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Gestational cytokines and the developmental expression of obesity in childhood

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Abstract

Objective: We examined the extent to which maternal immune activity during pregnancy is associated with childhood adiposity, and if so, whether associations at birth differ from those in infancy and childhood. We also examined sex-specific associations.

Methods: Participants were 1366 singleton pregnancies from the Collaborative Perinatal Project (1959–1966). Interleukin (IL)-1 β , IL-6, tumor necrosis factor alpha (TNF- α), IL-8, and IL-10 in maternal sera were assayed repeatedly during pregnancy. We calculated children's body mass index (BMI) repeatedly from birth through age 8 and derived age- and sex-normalized BMI z-scores (BMIZ). We used linear mixed models to estimate the cumulative concentration of each cytokine in 2nd and 3rd trimesters and then related this concentration to child BMIZ.

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Disclosure: Dr. Goldstein is a consultant for Cala Health, although there is no conflict of interest relevant to this article. All other authors also have no conflicts of interest relevant to this article.

Results: Children exposed to higher IL-1 β , IL-6, IL-8, and IL-10 concentrations had *lower* BMIz at birth but *higher* BMIz during childhood. Higher concentrations of IL-8 and IL-1 β were also associated with *higher* BMIz during infancy (B per log increase in IL-8=0.04, 95%CI:0.02, 0.07; and IL-1 β =0.03, 95%CI:0.001, 0.06). The associations between TNF- α and BMIz were in opposing direction in boys (B=-0.13, 95%CI: -0.31, 0.04) and girls (B=0.14, 95%CI:0.02, 0.26) during childhood.

Conclusions: Maternal prenatal inflammation contributes to the age- and sex-specific programming of obesity risk in childhood.

Keywords

cytokines; pregnancy; body mass index; inflammation

Introduction

Obesity is a major public health concern among US children with a steady increase across all age groups, particularly among 2- to 5-year-old children.^{1,2} Childhood obesity is likely to be sustained through young adulthood and contributes to suboptimal trajectories of both physical and mental development.³ In addition to energy intake and sedentary lifestyle, *in utero* environment might contribute to the programming of childhood obesity. *In utero* environment predisposes the fetus toward adiposity and weight gain in childhood, in part due to inflammatory processes associated with maternal obesity, poor diet, or psychosocial stress.⁴⁻⁶ In a normally progressing pregnancy, immune processes are essential for trophoblast invasion in the uterus, fetal implantation, continuation of pregnancy, and survival of the fetus.⁷ However, maladaptive deviations—occurring due to prenatal insults such as stress that lead to immune activation in maternal periphery and potentially placenta—are associated with intrauterine growth restriction, low birth weight, and small birth size in the offspring.^{8,9}

Emerging evidence suggests that maternal inflammation during pregnancy leads to excess childhood adiposity, but findings have remained mixed with regard to adiposity in different life stages. Earlier studies also substantially differed in their length of follow-up and measures of inflammation biomarkers.¹⁰⁻¹² For example, higher concentrations of maternal interleukin (IL)-6 during late pregnancy were associated with lower measures of adiposity, but only in female neonates.¹⁰ Also, among girls, tumor necrosis factor (TNF)- α and IL-6 concentrations during early pregnancy were positively associated with total and central adiposity at age 6 months.¹⁰ In another study, maternal IL-4 and IL-13 but not IL-6, IL-10, IL-8 and TNF- α were associated with a *decreased* risk for overweight development in early childhood.¹¹ Higher concentration of c-reactive protein (CRP) during mid-pregnancy was associated with higher overall fat mass and trunk fat mass based on dual-energy X-ray absorptiometry scans in 7- to 10-year-old children and with higher body mass index (BMI) at 3–5 years and 7–10 years.¹² Higher maternal CRP during mid-pregnancy was associated with large-for-gestational age at birth in one study,¹² though –interestingly– it was associated with lower weight at birth and small for gestational age in a different study.¹³ Therefore, the question remains how fetal exposure to maternal inflammatory processes may contribute to the risk of obesity in the offspring during different developmental stages.

A major limitation of earlier studies has been the measurement of inflammatory markers at a single time point during gestation, despite the importance of maternal immune activation throughout pregnancy for fetal development. Maternal peripheral cytokine concentrations change across the course of pregnancy.¹⁴ Moreover, hormonal changes during the course of healthy gestation and physiological adaptations for the continuation of pregnancy and initiation of labor are associated with variations in immune markers.¹⁵ Although exposures at specific periods of gestation may be critical in understanding the impact on fetal development, a single measure might not capture cumulative exposure of the fetus to maternal inflammation throughout gestation. We aimed to determine the extent to which cumulative exposure to maternal cytokines during pregnancy is associated with BMI in children from birth through age 8 years; and if so, to characterize the pattern and its impact on growth parameters at birth, in infancy, and during early childhood. Earlier studies of childhood obesity suggested a differential vulnerability by sex of the offspring with *in utero* exposure to inflammation.^{6,10} Thus, we examined if the associations differed in boys and girls.

Methods

Participants

We used data from the New England cohorts (Boston, MA; Providence, RI) of the United States Collaborative Perinatal Project (CPP, enrollment between January 1959 – September 1966). The New England Family Study comprises a series of follow-up studies of several thousands of CPP offspring; as described previously, we obtained the prenatal serum from the mothers of a subset of these participants for conducting assays of gestational cytokines.¹⁶ Children were followed through age 8 years with systematic assessments of development, growth, and health outcomes. For this analysis, we excluded twins (n=64) and mother-child pairs with cytokine measurements before 14 weeks of gestation due to few numbers (n=8). From the remaining 1596 children, we included 1366 singletons pregnancies with one or more measures of cytokines during 2nd and 3rd trimesters and data on childhood BMI and covariates. The sample includes a small number of women who were enrolled across successive pregnancies (101 women twice, six with three, and two women across four pregnancies). This study was granted an exemption from ethics review because the CPP data are de-identified for public use.

Cytokine measurements

Pregnant women provided up to four serum samples, which were stored at the National Institutes of Health biorepositories at -20°C. During a two-month period, serum concentrations of IL-1 β , IL-8, IL-6, IL-10, and TNF- α were measured using a multiplexed, bead-based immunoassay (Milliplex™ human cytokine panel, MPXHCYTO-60K, Millipore, MO) on a Luminex 3D™ detection platform (Luminex Corporation, TX) (n=2367).¹⁷ From the total serum samples selected for cytokine assays, less than 1% had been thawed one or more times prior to shipment for the cytokine measurements and thus the majority of samples had the same number of freeze-thaw cycles. To further minimize potential bias, all study samples were assigned randomly across assay plates. Samples were treated similarly, using assay kits and reagents from a single lot. The total number of freeze-

thaw cycles for samples run once was the number prior to shipment from the repository to the lab plus 1. The total number of freeze-thaw cycles for any samples that we ran more than once (less than 5% of samples) was the number prior to shipment plus 2. Assay sensitivities ranged from 0.1–0.4 pg/mL. Assays were completed according to manufacturers' protocols, with overnight incubation at 4°C on a shaker prior to detection of mean fluorescence intensities of analyte-specific immunoassay beads on the Luminex 3D. Raw data on mean fluorescence intensities were captured using Luminex xPONENT™ software (v.4.0.846.0) and concentrations of immune factors in each sample were interpolated from standard curves using a 5-parameter, weighted, logistic regression curve equation in Milliplex Analyst™ (v.3.5.5.0). The laboratory's intra-assay CV was within the manufacturer's range of <10%. We used the machine read values unless the machine-read values were null in which case we substituted the mid-point between zero and the limit of detection of that analyte. Eighteen women (1%) contributed with four measures of cytokine, 145 (10%) had three measures, 657 (48%) women had two measures, and 546 (40%) had one measure of cytokines. Women with one, two, three, or four assays were comparable regarding their age, BMI, and preeclampsia; but women who had cytokine data from one time point were more likely to have high socioeconomic disadvantage compared to women with more measurements.

Child growth

Neonates were weighted and their recumbent lengths were measured within an hour after birth and values were recorded to the nearest ounce (later converted to gram) and centimeter, respectively. Trained research assistants measured weight and height (recumbent length through 20 months and standing afterward) with calibrated weighting scale and stadiometer when children were wearing light clothing and no shoes. We calculated children's BMI (weight/height squared) using weight (kg) and height/length (m) serially from birth through age 8 years (up to 9 assessments per child). Assessments occurred mostly around birth, around age 1 year, 3–4 years, and 7–8 years. We excluded 29 observations of height and/or weight with biologically implausible values according to the Centers for Disease Control and Prevention (CDC) definition. Following others,¹⁸ we used 2000 CDC growth charts to standardize BMI data in the CPP. Growth charts are mainly revised periodically because more recent and comprehensive data and improved statistical procedures are available. We determined age- and sex-normalized BMI z-scores (BMIz) according to the World Health Organization Child Growth Standards (up to age 2 years) and the CDC.¹⁹ We defined weight gain in the first year of life as the change in weight-for-age z scores between birth and the study assessment date closest to age one year (between 11 and 13 months). Following previous literature, a gain 0.67 weight-for-age z scores between birth and age one was considered *rapid weight gain* in infancy as this amount of gain is associated with later central adiposity and cardiometabolic diseases.^{20,21}

Covariates

During in-person interviews at enrollment, pregnant women reported demographic characteristics including age, years of education, and race/ethnicity, number of cigarettes smoked per day during pregnancy, and history of treatment for psychiatric disorders. We used self-reported pre-pregnancy weight and height to calculate maternal BMI. We obtained information on preeclampsia from study records.²² We calculated a socioeconomic

disadvantage score using self-reported information on education and occupation for women and their partners, household income, and marital status, with higher scores indicating greater disadvantage. The scores were categorized as low (0–2), medium (3–5) or high (> 6) disadvantage to reflect the number of indicators of disadvantage present in each family.²³ An earlier study in the CPP reported associations of socioeconomic disadvantage with immune markers in pregnant women.²⁴

Statistical analysis

We applied a two-step regression calibration approach to examine the association between cytokine concentrations and children's BMI through age 8 years. First, as described previously,¹⁷ we estimated the cumulative concentrations for each cytokine during 2nd and 3rd trimesters of pregnancy using linear piecewise mixed models with pregnancy-level random intercepts and slopes. These models were used to make predictions of the concentration curve for each pregnancy defined by two lines with potentially different slopes (in log linear scale of pg/ml concentrations) joined at the end of 27 weeks of gestation (end of the 2nd trimester and the beginning of the 3rd trimester). We selected mid-point of the two trimesters because each trimester in pregnancy is marked by specific fetal developmental stages. Cytokines increase substantially during labor and delivery; therefore, models were adjusted for an indicator variable (D) if samples were provided on the delivery date.

Effect estimates were calculated using the following:

$$Y_{ij} = \beta_0 + \beta_1 \text{Gestaional Age}(if \leq 27 \text{ wks}) + \beta_2 \text{Gestaional Age}(if > 27 \text{ wks}) + \beta_3 D + b_i + \epsilon_{ij},$$

where i indicates measurements of cytokines in pregnancy and j refers to the number of pregnancies for each woman. We then computed the area below the concentration curve and used it as a measure of the cumulative inflammatory burden over 2nd and 3rd trimesters. The underlying assumption was that the number of cytokine measures is not related to the cytokine cumulative concentration. This method allowed us to estimate a cumulative inflammatory burden for each pregnancy even for pregnancies with only a single measurement (40% of pregnancies).

Second, we applied generalized linear mixed models with an unstructured covariance matrix to examine the association between cumulative cytokine concentrations (log-transformed) and repeated measures of child BMIz from birth through age 8 years. Models included a child-level random effect for intercept to account for the correlation between repeated measures of BMIz across all ages and also a random effect for women participating with more than one pregnancy. Models did not include age since we used age and sex-specific z scores. To examine the association between cytokines and accelerated growth during infancy, we used mixed-effects logistic regression to estimate the odds ratio (OR) and 95% confidence intervals (CI) of the association between cumulative cytokines and *rapid weight gain*.

We included an interaction term of each cumulative cytokine measure and age categories (at birth, during infancy, and age one to eight years) to explore if cytokines were associated with

child BMIz differently at birth than in infancy and early childhood. We tested the interaction between maternal inflammation and sex in the analyses of childhood BMI, and stratified the analysis where the sex-interaction was present. We used restricted cubic splines with four knots to examine the non-linearity in the associations of five cytokine concentrations with child BMIz.

Selection of confounders was based on the directed acyclic graph of the study questions. Models were adjusted for maternal age, education, socioeconomic disadvantage, cigarette smoked during pregnancy, pre-pregnancy BMI, and treatment for psychiatric illnesses, and child race/ethnicity. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

Table 1 summarizes participants characteristics. In total, 89% of participating women were white and 50% reported medium to high socioeconomic disadvantage during pregnancy. Maternal mean age at enrollment was 24.8 years [standard deviation (SD) = 5.7] and mean pre-pregnancy BMI was 22.9 kg/m² (SD=4.2). Fifty-six percent of children were girls and 530 (44%) exceeded the threshold of rapid infant weight gain. Maternal and child characteristics in this subsample were comparable to all Boston and Providence participants in the CPP (Supplementary Table S1). There were moderate correlations between concentrations of cytokines (Pearson *r* for log-transformed cumulative cytokine concentrations varied between 0.36 and 0.75) while IL-10 had weak positive correlations with other cytokine measures (*r*=0.003–0.13). Figure 1 presents observed BMIz from birth through age seven years by maternal cumulative cytokines (log-transformed) in boys and girls.

Higher cumulative concentration of maternal IL-8 was associated with higher BMIz repeatedly measured from birth through age 8 years (B=0.04, 95%CI: 0.01, 0.06), in a pattern that was similar for boys and girls. Higher maternal TNF- α was associated with lower BMIz in boys but higher BMIz in girls (Table 2, unadjusted analysis in Supplementary Table S2). Results using cytokine values categorized in quintiles revealed no threshold in the associations (data not shown).

We found significant variability in the association between gestational cytokine concentrations and offspring BMIz across time periods at birth, during infancy, and through childhood (Table 2). Higher concentrations of all five cytokines were associated with lower BMIz at birth. During infancy, the association of maternal IL-8 and IL-1 β with offspring BMIz changed to a positive direction. Between ages 1–8 years, coefficients pointed to higher childhood growth following exposure to higher maternal inflammation prenatally (Table 2).

The associations of maternal TNF- α with BMIz differed between boys and girls, such that higher TNF- α was associated with lower BMIz at birth in boys but not in girls. Higher maternal TNF- α was also associated with lower BMIz in boys during infancy and childhood (B for infancy=−0.19, 95%CI: −0.36, −0.01; for childhood=−0.13, 95%CI: −0.31, 0.04). But in girls, higher maternal TNF- α was associated with higher BMIz at both time points (B for

infancy= 0.12, 95%CI: 0.00, 0.23; and for childhood=0.14, 95%CI: 0.02, 0.26). Predicted BMIz from fully adjusted linear mixed models per quintiles of maternal cumulative cytokine concentrations are plotted against child age in Figure 2.

Children prenatally exposed to higher concentrations of maternal IL-8 had also higher odds of rapid weight gain during infancy (Table 3, unadjusted analysis in Supplementary Table S3). In contrast, there was an association between higher TNF- α and lower odds of rapid weight gain during infancy, but for boys only. We found no associations between concentrations of other cytokines and rapid weight gain during infancy. There was no indication of non-linearity in the association of cytokines with child BMIz (data not shown).

Discussion

We found a developmental pattern in the association of gestational inflammation with offspring BMI through age 8 years, with some associations influenced by sex. Higher cumulative concentrations of IL-1 β , IL-6, IL-8, and IL-10 during 2nd and 3rd trimesters were associated with lower BMIz at birth whereas higher levels of these cytokines were associated with higher BMI through mid-childhood. Maternal TNF- α showed sex-dependent associations. At birth, higher TNF- α was associated with lower BMIz in boys; during infancy and childhood, higher TNF- α was also associated with lower BMIz among boys but with higher BMIz in girls. The associations were small to medium in size, with the largest associations observed with TNF- α and in boys (a decrease of 0.20 BMIz in boys, which translates into one-fifth standard deviation of the population).

Activation of a network of cytokines at the feto-maternal interface is required for successful implantation and progression of a healthy pregnancy. Nonetheless, prenatal inflammation is a risk factor for intrauterine growth retardation and low birth weight.^{13,25,26} Cytokines with pro-inflammatory role, such as TNF- α and IL-6, as well as IL-8 are elevated in serum of women with fetal growth restriction.^{25,27–29} Three earlier studies addressing the question of whether prenatal inflammation is associated with child obesity included a single measure of IL-4, IL-13, TNF- α , IL-6 or CRP, with varying follow-up periods, i.e., 6 months and mid-childhood.^{10–12} Two of these studies reported positive associations between higher cytokine levels and measures of adiposity and BMI in infancy and mid-childhood.^{10,12} For T helper 2 cytokines, i.e., IL-4 and IL-13, Englich and colleagues reported a decreased risk of overweight development in offspring.¹¹ Our study extended these findings and demonstrated positive associations between cumulative maternal IL-1 β , IL-8, IL-6 and IL-10 and childhood BMI in both boys and girls, and between cumulative TNF- α measures and BMI in girls. Here, we also report the association between maternal IL-8 and rapid infant weight gain, suggesting that a transition from lower BMI at birth to higher BMI in later childhood might follow a period of greater weight gain and attainment of higher body mass after birth and during infancy. IL-8 is consistently reported to be higher in obese individuals compared to normal weight people,^{30,31} but its role in obesity, lipolysis activity or energy expenditure is understudied. Evidence supporting the role of IL-8 in insulin resistance suggests that IL-8 may also help explain the observation that the offspring of women with higher concentrations of IL-8 have increased adiposity levels.³²

Several investigations have focused on obesity as a low-grade inflammatory status and shown direct associations between obesity and inflammation, e.g., production of TNF- α , IL-6, and IL-1 β by macrophages in adipose tissues, or leptin-induced production of IL-6 and CRP.^{33,34} These studies further suggest the role of immune system activation in obesity-related insulin resistance. However, the mechanisms for the association between maternal inflammatory markers and childhood adiposity are less understood. Longitudinal profiling of cytokines in pregnancy demonstrates that increases in concentrations of IL-6 and IL-10 are associated with adverse pregnancy outcomes and placental dysfunction.³⁵ Indirect effect of maternal IL-6 and IL-10 on placental function can translate into lower birth weight, in line with the observed associations between both pro- and anti-inflammatory cytokines and lower birth weight. IL-10 may be involved not only in the regulation of placental vascular function, but may also play a role in fetal development. The weak but positive correlation between IL-10 and pro-inflammatory cytokines in our study and other pregnancy samples³⁵ supports this hypothesis. The complex effect of IL-6 on the metabolism and pathogenesis of obesity is likely affected by its neuroendocrine activities, including regulation of the hypothalamus–pituitary–adrenal axis.³⁶

Our finding of sex differences in the associations between gestational TNF- α and BMI at birth and during childhood needs further investigation. We found that boys exposed to higher TNF- α during the 2nd and 3rd trimesters of pregnancy had lower BMI at birth and *continued* to have lower BMI, as opposed to girls in whom gestational TNF- α was associated with higher BMI during childhood. These results are in line with another report showing that infant girls are at risk of adiposity if they had been exposed to prenatal inflammation—in particular, higher concentrations of TNF- α .¹⁰ TNF- α is involved in obesity-linked insulin resistance, but it is also shown to induce lipolysis and to reduce both adiponectin gene expression and protein levels *in vitro*.³⁷ Women pregnant with female fetuses show lower pro-inflammatory and higher anti-inflammatory cytokine production as compared with women pregnant with male fetuses.³⁸ Due to minimal transfer through the placenta, concentrations of TNF- α in fetal components are not a direct reflection of TNF- α levels in maternal circulation.³⁹ While higher maternal levels of TNF- α during gestation may induce changes at the maternal-fetal interface that subsequently influence placenta and/or increase TNF- α in fetal compartments, thereby contributing to higher obesity risk in girls, female-specific obesity risk might also relate to other sex-dependent factors associated with maternal inflammatory molecules, such as mood and anxiety disturbances, dysregulation of stress response circuitry, steroid hormones, and the cardiovascular system.⁶ Sex-specific associations involving gestational exposures likely depend on the timing of the exposure and its intersection with the development of male and female fetuses across gestation. Future work is needed to examine the role of inflammation —across developmental stages of pregnancy and during each trimester— in sex-specific developmental programming of adiposity.⁴⁰

We derived cumulative concentrations of each cytokine during 2nd and 3rd trimesters. There were high correlations between median concentrations of each cytokine across pregnancy and the cumulative concentrations estimated with mixed models; yet, the advantage of the later approach is that it reduces the impact of measurement error in cytokine immunoassays and focuses on sustained changes in cytokine concentrations across gestation rather than

short-term variability.⁴¹ The disadvantage may be the loss of precision in relating the association of exposures at specific times of gestation with child outcomes; however, this would not discount our investigation of the associations between cumulative inflammatory burden over gestation and child BMI. We acknowledge that the limited availability of two or more samples for about 40% of participants could influence the estimates of cumulative cytokine concentrations in this population. Further, while long-term storage of serum samples may be associated with cytokine degradation, we treated all samples equally to prevent any non-differential degradation with respect to child BMI. We used measures of maternal peripheral cytokines as proxies of fetal exposure and that can be a limitation for cytokines with low fetal transfer (e.g., TNF- α). Measures in matrices more proximal to the maternal-fetal interface, such as amniotic fluid—if available, can provide a better representation of fetal exposure. Another limitation of this study was that we were not able to include measures of cytokines in the 1st trimester and had limited measures in each of the 2nd and 3rd trimesters. Cytokine variation across the course of pregnancy is under the influence of several factors (e.g., hypothalamic pituitary axis and infections) that can act beyond the boundaries of trimesters. Future studies with several repeated measures of cytokines during each trimester of pregnancy would more fully characterize the inflammatory burden across the entire course of pregnancy. While we accounted for several risk factors of higher inflammation in pregnancy, such as smoking and prenatal distress, we did not have information on important confounders, e.g., maternal diet during pregnancy. Some dietary factors, e.g., fatty acids, are shown to induce inflammatory response,⁴² while prenatal status in mother is also associated with child adiposity.⁴³ This sample of pregnant women had a large number of smokers which differentiates the CPP from more contemporary cohorts; however, our analysis did not reveal any association between cigarette smoking and cytokines nor did the association changed with adjustment for cigarette smoking. Finally, while the same standard curve can be used to standardize BMI in participants of CPP as contemporary cohorts, the number of children with obesity is substantially higher now than 1959–1966. We also expect considerable changes in the nature of childhood obesity. Future studies of immune marker-adiposity associations would also benefit from inclusion of more detailed measures of adiposity such as fat percentage.

In summary, we showed that maternal cytokine concentrations were associated with lower BMI at birth but higher BMI during childhood. Rapid weight gain during infancy, an important predictor of adiposity and obesity later in life, was associated with maternal IL-8 concentrations. Increased cumulative gestational TNF- α was also associated with higher childhood BMI in girls. Immune markers act in network; therefore, future studies are warranted to examine the immune markers in the network of action to further address the interactions of markers in relation to adiposity in children. Also, further follow-up through adolescents and later are needed to establish if sex-differences in findings will replicate after puberty. A recent follow-up study in more than 50,000 children from the general population demonstrated that excessive weight gain in preschool children put children on the path to obesity in adolescence more definitively than the amount weight gained during late childhood.⁴⁴ Our results suggest that maternal inflammation during pregnancy contributes to the development of obesity risk in early childhood, and that some of these associations may be sex-specific. A potential implication of these results, if replicated further, would be a

beneficial effect of minimizing risk factors associated with prenatal inflammation, such as maternal infection, maternal obesity, and multiple exogenous and endogenous factors associated with stress during pregnancy.^{24,45}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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Study Importance

What is already known about this subject?

- *In utero* environment might predispose the fetus toward adiposity and weight gain in childhood.

What are the new findings in this manuscript?

- Children who were prenatally exposed to higher interleukin (IL)-1 β , IL-6, IL-8, and IL-10 concentrations had *lower* body mass index (BMI) at birth and *higher* BMI during childhood.
- The associations between tumor necrosis factor (TNF)- α and child BMI were in opposing direction in boys and girls at age 1–8 years.

How might these results change the direction of research or the focus of clinical practice?

- Our results suggest a developmental pattern in the association of gestational inflammation with offspring BMI through childhood, with some associations influenced by sex.
- There might a beneficial effect of minimizing risk factors associated with prenatal inflammation for childhood obesity.

Figure 1. Observed body mass index z score (BMIz) from birth through age seven by maternal cumulative cytokine concentrations in 2nd and 3rd trimesters.

Observed BMIz (y axis) are presented at birth, and at ages one, four, and seven years (horizontal panels) in boys and girls (vertical panels) for each measurement of cumulative concentrations of (a) Interleukin (IL)-6; (b) IL-8; (c) IL-10; (d) IL-1 β ; (e) Tumor Necrosis Factor (TNF)- α (x axis).

Figure 2. Predicted body mass index z score (BMIz) for quintiles of maternal cumulative cytokine concentrations in 2nd and 3rd trimesters.

Predicted BMIz (y axis) were derived from the generalized linear mixed models with cumulative cytokines and are presented by child age in months (x axis) per quintiles of maternal cumulative cytokine concentrations. Panels are (a) Interleukin (IL)-6; (b) IL-8; (c) IL-10; (d) IL-1 β ; (e) Tumor Necrosis Factor (TNF)- α . Models were adjusted for maternal age (year), race (White/non-White), education (year), socioeconomic disadvantage, number of cigarette smoked/day during pregnancy, pre-pregnancy BMI, and treatment for psychiatric illnesses (yes/no).

Table 1.

Study participants' characteristics (from the United States Collaborative Perinatal Project, 1959–1966).

Maternal and child characteristics (n=1366)	
	Mean (SD)
Maternal age at enrollment, year	24.8 (5.7)
Maternal education, year	11.1 (2.2)
Maternal pre-pregnancy BMI	22.9 (4.2)
Maximum number of cigarettes per day in pregnancy	11.4 (12.9)
	N (%)
Child race	
White	1213 (88.8)
Others	153 (11.2)
Prenatal socioeconomic disadvantage	
0–1	677 (49.5)
1.5–2.5	457 (33.5)
≥3	232 (17.0)
Maternal history of treatment for psychiatric disorders, yes	155 (11.4)
Preeclampsia, yes	20 (1.5)
Child female sex	766 (56.1)
	Median (25 th , 75 th percentile)
Birth weight, gram	3260 (2948, 3600)
Gestational age at birth, weeks	40 (38, 42)
Cumulative cytokine concentration in pregnancy, pg/ml	
IL-1 β	39.3 (15.6, 112.4)
IL-8	511.4 (205.5, 2137.5)
IL-6	36.1 (21.2, 62.9)
IL-10	67.3 (45.5, 96.1)
TNF- α	128.3 (105.1, 152.4)

Socioeconomic disadvantage score was calculated using information on education and occupation, household income, and marital status, with higher scores indicating more disadvantage.

The cumulative concentrations of cytokines during the 2nd and 3rd trimesters were estimated using linear piecewise mixed models with a pregnancy-level random intercept and slope to estimate the variation in the concentration of each cytokine across pregnancy (area below the estimate line, pg/ml)

BMI: body mass index; IL: interleukin; SD: standard deviation; TNF: tumor necrosis factor

Table 2.

Associations of cumulative cytokine concentrations during the 2nd and 3rd trimesters of pregnancy with children's body mass index z-score from birth through age 8 years (The United States Collaborative Perinatal Project, 1959–1966).

BMI z-score (all children), n=1366				
	From birth to 8 years ^a B (95%CI)	At birth ^b B (95%CI)	During infancy ^b B (95%CI)	Age 1–8 years ^b B (95%CI)
IL-1β	0.01 (−0.02, 0.04)	−0.12 (−0.15, −0.09)	0.03 (0.001, 0.06)	0.05 (0.02, 0.09)
TNF-α	−0.002 (−0.10, 0.10)	−0.12 (−0.22, −0.02)	0.02 (−0.08, 0.12)	0.06 (−0.04, 0.15)
IL-6	−0.01 (−0.04, 0.03)	−0.15 (−0.19, −0.11)	0.01 (−0.03, 0.05)	0.05 (0.01, 0.09)
IL-8	0.04 (0.01, 0.06)	−0.06 (−0.09, −0.03)	0.04 (0.02, 0.07)	0.09 (0.06, 0.11)
IL-10	−0.004 (−0.06, 0.05)	−0.14 (−0.19, −0.08)	0.01 (−0.04, 0.07)	0.05 (0.00, 0.11)
BMI z-score (boys), n=600				
IL-1β	0.01 (−0.03, 0.06)	−0.11 (−0.16, −0.06)	0.04 (−0.01, 0.08)	0.09 (0.07, 0.11)
TNF-α	−0.20 (−0.37, −0.03)	−0.33 (−0.50, −0.15)	−0.19 (−0.36, −0.01)	−0.13 (−0.31, 0.04)
IL-6	−0.03 (−0.09, 0.02)	−0.18 (−0.24, −0.12)	−0.02 (−0.08, 0.04)	0.03 (−0.03, 0.09)
IL-8	0.02 (−0.02, 0.07)	−0.08 (−0.12, −0.03)	0.03 (−0.02, 0.07)	0.09 (0.04, 0.13)
IL-10	−0.05 (−0.12, 0.03)	−0.19 (−0.27, −0.11)	−0.04 (−0.12, 0.04)	0.03 (−0.06, 0.11)
BMI z-score (girls), n=766				
IL-1β	0.01 (−0.03, 0.05)	−0.13 (−0.17, −0.08)	0.03 (−0.01, 0.07)	0.05 (0.01, 0.09)
TNF-α	0.09 (−0.02, 0.21)	−0.02 (−0.14, 0.10)	0.12 (0.00, 0.23)	0.14 (0.02, 0.26)
IL-6	0.01 (−0.03, 0.06)	−0.13 (−0.18, −0.08)	0.03 (−0.01, 0.08)	0.06 (0.01, 0.11)
IL-8	0.04 (0.01, 0.08)	−0.05 (−0.08, −0.01)	0.05 (0.02, 0.09)	0.08 (0.05, 0.12)
IL-10	0.04 (−0.04, 0.11)	−0.09 (−0.17, −0.02)	0.06 (−0.01, 0.13)	0.08 (0.01, 0.16)

^aEstimates based on generalized linear mixed models to examine the association between cumulative cytokine concentrations (log-transformed) and repeated measures of child BMIz from birth through age 8 years.

^bEstimates based on generalized linear mixed models with an interaction term of each cumulative cytokine measure and age categories (at birth, during infancy, and age one to eight years). B's are reported per log unit increase in the cumulative cytokine concentration (pg/ml) of each cytokine during the 2nd and 3rd trimesters.

Models were adjusted for maternal age (year), race (White/non-White), education (year), socioeconomic disadvantage, number of cigarette smoked/day during pregnancy, pre-pregnancy BMI, and treatment for psychiatric illnesses (yes/no).

BMI: body mass index; CI: confidence Interval; CI: Interleukin; IL; Tumor Necrosis Factor: TNF

Table 3.

Associations of cumulative cytokine concentrations during the 2nd and 3rd trimesters of pregnancy with infant rapid weight gain years (The United States Collaborative Perinatal Project, 1959–1966).

	Infant rapid weight gain		
	All children n=1366 OR (95%CI)	Boys n=600 OR (95%CI)	Girls n=766 OR (95%CI)
IL-1β	0.95 (0.87, 1.04)	0.94 (0.82, 1.07)	0.97 (0.86, 1.09)
TNF-α	0.85 (0.63, 1.14)	0.63 (0.37, 1.07)	1.01 (0.70, 1.47)
IL-6	0.94 (0.84, 1.04)	0.89 (0.75, 1.07)	0.97 (0.84, 1.11)
IL-8	1.08 (1.00, 1.17)	1.13 (0.99, 1.28)	1.05 (0.95, 1.16)
IL-10	0.97 (0.83, 1.14)	1.03 (0.82, 1.29)	0.92 (0.74, 1.15)

Models were adjusted for maternal age (year), race (White/non-White), education (year), socioeconomic disadvantage, number of cigarette smoked/day during pregnancy, pre-pregnancy BMI, and treatment for psychiatric illnesses (yes/no).

ORs are reported per log unit increase in the cumulative cytokine concentration (pg/ml) of each cytokine during the 2nd and 3rd trimesters.

We defined weight gain in the first year of life as the change in weight-for-age z scores between the birth and the end of the first year (or the closest date to age one year). A gain ≥ 0.67 between birth and age one year was considered *rapid weight gain* in infancy.

BMI: body mass index; CI: confidence interval; IL: interleukin; OR: odds ratio; TNF: tumor necrosis factor