 1–3 days) (N = 861), 7 (range: 5–8 days) (N = 867), and 14 (range: 12–15 days) (N = 786). All blood was from what remained after specimens were obtained for clinical indications. Dried blood spots were stored at −70 °C in sealed bags with desiccant until processed.

2.7. Elution of proteins from blood spots

For protein elution, 12 mm punched biopsies of the frozen blood spots were submerged in 300 μL phosphate buffered saline containing 0.1% Triton X100 (Sigma-Aldrich, St. Louis, MO) and 0.03% Tween-20 (Fisher, Hampton, NH), vortexed for 30 s, and incubated on a shaker for 1 h at 4 °C. The buffer and biopsy were then transferred over the filter of a SpinX tube (Corning Fisher), centrifuged at 2000 ×g followed by collection of the filtered eluted blood. An additional wash of the punch was performed in 100 μL for a final elution volume of 400 μL.

2.8. Protein measurements

Proteins were measured in duplicate in the Laboratory of Genital Tract Biology of the Department of Obstetrics, Gynecology and Reproductive Biology at Brigham and Women's Hospital, Boston using the Meso Scale Discovery (MSD) multiplex platform and Sector Imager 2400 (MSD, Gaithersburg, MD). This electrochemiluminescence system has been validated by comparisons with traditional ELISA [40,41] and produces measurements that have high content validity [35,36,42,43].

The multiplex assays measuring up to 10 proteins simultaneously were optimized to allow detection of each biomarker within the linearity range of the eluted samples. The MSD Discovery Workbench Software was used to convert relative luminescent units into protein concentrations using interpolation from several log calibrator curves. Split quality control blood pools tested on each plate showed inter-assay variation of <20% in the linearity range customized for the blood spot elution samples. The total protein concentration in each eluted sample was determined by BCA assay (Thermo Scientific, Rockford, IL) using a multi-label Victor 2 counter (Perkin Elmer, Boston, MA) and the measurements of each analyte normalized to mg total protein.

We measured the following 25 proteins as well known and well measurable representatives of the major classes of inflammation-associated proteins e.g. acute phase proteins, cytokines and their receptors, chemokines, adhesion molecules, factors regulating vascular permeability and angiogenesis: C-Reactive Protein (CRP), Serum Amyloid A (SAA), Myeloperoxidase (MPO), Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-6 Receptor (IL-6R), Tumor Necrosis Factor-α (TNF-α), Tumor Necrosis Factor Receptor-1 (TNF-R1), Tumor Necrosis Factor Receptor-2 (TNF-R2), Interleukin-8 (IL-8; CXCL8), Monocyte Chemotactic Protein-1 (MCP-1; CCL2), Monocyte Chemotactic Protein-4 (MCP-4; CCL13), Macrophage Inflammatory Protein-1β (MIP-1β;