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Original article

Maternal Inflammation, Offspring Neurobehavioral Health, and Perinatal Correlates in the Healthy Start Study

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Abstract

Background: *In utero* exposure to increased inflammation caused by acute experiences may negatively impact on child neurodevelopment, but little is known about the effects of low-grade chronic inflammation. We wanted to investigate prenatal inflammation, as indicated by biomarkers of low-grade chronic inflammation, as an *in utero* programming method for neurodevelopment, and see how much of these associations are explained by perinatal factors.

Methods: We utilized linear regression to analyze the relationship between prenatal C-reactive protein, interleukin-6, and tumor necrosis factor-α and offspring Child Behavior Check List scores for total problems, externalizing and internalizing behaviors in 489 mother-offspring pairs from the Healthy Start cohort, based in Denver, Colorado, USA. We made sequential adjustments for perinatal correlates. Model 1 considered maternal age, gestational age at blood draw, and child age. Model 2 included further adjustments for mother race/ethnicity, followed by household income in Model 3 and pre-pregnancy body mass index (BMI) in Model 4. Finally, we used the maternal Edinburgh Postnatal Depression Scale and Perceived Stress Scale scores as measures of psychosocial stress.

Results: In Model 1, children of mothers with C-reactive protein in the fourth quartile had a 3.82 (95% CI: 0.94, 6.70) unit higher t-score for total problems compared to those in the first to third quartiles. The inclusion of household income and pre-pregnancy BMI in the fully adjusted model reduced the impact estimate to 3.11 (-0.45, 6.67). We found a similar pattern in externalizing behavior and across models for interleukin 6.

Conclusions: Maternal inflammation is associated with worse neurobehavioral outcomes in children. This association was influenced by higher pre-pregnancy BMI and lower household income.

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Keywords: Prenatal inflammation, Offspring neurobehavioral health, Psychosocial stress, *in utero*, BMI, IL-6, CRP, TNF-α, Offspring Child Behavior Checklist scores, Maternal inflammation, USA

Introduction

Gestation is a sensitive stage of development for neurobehavioral programming (1), and exposure to an inflammatory *in utero* environment may negatively impact on neurodevelopment via influencing fetal brain development. For example, maternal inflammation caused by an acute infection has been related to a variety of adverse neurodevelopmental disorders in children, including bipolar disorder and schizophrenia (2-5). Animal models indicate that the adverse impact on offspring neurobehavioral health is caused by the maternal immune response on neural connections and central nervous system function in the developing fetal brain, rather than the invading virus itself (6,7). Less is known about the impact of low-grade, chronic inflammation during pregnancy, which is caused by a variety of exposures and experiences such as maternal psychosocial stress, exposure to environmental toxins, and excess adiposity prior to and during pregnancy.

To date, only a few research have looked into the link between prenatal inflammation in otherwise healthy pregnant women and offspring neurobehavioral outcomes. In a study of 86 mother-offspring pairs, higher maternal interleukin-6 (IL-6), an inflammatory cytokine that stimulates other acute phase proteins, was linked to larger right amygdala volume and stronger left amygdala connection at four weeks of age. These structural changes to the brain were later linked to poorer impulse control at the age of two (8). In another study of the same cohort, greater maternal IL-6 levels during pregnancy were associated with reduced white matter integrity in the neonate's brain at birth and lower cognitive scores at 12 months of age using the Bayley Scales of Infant Development (9). Elevated maternal serum IL-6 during pregnancy negatively impacts fetal brain development and neurocognitive outcomes during childhood. However, the inflammatory cascade includes multiple cytokines and proteins, such as CRP and TNF-α, that represent distinct aspects of the inflammatory response and have also been implicated in *in utero* programming of suboptimal neurodevelopment. For example, a meta-analysis of 15 studies found a strong link between greater maternal C-reactive protein (CRP) and the risk of schizophrenia in children (10). In another investigation, cytokines such as IL-6 and TNF-α, as a latent variable (11), were found to be associated with ADHD in early childhood (12). Little is known about how biomarkers of low-grade chronic inflammation, measured separately or in combination during the prenatal period, relate to subclinical progression of neurodevelopmental disorders.

In this study, we investigated associations between maternal inflammation during mid-pregnancy, as indicated by CRP, IL-6, and TNF-α - individually and as a composite z-score to capture overall inflammatory potential - and neurobehavioral outcomes in offspring during early childhood, based on maternal responses to the Child Behavior Check List. Subsequently, we investigated the extent to which the associations between maternal inflammation and child neurobehavioral health are explained by maternal perinatal characteristics, such as psychosocial stress during pregnancy, which has been consistently linked to poor neurodevelopmental outcomes in offspring (13-17), including our own research (18). We hypothesized that increased maternal inflammation would be related with poorer neurobehavioral outcomes in young children, and that maternal psychosocial stress during pregnancy would be a critical upstream driver of this association.

Methods

Study Population

The Healthy Start Study is a prospective, population-based cohort study of 1,410 pregnant women and their infants recruited at the University of Colorado Hospital in Aurora, Colorado, USA, between 2010 and 2014. Women were excluded if they were expecting multiple babies, had a history of stillbirth or premature birth <25 gestational weeks, had pre-existing diabetes, asthma, cancer, or psychiatric illness, were younger than 16 years old, or had already completed 24 weeks of gestation (19). During pregnancy, women participated in two study visits at about 17 ("early pregnancy visit") and 27 ("mid-pregnancy visit") gestational weeks. During these appointments, they filled out questionnaires about their sociodemographic and health behaviors. During the mid-pregnancy appointment, women supplied serum samples that were utilized to assess inflammation biomarkers. Following delivery, mother and child pairs participated in subsequent study visits, including one between the ages of 4 and 7 ("early childhood visit"). During the early childhood visit, the mother completed questionnaires about her child's health history, health behaviors, and neurocognitive and behavioral health.

For this study, we excluded mother-offspring pairs who did not complete the Edinburgh Postnatal

Depression Scale (EPDS) or Perceived Stress Scale (PSS) questionnaires during early pregnancy, the Child Behavior Checklist (CBCL) questionnaire during early childhood or did not have data on at least one inflammation biomarker during mid-pregnancy (Figure 1). This yielded a sample of 489 mother-offspring pairings for the current investigation. Our subsample was comparable to the overall sample in terms of demographic and perinatal features, except for a lower prevalence of smoking during pregnancy (4.3% vs. 8.8%) (Supplemental Table 2).

Assessment of maternal inflammation during pregnancy

We measured three maternal inflammation indicators in serum obtained during mid-pregnancy: CRP, IL-6, and TNF-α. CRP was evaluated using immunoturbidimetric methods (Beckman Coulter, Inc.), whereas IL-6 was assessed with Luminex MAP technology (R&D Systems, Inc.). CRP and IL-6 were measured in a subsample (N = 243) of women chosen for an auxiliary investigation. TNF-α was quantified with a multiplex panel immunoassay (20). TNF-α values were verified using repeated assays on the same serum sample. If the intra-assay coefficient of variation (CV) exceeded 20% or only one TNF-α measurement was obtained, the experiment was repeated. Each participant's TNF-α levels were reported as an average of two measurements.
We assessed each inflammatory biomarker in its natural unit. We also computed a composite inflammation z-score for each participant by averaging the internally-standardized z-scores for the three biomarkers, as has been done in prior research using several inflammation biomarkers to capture overall inflammatory potential (21,22).

Assessment of offspring neurobehavioral outcomes

During the early childhood visit, a parent gave demographic information about their child and filled out the CBCL questionnaire. The CBCL-5 was completed for children under the age of five, while the CBCL-6 was completed for children six years and older. The CBCL-5 and CBCL-6 are questionnaires of 106 and 118 items, respectively, that are used to assess social, emotional, and behavioral disorders. In this study, we focused on the three composite scale outcomes measured by the CBCL: total problems, externalizing behavior, and internalizing behavior. We opted to focus on these outcomes because they are higher order scales that reflect prevalent neurobehavioral illnesses, such as anxiety, depression, and attention disorders, which can be difficult to identify in early childhood but can be anchored in this developmental stage and persist into adulthood (23). The CBCL data were graded using a standard technique (24). We utilized t-scores to assess all three CBCL outcomes.

Perinatal determinants of prenatal inflammation

Sociodemographic characteristics

Self-reported sociodemographic factors included maternal race and ethnicity, education level, annual household income, household size, and receipt of public assistance. Women stated their race and ethnicity by choosing from the following options: Hispanic or Latina, White, Black, or African American, Asian or Pacific Islander, American Indian or Alaska Native, and others. We collapsed race and ethnicity options to Asian or Pacific Islander, Hispanic, non-Hispanic Black, non-Hispanic White, and Other. Women who identified as Hispanic or Latina and neither White nor Black were categorized as Hispanic or Latina. Women who identified with more than one race were classified as multi-race and then as "Other" due to the minimal number of participants in the multi-race category. We classified education levels as less than high school, high school diploma or GED, some college or associate degree, four years of college, or graduate degree. The birth country was classified as either in the United States or outside of it. The annual household income was divided into two categories: ≤$70,000 and >$70,000. Household sizes were classified as <5 and ≥5 persons. Women who reported getting food stamps or WIC benefits at the time of their early prenatal visit were considered to have received public assistance, as compared to those who did not.

Perinatal characteristics

Pre-pregnancy body mass index (BMI) (kg/m2) was obtained by measuring mother's height at the first study visit and maternal weight at the first prenatal visit, or by self-report at the first research visit. The pre-pregnancy BMI was then classified as underweight, normal weight, overweight, or obese using conventional thresholds (19). Women self-reported parity at enrollment, and gestational diabetes mellitus diagnosis was documented from the medical record (25).

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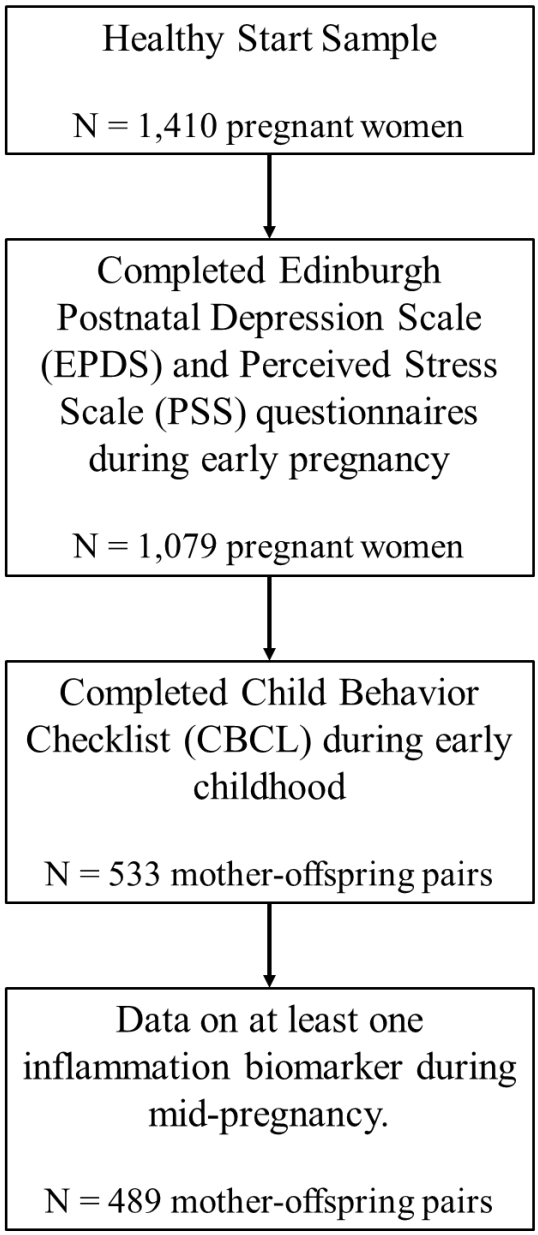

Figure 1. Sample inclusion criteria

Table 1. Associations of maternal characteristics and maternal inflammation biomarkers assessed during early pregnancy.

		C-reactive protein (CRP; mg/L)		Interleukin-6 (IL-6; pg/mL)		Tumor necrosis factor-α (TNF-α; pg/mL)	
Maternal Sociodemographic Characteristics	**N**	**Mean ± SD**	**p-value**	**Mean ± SD**	**p-value**	**Mean ± SD**	**p-value**
Maternal Age			0.16		**0.03**		0.23
16 - 24 years	125	6.47 ± 6.48		2.20 ± 3.31		1.50 ± 1.05	
25 - 29 years	140	8.25 ± 8.36		1.89 ± 1.54		1.72 ± 1.41	
30-34 years	152	5.64 ± 5.37		1.64 ± 1.10		1.61 ± 1.33	
35 years or older	72	5.21 ± 7.25		1.38 ± 0.99		1.79 ± 1.32	
Mother's race and ethnicity			**0.0009**		0.07		0.43
Hispanic	119	9.91 ± 10.19		2.35 ± 2.01		1.72 ± 1.2	
Non-Hispanic White	289	5.71 ± 5.08		1.56 ± 1.33		1.67 ± 1.37	
Non-Hispanic Black or African American	57	5.43 ± 6.35		2.23 ± 4.06		1.39 ± 1.14	
Asian	12	2.88 ± 1.31		1.05 ± 0.29		1.47 ± 1.09	
Other	12	5.78 ± 6.40		1.99 ± 0.91		1.33 ± 0.73	
Mother's birth country			0.27		0.79		0.85
United States	402	6.78 ± 7.2		1.84 ± 2.12		1.63 ± 1.31	
Outside United States	87	5.47 ± 5.54		1.75 ± 1.59		1.66 ± 1.18	

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Mother's education			**0.002**		**0.01**		0.89
Less than 12th grade	58	9.13 ± 8.10		1.83 ± 0.98		1.66 ± 1.21	
High school degree or GED	77	6.61 ± 6.28		2.65 ± 3.89		1.58 ± 1.19	
Some college or associate's degree	102	8.36 ± 8.92		2.07 ± 1.92		1.59 ± 1.27	
Four years of college (BA, BS)	118	5.97 ± 6.86		1.63 ± 1.53		1.76 ± 1.54	
Graduate degree	134	4.77 ± 4.62		1.37 ± 0.95		1.59 ± 1.15	
Household Income			**0.002**		**<.0001**		**0.03**
≤$20,000	55	9.60 ± 9.08		1.96 ± 1.14		1.39 ± 1.07	
$20,001 - 40,000	73	7.76 ± 7.44		2.22 ± 2.43		1.50 ± 0.89	
$40,001 - 70,000	89	7.58 ± 7.69		1.62 ± 1.17		1.54 ± 1.26	
>$70,000	188	4.61 ± 4.93		1.42 ± 1.01		1.77 ± 1.47	
Number of persons in household			0.81		0.48		**0.04**
<5	437	6.58 ± 7.04		1.86 ± 2.15		1.60 ± 1.25	
≥5	50	6.23 ± 6.33		1.56 ± 0.77		2.00 ± 1.57	
Receipt of public assistance			**<.0001**		0.22		0.3
Yes	140	9.56 ± 8.97		2.08 ± 1.53		1.54 ± 1.18	
No	346	5.38 ± 5.58		1.73 ± 2.21		1.68 ± 1.33	

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Maternal Perinatal Characteristics								
Pre-pregnancy body mass index			**<.0001**		**<.0001**		0.94	
Underweight	16	3.38 ± 1.99		1.23 ± 0.44		1.26 ± 0.83		
Normal weight	222	4.47 ± 4.83		1.31 ± 0.70		1.70 ± 1.40		
Overweight	152	6.46 ± 4.79		1.59 ± 0.93		1.58 ± 1.21		
Obese	99	12.32 ± 10.88		3.60 ± 4.01		1.64 ± 1.21		
Parity			0.99		0.36		0.3	
1	468	6.57 ± 6.86		1.85 ± 2.09		1.63 ± 1.28		
2	21	6.54 ± 8.95		1.30 ± 0.53		1.92 ± 1.55		
Gestational diabetes			0.11		0.63		0.47	
Yes	22	9.54 ± 9.63		2.10 ± 1.51		1.45 ± 0.71		
No	434	6.47 ± 6.82		1.82 ± 2.1		1.66 ± 1.34		
Smoked during pregnancy			0.52		0.49		**0.02**	
Did smoke	21	7.83 ± 8.53		1.43 ± 0.83		1.01 ± 0.52		
Did not smoke	468	6.5 ± 6.88		1.85 ± 2.08		1.67 ± 1.31		
Healthy Eating index (HEI) score			0.09		**0.03**		0.48	
HEI >57	254	7.28 ± 7.48		2.09 ± 2.52		1.61 ± 1.32		
HEI ≤57	230	5.78 ± 6.28		1.53 ± 1.25		1.69 ± 1.27		

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Physical activity			0.15		0.79	0.46
≥150 minutes/week	271	6.02 ± 6.64		1.86 ± 2.27	1.60 ± 1.31	
<150 minutes/week	218	7.30 ± 7.33		1.79 ± 1.69	1.69 ± 1.27	
Gestational age at delivery			0.35		0.57	0.72
<37 weeks	30	4.17 ± 3.04		1.40 ± 0.86	1.72 ± 1.34	
≥37 weeks	459	6.64 ± 7.03		1.84 ± 2.06	1.63 ± 1.29	
Offspring Characteristics						
Sex			0.56		0.15	0.13
Female	240	6.27 ± 5.94		1.62 ± 1.22	1.55 ± 1.29	
Male	249	6.79 ± 7.66		1.99 ± 2.49	1.73 ± 1.28	

SD = standard deviation; GED = general education diploma; BA = bachelor of arts; BS = bachelor of science

Table 2. Spearman Correlation Coefficients (Rho; ρ) among inflammation biomarkers C-reactive protein (CRP), Interleukin (IL)-6, and Tumor necrosis factor (TNF)-α measured at 27 weeks gestation.

		CRP	IL-6	TNF-α
CRP	ρ		0.52	0.09
	N		242	242
IL-6	ρ	0.52		0.12
	N	242		243
TNF-α	ρ	0.09	0.12	
	N	242	243	

ρ represents the Spearman correlation coefficient, indicating the strength and direction of the association between two variables.

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Table 3. Associations of maternal prenatal inflammation biomarkers, assessed as the highest (Q4) vs. lower quartiles (Q1-3) of inflammation biomarkers assessed at 27 weeks gestation with offspring Child Behavior Checklist (CBCL) scores assessed at age 4-7 y, adjusting for sociodemographic and perinatal characteristics

	Difference β [95% CI]) in offspring CBCL t-scores in early childhood for Q4 vs. Q1-3 of maternal inflammation during mid-pregnancy								
	Total problems			**Externalizing behavior**			**Internalizing behavior**		
Exposure: Q4 vs. Q1-3 (referent) of maternal inflammation	**N**	**β (95% CI)**	**p-value**	**N**	**β (95% CI)**	**p-value**	**N**	**β (95% CI)**	**p-value**
Model 1: maternal age and gestational week at the time of mid-pregnancy blood draw; and child's age at the time of CBCL assessment									
C-reactive protein (CRP)	239	3.82 (0.94, 6.70)	0.01	239	4.19 (1.27, 7.10)	0.005	239	2.26 (-0.65, 5.17)	0.13
Interleukin-6 (IL-6)	240	3.56 (0.67, 6.44)	0.02	240	4.80 (1.90, 7.70)	0.001	240	1.23 (-1.69, 4.15)	0.41
Tumor necrosis factor-α (TNF-α)	475	0.06 (-1.98, 2.10)	0.96	475	0.46 (-1.57, 2.48)	0.66	475	-0.54 (-2.62, 1.54)	0.61
Overall inflammation z-score	239	3.14 (0.28, 6.01)	0.03	239	3.77 (0.88, 6.66)	0.01	239	1.42 (-1.47, 4.31)	0.34
Model 2: Model 1 + maternal race and ethnicity									
C-reactive protein (CRP)	239	3.76 (0.85, 6.66)	0.01	239	4.33 (1.40, 7.26)	0.004	239	2.16 (-0.78, 5.09)	0.15
Interleukin-6 (IL-6)	240	3.48 (0.60, 6.37)	0.02	240	4.86 (1.97, 7.75)	0.001	240	1.15 (-1.77, 4.07)	0.44
Tumor necrosis factor-α (TNF-α)	475	-0.01 (-2.06, 2.03)	0.99	475	0.46 (-1.57, 2.49)	0.66	475	-0.67 (-2.74, 1.41)	0.53
Overall inflammation z-score	239	3.04 (0.17, 5.92)	0.04	239	3.82 (0.93, 6.72)	0.01	239	1.29 (-1.62, 4.19)	0.39

Model 3: Model 2 + annual household income during the prenatal period									
C-reactive protein (CRP)	195	3.31 (0.02, 6.60)	0.05	195	3.39 (0.09, 6.69)	0.04	195	2.73 (-0.66, 6.12)	0.11
Interleukin-6 (IL-6)	195	3.06 (-0.18, 6.30)	0.06	195	4.14 (0.92, 7.36)	0.01	195	0.74 (-2.61, 4.10)	0.66
Tumor necrosis factor-α (TNF-α)	394	-0.29 (-2.47, 1.89)	0.79	394	0.19 (-1.97, 2.36)	0.86	394	-1.36 (-3.61, 0.88)	0.23
Overall inflammation z-score	195	2.95 (-0.21, 6.11)	0.07	195	3.39 (0.24, 6.54)	0.04	195	1.44 (-1.83, 4.70)	0.39
Model 4: Model 3 + maternal pre-pregnancy body mass index									
C-reactive protein (CRP)	195	3.11 (-0.45, 6.67)	0.09	195	2.48 (-1.06, 6.03)	0.17	195	3.31 (-0.35, 6.97)	0.08
Interleukin-6 (IL-6)	195	2.85 (-0.69, 6.39)	0.11	195	3.36 (-0.14, 6.87)	0.06	195	1.01 (-2.65, 4.67)	0.59
Tumor necrosis factor-α (TNF-α)	394	-0.42 (-2.60, 1.76)	0.71	394	-0.02 (-2.18, 2.14)	0.99	394	-1.34 (-3.59, 0.91)	0.24
Overall inflammation z-score	195	2.72 (-0.70, 6.14)	0.12	195	2.54 (-0.87, 5.94)	0.14	195	1.79 (-1.74, 5.32)	0.32

CBCL = Child Behavior Checklist; Q1 = 1st quartile; Q2 = 2nd quartile; Q3 = 3rd quartile; Q4 = 4th quartile; CI = confidence interval
β represents the beta coefficient, which measures the change in the specified CBCL outcome for a one-unit change in the specified inflammation biomarker. There is a 95% probability that the true effect estimate of the population will lie within the 95% CI calculated from the study sample.

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Table 4. Associations of maternal prenatal inflammation biomarkers, assessed as the highest (Q4) vs. lower (Q1-3) quartiles of inflammation biomarkers assessed at 27 weeks gestation with offspring Child Behavior Checklist (CBCL) scores assessed in at age 4-7 y, adjusting for indicators of maternal psychosocial stress during pregnancy

	Difference β [95% CI]) in offspring CBCL t-scores in early childhood for Q4 vs. Q1-3 of maternal inflammation during mid-pregnancy								
	Total problems			**Externalizing behavior**			**Internalizing behavior**		
Exposure: Q4 vs. Q1-3 (referent) of maternal inflammation	**N**	**β (95% CI)**	**p-value**	**N**	**β (95% CI)**	**p-value**	**N**	**β (95% CI)**	**p-value**
Model 1[a]									
C-reactive protein (CRP)	195	3.11 (-0.45, 6.67)	0.09	195	2.48 (-1.06, 6.03)	0.17	195	3.31 (-0.35, 6.97)	0.08
Interleukin-6 (IL-6)	195	2.85 (-0.69, 6.39)	0.11	195	3.36 (-0.14, 6.87)	0.06	195	1.01 (-2.65, 4.67)	0.59
Tumor necrosis factor-α (TNF-α)	394	-0.42 (-2.60, 1.76)	0.71	394	-0.02 (-2.18, 2.14)	0.99	394	-1.34 (-3.59, 0.91)	0.24
Overall inflammation z-score	195	2.72 (-0.70, 6.14)	0.12	195	2.54 (-0.87, 5.94)	0.14	195	1.79 (-1.74, 5.32)	0.32
Model 1 + Domain 1 (Overwhelmed)									
C-reactive protein (CRP)	195	3.28 (-0.14, 6.70)	0.06	195	2.65 (-0.77, 6.07)	0.13	195	3.45 (-0.11, 7.02)	0.06
Interleukin-6 (IL-6)	195	2.71 (-0.70, 6.12)	0.12	195	3.24 (-0.15, 6.62)	0.06	195	0.89 (-2.68, 4.47)	0.62
Tumor necrosis factor-α (TNF-α)	394	-0.25 (-2.30, 1.81)	0.82	394	0.13 (-1.93, 2.19)	0.90	394	-1.17 (-3.31, 0.97)	0.28
Overall inflammation z-score	195	2.33 (-0.98, 5.63)	0.17	195	2.16 (-1.14, 5.45)	0.20	195	1.46 (-2.00, 4.91)	0.41

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Model 1 + Domain 2 (Anhedonia)									
C-reactive protein (CRP)	195	3.32 (-0.23, 6.88)	0.07	195	2.72 (-0.81, 6.26)	0.13	195	3.51 (-0.15, 7.17)	0.06
Interleukin-6 (IL-6)	195	3.04 (-0.49, 6.58)	0.09	195	3.59 (0.10, 7.09)	0.04	195	1.18 (-2.48, 4.84)	0.53
Tumor necrosis factor-α (TNF-α)	394	-0.45 (-2.64, 1.73)	0.68	394	-0.03 (-2.18, 2.13)	0.98	394	-1.37 (-3.62, 0.87)	0.23
Overall inflammation z-score	195	3.13 (-0.32, 6.57)	0.08	195	2.99 (-0.43, 6.41)	0.09	195	2.15 (-1.41, 5.71)	0.24
Model 1 + Domain 3 (Lack of control)									
C-reactive protein (CRP)	195	3.24 (-0.35, 6.82)	0.08	195	2.67 (-0.90, 6.24)	0.14	195	3.37 (-0.33, 7.06)	0.07
Interleukin-6 (IL-6)	195	2.84 (-0.70, 6.38)	0.12	195	3.34 (-0.16, 6.85)	0.06	195	1.01 (-2.66, 4.67)	0.59
Tumor necrosis factor-α (TNF-α)	394	-0.51 (-2.68, 1.67)	0.65	394	-0.13 (-2.27, 2.02)	0.91	394	-1.41 (-3.66, 0.83)	0.22
Overall inflammation z-score	195	2.75 (-0.67, 6.17)	0.12	195	2.59 (-0.82, 5.99)	0.14	195	1.80 (-1.74, 5.33)	0.32
Model 1 + EPDS z-score									
C-reactive protein (CRP)	195	3.02 (-0.48, 6.53)	0.09	195	2.40 (-1.10, 5.90)	0.18	195	3.23 (-0.39, 6.85)	0.08
Interleukin-6 (IL-6)	195	2.58 (-0.91, 6.08)	0.15	195	3.12 (-0.35, 6.59)	0.08	195	0.77 (-2.86, 4.41)	0.68
Tumor necrosis factor-α (TNF-α)	394	-0.54 (-2.62, 1.53)	0.61	394	-0.12 (-2.20, 1.95)	0.91	394	-1.46 (-3.61, 0.68)	0.18
Overall inflammation z-score	195	2.15 (-1.26, 5.56)	0.22	195	2.00 (-1.40, 5.40)	0.25	195	1.28 (-2.25, 4.82)	0.48

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Model 1 + PSS z-score									
C-reactive protein (CRP)	195	3.20 (-0.29, 6.68)	0.07	195	2.57 (-0.91, 6.05)	0.15	195	3.37 (-0.25, 6.99)	0.07
Interleukin-6 (IL-6)	195	2.79 (-0.68, 6.26)	0.12	195	3.31 (-0.14, 6.75)	0.06	195	0.96 (-2.66, 4.59)	0.60
Tumor necrosis factor-α (TNF-α)	394	-0.17 (-2.29, 1.94)	0.87	394	0.18 (-1.93, 2.29)	0.87	394	-1.13 (-3.34, 1.07)	0.31
Overall inflammation z-score	195	2.48 (-0.88, 5.84)	0.15	195	2.30 (-1.04, 5.65)	0.18	195	1.61 (-1.89, 5.11)	0.37

[a] **Model 4** is adjusted for gestational week at the time of mid-pregnancy blood draw, maternal age, race and ethnicity, annual household income, and pre-pregnancy body mass index.

CBCL = Child Behavior Checklist; Q1 = 1st quartile; Q2 = 2nd quartile; Q3 = 3rd quartile; Q4 = 4th quartile; CI = confidence interval
β represents the beta coefficient, which measures the change in the specified CBCL outcome for a one-unit change in the specified inflammation biomarker. There is a 95% probability that the true effect estimate of the population will lie within the 95% CI calculated from the study sample.

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Health behaviors during pregnancy

Women's food consumption data was gathered using the multiple-pass Automated Self-Administered 24-hour Dietary Recall (ASA24) questionnaire, which began in the first trimester and averaged three to four recalls per participant (26,27). We estimated the Healthy Eating Index (HEI)-2010 score and divided it into two categories: ≤57 and >57, indicating low vs good diet quality (26, 27). Women self-reported their smoking patterns throughout early pregnancy, and we classified them as having smoked during gestation and not smoking during gestation.

Maternal psychosocial stress

We utilized the Edinburgh Postnatal Depression Scale (EPDS) to assess likely prenatal depression and Cohen's Perceived Stress Scale (PSS) to assess perceived stress. We discuss data collection methods, as well as the validity and reliability of the EPDS and PSS, elsewhere (28).

In the current study, we measured maternal psychosocial stress using EPDS and PSS scores normalized as z-scores for use in regression models. We also incorporated three new categories of maternal psychosocial stress that we had previously identified through EPDS and PSS responses (28). These domains include Domain 1 (Overwhelmed), Domain 2 (Anhedonia), and Domain 3 (Lack of Control) and operationalized as continuous scores with mean ~0 and SD ~1.

Statistical Analysis

First, we used linear regression to assess the distribution of each inflammation biomarker across maternal sociodemographic, perinatal, and pregnancy-related health behaviors. At the time, we used Spearmen's correlation tests to investigate the relationships between inflammation biomarkers. Next, we utilized multivariable linear regression using the following formula: $y_i = x_{i1} + x_{i2} + x_{i3} + ... x_{in} + e_i$, where y_i is the offspring CBCL score, x_{i1} is the maternal inflammatory biomarker of interest, and subsequent x_{in} are adjustment covariates. We used a series of multivariable models to investigate the correlations between each maternal inflammation biomarker and child CBCL score. In these models, we classified inflammatory biomarkers as quartile 4 (Q4) against quartiles 1-3 (Q1-3; referent) based on a threshold effect discovered in exploratory analysis.

To account for variation caused by biological and logistical factors, Model 1 includes maternal and gestational age at the time of mid-pregnancy blood draw, as well as child age at the time of CBCL assessment. Model 2 further accounted for maternal race and ethnicity. Model 3 also accounted for household income, a form of stress that is likely linked to other perinatal factors associated with maternal inflammation (for example, education level and receipt of public assistance). Finally, in Model 4, we included Model 3 covariates with pre-pregnancy BMI to capture physiological parameters that have a direct impact on inflammation throughout pregnancy and child outcomes. Finally, we added each indicator of maternal psychosocial stress (EPDS z-score, PSS z-score, Overwhelmed, Anhedonia, Lack of Control) separately to Model 4 to determine how much variation in the association between maternal inflammation and offspring neurobehavioral health is explained by maternal experiences of psychosocial stress. Prior to doing this, we assessed correlations between the psychosocial stress and we found strong positive associations between Overwhelmed (Domain 1) and EPDS z-score (Spearman $\rho = 0.77$), as well as Overwhelmed and PSS-z-score ($\rho = 0.72$) (Supplementary Table 1). We examined how correction for variables altered the direction, size, and significance of each inflammation biomarker across the models. An attenuation of ≥10% in the impact size of an inflammatory biomarker indicates that a specific covariate (or collection of covariates) is a significant upstream driver of the relationship of interest.

Because the relationship between maternal psychosocial stress and offspring outcomes may differ fundamentally among racial/ethnic groups, we incorporated a product term for continuous maternal inflammation and maternal race/ethnicity into the unadjusted models. We found no indication of effect modification by race/ethnicity (all P-interactions exceeded our alpha criterion of 0.05), hence we did not stratify analyses by race/ethnicity. Similarly, we investigated effect modification by child sex, based on existing evidence demonstrating sex differences in the prenatal response to maternal psychosocial stress (29) and inflammation (30-32). We found no indication of effect modification by child sex (all P-interactions > 0.05), hence we present results for boys and girls together.

In sensitivity analyses, we adjusted for a child's sex to account for potential disparities in neurodevelopment between boys and girls. Because doing so did not impact the results, we did not include child's sex in the models for the sake of parsimony (33) - especially as we found no variations in inflammatory biomarkers based on offspring sex. Finally, while there are no set criteria for inflammation biomarkers in pregnant populations, a CRP level greater than 10 mg/dL indicates an acute infection in non-pregnant groups (34). There were no

women in our sample who met this criterion, hence we did not conduct any sensitivity analyses excluding mother-offspring pairs with high inflammation biomarker values. In all analyses, we used alpha = 0.05 as the statistical significance level. We used jackknife residuals to assess deviations from linear regression assumptions in all models. SAS version 9.4 (Cary, North Carolina, USA) was used for all statistical analyses.

Results

Women in our sample had a mean age (SD) of 29 (6) years at the time of the early pregnancy visit. Many women in our sample identified as non-Hispanic White (58.4%), were born in the United States (83.4%), and had attended college or gotten a higher degree (71.8%). Over half of the women (55%) had yearly household earnings of less than $70,000, and about 29% received food stamps or WIC program benefits. The average (SD) age of the children at the early childhood visit was 5 (0.6), with half (49.3%) being female.

During the mid-pregnancy visit, the maternal CRP level was 3.3 (0.4) mg/L, IL-6 was 1.8 (2) pg/mL, and TNF-α was 1.6 (1.3) pg/mL. Table 1 displays the distribution of CRP, IL-6, and TNF-α based on maternal and perinatal factors. Women who identified as Hispanic, had lower educational attainment, lower household income, received public assistance, and had a greater BMI had higher CRP levels. Younger maternal age, lower educational attainment, lower household income, and increasing BMI were associated with higher IL-6. Lower household income, household size more than four people, and smoking during pregnancy were associated with higher TNF-α.

Table 2 demonstrates the correlations between inflammatory biomarkers. CRP and IL-6 exhibited a moderately positive correlation (Spearman $\rho = 0.52$). TNF-α showed positive correlations with IL-6 ($\rho = 0.12$) and CRP ($\rho = 0.09$), however the magnitude was modest to moderate.

Table 3 displays the findings of multivariable models. In our base model (Model 1), which we adjusted for gestational week at the time of mid-pregnancy blood draw, maternal age, and child age at the time of CBCL assessment, we found statistically significant associations between CRP, IL-6, and the inflammation z-score with total problems and externalizing behavior. Children of mothers in Q4 had 3.82 (95% CI: 0.94, 6.70) units higher t-scores for total problems and 4.19 (95% CI: 1.27, 7.10) units higher t-scores for externalizing behavior than children of moms in Q1-3 of CRP. We found comparable relationships for IL-6 but not for TNF-α (Table 3). When the inflammatory z-score was used as the exposure, children born to women in Q4 had 3.14 (95% CI: 0.28, 6.01) units higher t-scores for total problems and 3.77 (95% CI: 0.88, 6.66) units higher t-scores for externalizing behavior than those born to moms in Q1-3.

Model 2 adjusted for maternal race and ethnicity, but the effect estimates remained unchanged. Accounting for annual household income in Model 3 attenuated the estimate for CRP and IL-6 by approximately 12%. For example, children of moms in Q4 vs. Q1-3 of CRP had 3.31 (95% CI: 0.02, 6.60) units higher t-score for total problems, or a 12% attenuation. (Table 3, Model 3). Adjusting for maternal pre-pregnancy BMI in Model 4 resulted in the highest attenuation of impact estimates: children of women with prenatal CRP in Q4 vs. Q1-3 had 3.11 (95% CI: -0.45, 6.67) units higher t-score for total problems, as opposed to 3.31 (95% CI: 0.02, 6.60) units higher t-score. Similar results were seen for IL-6 and the inflammatory z-score (Table 3, Model 4).

In Table 4, we refer to the fully adjusted model from Table 3 as Model 1 and add each maternal stress indicator to this model to see how much of the association of interest was explained by maternal psychosocial stress during pregnancy. Contrary to our expectations, we did not see any further attenuation in estimates after correcting for maternal stress.

Discussion

In this study of 489 mother-offspring pairs, we examined associations between maternal prenatal inflammation, according to mid-pregnancy serum levels of CRP, IL-6, TNF-α, and a composite inflammation z-score, and offspring total problems, externalizing behavior, and internalizing behavior assessed by the Child Behavior Check List (CBCL) at age 5 years. We identified relationships between CRP, IL-6, and overall inflammation z-scores and higher (worse) ratings for total problems and externalizing behaviors. Contrary to our expectations, these associations were partly driven by annual household income and pre-pregnancy BMI, but not by markers of maternal psychosocial stress.

Prenatal inflammation and offspring neurodevelopmental outcomes

Our findings that maternal prenatal inflammation is associated with poorer offspring neurodevelopmental outcomes are consistent with those of Rasmussen and colleagues and Graham and colleagues (8,9), who reported that higher maternal IL-6 levels during pregnancy were associated with poorer offspring cognition and impulse control according to the

Bayley Scales of Infant and Toddler Development (35) and the snack delay task (36,37) at 12 and 24 months of age, respectively. The current study contributes to these findings in two important ways. First, we measured not just IL-6, but also CRP, an acute phase protein produced in response to and regulated by IL-6 production (2,38), and TNF-α, an indicator of strength, efficacy, and duration of immune responses, which has been related to mood and psychosocial stress (2,38). Furthermore, we evaluated the average of all three biomarkers, which may more comprehensively reflect an inflammatory milieu than any single biomarker alone (11, 39). Second, while we did not measure structural or functional aspects of brain development in neonates, as Rasmussen and colleagues (9) and Graham and colleagues (8) did, we were able to detect significant differences in composite CBCL scores that have been linked to risk of leading mental health conditions (e.g., ADHD, anxiety, depression) (23) during a life stage when many chronic disease precursors take root and track across the life course (40).

There are several interpretations for the preceding finding. First, inflammation is linked to a variety of maternal health conditions, including obesity, gestational diabetes, and psychosocial stress. These features and experiences are also associated with less desirable neurobehavioral outcomes in children (7). As a result, it is possible that the link between maternal prenatal inflammation and offspring outcomes reflects the influence of these upstream factors. This notion, which we explicitly evaluated in our analysis and explain in the following section, is supported by the finding that adjusting for specific maternal features reduced effect estimates for inflammation biomarkers. Another explanation is that an inflammatory *in utero* environment has a direct impact on offspring neurodevelopment. One potential explanation is that maternal inflammation during pregnancy triggers an immunological response, which alters the developing central nervous system, including the brain and spinal cord (6,7). Furthermore, in mouse models, researchers discovered that even mild maternal inflammation might change placental synthesis of neurotransmitters (e.g., serotonin), which play important roles in fetal brain development (7,41). Future research is needed to determine whether the link between fetal inflammation and poor neurodevelopmental outcomes is causal or the result of confounding by upstream factors.

Drivers of the relationship between maternal prenatal inflammation and offspring neurodevelopment

Recognizing that sociodemographic, behavioral, and perinatal characteristics can provide possibilities for intervention, we parsed our multivariable models to uncover maternal traits that could explain the link between prenatal inflammation and child neurobehavioral outcomes. Our main conclusion is that pre-pregnancy BMI and household income were the biggest upstream predictors of this relationship. These findings are validated by literature. For example, higher circulating CRP has been associated with lower income (42) and educational attainment (43,44). Lower socioeconomic level is related with poor neurodevelopmental outcomes in children, such as unfavorable fetal brain morphology (45) and lower language scores at 24 months (46). Pre-pregnancy obesity, a risk factor for ADHD, autism spectrum disorder (ASD), and internalizing or externalizing behaviors in offspring (47) has been linked to higher serum CRP (48), IL-6, TNF-α, and monocyte chemoattractant protein-1 (39). In the Healthy Start cohort, there is a moderate inverse correlation between pre-pregnancy BMI and household income (Spearman $\rho = -0.24$). This likely reflects a tendency for higher BMI to cluster with lower income in most developed countries, which further highlights the vulnerability of women and children living under low socioeconomic status (49).

Contrary to our expectations and some prior literature (2,11,50), controlling for indicators of maternal psychosocial stress during pregnancy (EPDS, PSS, and the three novel domains of maternal prenatal stress) did not reduce the association between inflammation biomarkers and offspring neurobehavioral outcomes, implying that maternal psychosocial stress is not a driver of this relationship. There are several possibilities for this unexpected discovery. First, prior studies that identified maternal psychosocial stress as a shared common cause of prenatal inflammation and offspring neurodevelopmental outcomes, such as those by Gustafsson and colleagues (11), included a large proportion (73%) of offspring with at least one ADHD-positive parent. A high prevalence of parental mental health diagnoses may increase offspring predisposition for neurobehavioral outcomes (and thus improve statistical power to detect associations with these outcomes) via genetic predisposition, an effect of parental behaviors, or the shared home environment. Second, and related to the preceding point, women in Healthy Start represent a general-risk population with a low prevalence of mental health concerns, with 3% meeting the criteria for depression (EPDS score >13) (51), compared to nearly 30% of women in Gustafsson and colleagues' sample (11). Third, many women in our sample identified as White (59%), followed by

Hispanic/Latina (24%), and Black or African American (11%). The Hispanic/Latina paradox observed in maternal-child health studies suggests that we may not observe expected associations between risk factors and poor offspring health (52,53) when all Hispanic/Latina women are lumped together rather than aggregated by nativity and origin, among other characteristics (54). Furthermore, given the extensive literature on the relationships between social stress (55,56), inflammation (57), and adverse health outcomes in Black or African American people, it is possible that we would have found results consistent with our hypothesis about the role of maternal psychosocial stress in a setting with a higher proportion of Black or African American participants. This possibility calls for further exploration in different cohorts with more detailed racial and ethnic data.

Strengths and limitations

This study had various strengths. First, the Healthy Start Study provides an intergenerational dataset with extensive prenatal and offspring data, allowing us to investigate the associations of interest while controlling for confounding bias. Second, we examined the role of both singular (e.g., EPDS and PSS scores) and multidimensional measures of psychosocial stress. The latter allows us to measure psychosocial stress experiences based on women's responses to both the EPDS and the PSS, rather than taking each evaluation score separately. Finally, the comprehensive covariate data allowed us to correct for confounding bias while also progressively adjusting for key covariates that we believed were important to the relationship of interest.

This study had some limitations. CRP and IL-6 were only assessed in a subset of the analytical sample (N = 243), while TNF-α was measured in 489 women. This significantly reduced our sample size for models with CRP, IL-6, or overall prenatal inflammation z-score as the independent variable. The excluded participants were demographically like the analytical sample, except for a lower prevalence of prenatal smoking than the whole recruited group (4.3 vs 8.8%). Given that maternal prenatal smoking is linked to increased inflammation and poorer behavioral outcomes in offspring (58), our findings are likely conservative. There are likely other forms of psychosocial stress, such as that caused by racism and immigration, which were not collected in the Healthy Start cohort. Third, despite the Healthy Start study's racial and ethnic diversity, only 10% of the analytical sample identified as Black, a demographic for which there is extensive literature demonstrating lifetime exposure to psychosocial stress, discrimination, and bias that affect maternal-child health. Fourth, we acknowledge the possibility of chance findings, but we point out that the goal of this study was not to identify a set of statistically significant predictors for offspring neurobehavioral outcomes, but rather to test for consistency in the direction and magnitude of associations across a set of correlated biomarkers using a set of sequential models. Finally, the threshold used to indicate attenuation of effect estimates (≥10% change in estimate for an inflammation biomarker) was arbitrary. In general, the degree of attenuation in relationships after accounting for upstream variables was rather small, implying that other factors are likely influencing the link between maternal prenatal inflammation and offspring neurodevelopment. Specifically, we believe that maternal experiences with racism and discrimination may be a significant upstream driver of the link between maternal prenatal inflammation and child neurodevelopment - a topic that deserves further examination in future research.

Conclusion

In this study of 489 mother-offspring pairs from the Healthy Start pre-birth cohort, greater CRP, IL-6, and total prenatal inflammation were related with poorer neurobehavioral outcomes in offspring during early life. We also identified pre-pregnancy BMI and household income as potential drivers of the relationship between prenatal inflammation and poor offspring neurobehavioral health. These upstream characteristics can help inform system- and individual-level strategies to regulate or reduce inflammation during pregnancy, resulting in better mother and child health outcomes.

Conflict of interest disclosures: The authors have no conflicts of interest relevant to this article to disclose.

Authors' contribution: Drs. Dhaliwal and Perng conceptualized the research idea and approach. Dr. Dhaliwal implemented the analysis, drafted the initial article, and incorporated co-author feedback. Dr. Dabelea obtained funding for the data used in this analysis. Drs. Perng, Dabelea, Glueck, Lee-Winn, and Wilkening provided critical intellectual feedback on the article. All authors approved the final version of the article.

Clinical trial registry

The Healthy Start study is registered as an observational study at clinicaltrials.gov (NCT #002273297).

Ethics approval

All women provided written informed consent at study enrollment. The study protocol for the Healthy Start cohort was approved by the Colorado Multiple Institutional Reviewer Board (COMIRB protocol #09-0563).

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Supplemental Table 1. Spearman Correlation Coefficients (Rho; ρ) among maternal stress measures Domain 1 (Overwhelmed), Domain 2 (Anhedonia), Domain 3 (Lack of Control), Edinburgh Postnatal Depression Scale (EPDS), and Perceived Stress Scale (PSS) scores measured at 17 weeks gestation.

		Domain 1	Domain 2	Domain 3	EPDS z-score	PSS z-score
Domain 1 (Overwhelmed)	ρ		-0.20941	0.09436	0.76889	0.72378
	N		536	536	536	536
Domain 2 (Anhedonia)	ρ	-0.20941		-0.09544	0.18466	0.09479
	N	536		536	536	536
Domain 3 (Lack of control)	ρ	0.09436	-0.09544		0.34934	-0.28845
	N	536	536		536	536
EPDS z-score	ρ	0.76889	0.18466	0.34934		0.44144
	N	536	536	536		536
PSS z-score	ρ	0.72378	0.09479	-0.28845	0.44144	
	N	536	536	536	536	

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Supplemental Table 2. Maternal sociodemographic and perinatal characteristics in the Healthy Start Study and the study sample

Maternal Sociodemographic Characteristics	Healthy Start (N = 1,410)		Study Sample (N = 489)	
Maternal Age at Early Pregnancy Interview: Mean(SD)	28 (3.3)		28.6 (5.8)	
Mother's race/ethnicity: N (%)				
Hispanic	349	24.8	119	24.3
Non-Hispanic White	752	53.3	289	59.1
Non-Hispanic Black or African American	220	15.6	57	11.7
Non-Hispanic Asian	43	3.1	12	2.5
Other	45	3.2	12	2.5
Mother's birth country: N (%)				
United States	1207	85.6	402	82.2
Outside United States	202	14.3	87	17.8
Mother's education: N (%)				
Less than 12th grade	204	14.5	58	11.9
High school degree or GED	259	18.4	77	15.7
Some college or associate's degree	334	23.7	102	20.9
Four years of college (BA, BS)	309	21.9	118	24.1
Graduate degree	304	21.6	134	27.4
Household income: N (%)				
$20,000 or less	218	15.5	55	11.2
$20,001 - 40,000	196	18.4	73	14.9
$40,001 - 70,000	260	18.4	89	18.2
>$70,000	460	32.6	188	38.4

Maternal Perinatal Characteristics				
Pre-pregnancy BMI: Mean (SD), Median (IQR)	25.7 (6.2)		26.2 (6.5)	
Parity (pregnancy index): N (%)				
1	1369	97.1	468	95.7
2	40	2.8	21	4.3
3	1	0.1	0	
Smoked during pregnancy: N (%)				
Did smoke	124	8.8	21	4.3
Did not smoke	1285	91.1	468	95.7
Gestational Age at Delivery (weeks): Mean (SD), Median (IQR)	39 (1.9)		39.3 (1.9)	

GED = general education diploma; BA = bachelor of arts; BS = bachelor of science