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Associations of Maternal Prenatal Smoking with Umbilical Cord Blood Hormones: The Project Viva Cohort

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Abstract

Background—Maternal smoking during pregnancy is associated with low fetal growth and adverse cardiometabolic health in offspring. However, hormonal pathways underlying these associations are unclear. Therefore, we examined maternal smoking habits and umbilical cord blood hormone profiles in a large, prospective cohort.

Methods—We studied 978 mother/infant pairs in Project Viva, a Boston-area cohort recruited 1999–2002. We categorized mothers as early pregnancy smokers, former smokers, or never smokers. Outcomes were cord blood concentrations of IGF-1, IGF-2, IGFBP-3, leptin, adiponectin, insulin, and C-peptide. We used linear regression models adjusted for maternal pre-pregnancy body mass index (BMI), race/ethnicity, parity, education, and infant sex. We conducted analyses in the full cohort and stratified by infant sex.

Results—Thirteen percent of women were early pregnancy smokers, 20% former smokers, and 68% never smokers. Infants of early pregnancy smokers had lower IGF-1 adjusted for IGFBP-3 [−5.2 ng/mL (95% CI: −8.6, −1.7)], with more pronounced associations in girls [−10.7 ng/mL

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DISCLOSURE STATEMENT

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

AFF designed the analysis and drafted the manuscript. SLR-S performed the analysis. All authors critically reviewed the manuscript, contributed to interpretation of data, and approved the final version for submission.

(95% CI: -18.5, -2.9) vs. -4.0 ng/mL (95% CI: -8.4, 0.4) for boys]. Early pregnancy smoking was not associated with cord blood hormones other than IGF-1. Infants of former smokers had a cord blood hormone profile similar to infants of never smokers.

Conclusions—As compared to mothers who never smoked, early pregnancy smokers had infants with lower cord blood IGF-1 which could prime adverse metabolic outcomes. This provides further reason to support smoking cessation programs in women of reproductive age.

Keywords

smoking; pregnancy; cord blood; hormones; insulin-like growth factor (IGF); leptin

Introduction

Cigarette smoking continues to be a major public health threat. It is the leading preventable cause of death in the United States and was estimated to have caused over 7 million deaths globally in 2015 [1]. Maternal smoking during pregnancy has been consistently associated with offspring morbidity, including lower birth weight by 175–200 g in infants of mid to late pregnancy smokers [2] and adiposity and insulin resistance in children of early to late pregnancy smokers [3, 4].

Prenatal exposure to nicotine, a major component of cigarette smoke, reduces placental blood flow which leads to fetal hypoxia and low fetal growth [5]. Similarly, rodents prenatally exposed to nicotine develop greater adiposity and abnormal glucose homeostasis in adulthood [6]. However, the hormonal pathways through which nicotine or other components of cigarette smoke may lead to these metabolic health outcomes are unclear.

Examining the extent to which prenatal smoking is associated with the umbilical cord blood hormonal milieu may provide additional insight into potential pathways of action. Lower concentrations of cord blood growth factors, particularly insulin-like growth factor-1 (IGF-1), are associated with smaller size at birth [7]. Lower cord blood adipokines, leptin and adiponectin, have been associated with early life weight gain [8]. Also, higher cord blood insulin has been associated with higher plasma insulin in early childhood which may have implications for development of insulin resistance in later life[9].

Although there have been several prior studies of prenatal smoking and metabolic cord blood hormones [10–22], these typically assessed only IGF-1 or adipokines, often did not account for potentially important confounding variables like socioeconomic status, and frequently included small sample sizes ($n \leq 150$). Also, only one prior study included women who were former smokers [17], and none have evaluated sex-specific associations, despite growing evidence that prenatal toxic exposures including smoking may have sex-specific effects [23].

In the present analysis, we used data from a large, prospective cohort to examine the extent to which maternal smoking habits were associated with IGF-1, insulin-like growth factor 2 (IGF-2), insulin-like growth factor binding protein 3 (IGFBP-3), leptin, adiponectin, insulin, and C-peptide in cord blood. Based on known associations of prenatal smoking with low

fetal growth and higher offspring adiposity and insulin resistance, we hypothesized that prenatal smoking would be associated with lower IGF-1, lower adipokines, and higher insulin and C-peptide in cord blood, and that associations would differ by infant sex.

Methods

Study population and design

From 1999 to 2002, we recruited pregnant women to the prospective cohort Project Viva during their first prenatal visit (median 9.9 weeks of gestation) at Atrius Harvard Vanguard Medical Associates, a multi-specialty group practice in eastern Massachusetts [24]. A total of 2,128 women with a live singleton birth were included in the cohort. For the present analysis, we excluded 16 women with pre-existing type 1 or type 2 diabetes mellitus, 1,124 women who did not have cord blood hormones measured, and 10 women without information on prenatal smoking status. We obtained cord blood only from about one-half of the total cohort because obstetric providers, whose primary focus was on clinical care, not research, were responsible for the blood collection, and because we collected cord blood at only one of the two main delivery hospitals. Women included (n=978) in the present analysis versus excluded (n=1,150) were more likely to be white (70% vs. 63%), and their children were more likely to have a longer gestational length (mean 39.6 v. 39.2 weeks) and greater birth weight for gestational age z-score (mean 0.24 v. 0.11 units). Women included versus excluded had similar rates of smoking during early pregnancy (13% vs. 12%) (Supplementary Table 1). All women provided written informed consent, and Institutional Review Boards of participating institutions approved the study.

Measurement of exposure

We collected data on maternal smoking habits during pregnancy based on self-report and categorized participants into three groups: (1) never smokers, (2) former smokers, and (3) early pregnancy smokers. At the time of study enrollment (median 9.9 weeks of gestation) we asked participants whether they had ever smoked, and women who smoked < 100 cigarettes in their lifetime were categorized as “never smokers.” Next, we asked women who smoked >100 cigarettes in their lifetime whether they had smoked in the 3 months before learning they were pregnant. We categorized women who stopped smoking 3 months or more before learning they were pregnant as “former smokers.” We categorized women who smoked during the 3 months before learning they were pregnant or reported smoking on the first or second trimester questionnaire, during their delivery interview, or in their prenatal medical record as “early pregnancy smokers.” Of 125 early pregnancy smokers, 72 smoked in the 3 months before learning they were pregnant and reported having quit by the time of the first trimester questionnaire (9.9 weeks gestation). We categorized these women as early pregnancy smokers because they may have smoked after the time of conception. We refer to all 125 women as “early pregnancy smokers” because the majority reported having quit by the end of the first trimester. This categorization is consistent with prior analyses in this cohort (e.g., [3]).

We also collected information about ongoing passive smoke exposure (hours per week at home, work, or in restaurants) on the first trimester questionnaire. We considered women to

be exposed if they reported greater than or equal to two hours of passive smoke exposure per week.

Measurement of outcome

The delivering obstetrician or midwife collected cord blood samples from the umbilical vein immediately after delivery. We refrigerated whole blood for less than 24 hours, spun and aliquoted samples, and stored in liquid nitrogen. In the stored plasma, we measured concentrations of IGF-1 (ng/mL), IGF-2 (ng/mL), and IGFBP-3 (ng/mL) by ELISA assay (IGF-1 and IGFBP-3: R&D Systems, Minneapolis, MN; IGF-2: Alpco Diagnostics, Salem, NH). We measured leptin (ng/mL) and adiponectin (μ g/mL) by radioimmunoassay (Millipore Co, Darmstadt, Germany). We measured insulin (μ U/mL) and C-peptide (ng/mL) by competitive electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN). Lower limit of detection (intraassay coefficient of variation; interassay coefficient of variation) was 0.06 ng/mL (3.5–4.3%; 7.5–8.3%) for IGF-1, 0.02 ng/mL (3.1–6.6%; 7.0–7.2%) for IGF-2, 0.14 ng/mL (2.3–5%; 5.4–8%) for IGFBP-3, 0.5 ng/mL (3.4–8.3%; 3.6–6.2%) for leptin, 1.0 μ g/mL (1.8–6.2%; 6.9–9.3%) for adiponectin, 0.2 μ U/mL (1.9–2.0%; 2.5–2.8%) for insulin, and 0.01 ng/mL (1.3–4.6%; 1.8–5%) for C-peptide.

Measurement of covariates

We collected data on maternal age, education, race/ethnicity, parity, alcohol intake, and household income using questionnaires at study enrollment. We calculated pre-pregnancy body mass index (BMI) from self-reported weight and height. Participants underwent a two-tiered glucose screening test during pregnancy, as previously described [25]. We abstracted infant birth date, weight, and sex from the hospital medical record. We calculated gestational age at birth using the date of the last menstrual period and updated with mid-pregnancy ultrasound if the two estimates differed by > 10 days. We used US reference data [26] to determine sex-specific infant birth weight for-gestational-age z scores.

Statistical analyses

In primary analyses, we first fit unadjusted models followed by multivariable linear regression models to examine associations of maternal smoking habits (early pregnancy, former, and never) with cord blood hormone concentrations (IGF-1, IGF-2, IGFBP-3, leptin, adiponectin, insulin, and C-peptide). We examined each hormone outcome in separate models, in which we included the same set of covariates potentially associated with maternal smoking [27] and/or cord blood hormone concentrations [28, 29]. These covariates included maternal pre-pregnancy BMI (continuous), race/ethnicity (black, Hispanic, white, or other), parity (nulliparous or multiparous), education (with or without college degree), and infant sex. Additional adjustment for maternal age, prenatal alcohol intake, gestational glucose tolerance, and household income did not appreciably alter findings, so we did not include these measures in our final models. We did not adjust for gestational weight gain, birth size, or gestational age because these factors might be on the causal pathway and would be likely to introduce collider bias [30]. In models with IGF-1 or IGF-2 as the outcome, we ran additional models adjusted for IGFBP-3 concentration, to estimate the effect of IGF-1 or IGF-2 independent of the level of the binding protein. We also examined the association between passive smoke exposure in early pregnancy and cord blood hormones.

Because associations of prenatal smoking with birth size vary by child sex [23], we stratified models by child sex and examined interaction p-values. In addition, we excluded 6% of women (n=58) with gestational diabetes mellitus (GDM) in a sensitivity analysis. We have shown sex-specific associations between maternal GDM and cord blood hormones in this cohort [25], and this sensitivity analysis allowed us to investigate whether the small number of participants who both smoked and had GDM were driving our observed associations, particularly in stratified analyses where sample sizes were small.

To roughly assess the extent to which changes in IGF-1 may mediate the association between prenatal smoking and fetal growth, we also examined the covariate-adjusted (maternal pre-pregnancy BMI, race/ethnicity, parity, education, and infant sex) association between early pregnancy smoking and birth weight-for-gestational age z-score with and without additional adjustment for IGF-1.

Because the distributions of insulin and C-peptide were right-skewed, we log-transformed those outcomes and reran sex-specific and full cohort analyses. The directions and significance of estimates were very similar, and therefore we present the untransformed values for ease of interpretation and for comparability across outcomes. We performed complete case analyses using data from the 99% of participants who did not have any missing covariate information. We performed all data analysis with SAS version 9.3 (SAS Institute, Cary NC).

RESULTS

Population characteristics

Of the 978 women included in analyses, 13% were early pregnancy smokers, 20% former smokers, and 68% never smokers. The mean [standard deviation (SD)] maternal age at the time of study enrollment was 31.8 (5.2) years, and mean (SD) pre-pregnancy BMI was 24.9 (5.5) kg/m². Forty-six percent of women were nulliparous, 70% were white, and 63% were college graduates; 48% of offspring were female (Table 1).

Details on cord blood hormone concentrations and correlations are presented in Table 1 and Supplementary Table 2. Correlations between cord blood hormone concentrations were variable, with the strongest correlations between IGF-1 and IGFBP-3 ($r=0.65$), IGF-2 and IGFBP-3 ($r=0.65$), and insulin and C-peptide ($r=0.80$). Leptin and adiponectin were moderately correlated ($r=0.39$), as were IGF-1 and insulin ($r=0.38$), and IGF-1 and IGF-2 ($r=0.27$) (Supplementary Table 2).

As compared to women who never smoked, women who smoked during early pregnancy were more likely to be younger, heavier, black or Hispanic, and less educated. Concentrations of IGF-1 were lower in women who smoked during early pregnancy (Table 1).

Maternal smoking habits and cord blood hormone concentrations

Prenatal smoking was associated with lower cord blood IGF-1 concentration with similar effect estimates in unadjusted (data not shown) and covariate-adjusted analyses. As

compared to infants of mothers who never smoked, infants of mothers who smoked during early pregnancy had a cord blood IGF-1 concentration that was 8.0 ng/mL lower (95% CI: -12.6, -3.3) in adjusted analyses. When we further adjusted for concentrations of the binding protein, IGFBP-3, the association of prenatal smoking with IGF-1 was somewhat attenuated [-5.2 ng/mL (95% CI: -8.6, -1.7) vs. infants of mothers who never smoked]. Smoking during early pregnancy was associated with lower IGFBP-3, but confidence intervals were wide and included the null [-56.0 ng/mL (95% CI: -119.0, 6.6)]. Smoking was not associated with concentrations of any other cord blood hormones (Table 2). The cord blood hormone profile of infants of former smokers was similar to that of infants of never smokers (Table 2). Fifteen percent of early pregnancy smokers reported concomitant passive smoke exposure in early pregnancy compared with 12% of former smokers and 10% of never smokers. Passive smoke exposure in early pregnancy was not associated with any of the cord blood hormones (data not shown).

Analyses excluding mothers with GDM

When we excluded mothers with GDM and re-examined the association between prenatal smoking and cord blood hormone profile (n=920), adjusted effect estimates were similar to those for the full cohort (Table 3).

Effect modification and stratification by infant sex

There was a tendency towards a stronger inverse association between prenatal smoking and cord blood IGF-1 in girls as compared to boys, although the interaction term was not significant. In adjusted models, IGF-1 was 10.7 ng/mL lower (95% CI: -18.5, -2.9) in girls of mothers who smoked during early pregnancy versus never smoked, whereas in boys, it was 6.3 ng/mL lower (95% CI: -12.1, -0.5) (interaction $p=0.34$). After additional adjustment for IGFBP-3, the association between smoking during early pregnancy and cord blood IGF-1 was attenuated in girls [-7.3 ng/mL (95% CI: -13.0, -1.6)] and boys [-4.0 ng/mL (95% CI: -8.4, 0.4)] (interaction $p=0.49$) (Table 2).

We also observed sex-specific patterns in the associations of prenatal smoking with cord blood insulin and C-peptide concentrations. For example, in boys of mothers who smoked during early pregnancy versus never smoked, cord blood C-peptide was 0.2 ng/mL higher (95% CI: 0.1, 0.4), whereas the association was null in girls [-0.2 ng/mL (95% CI: -0.4, 0.1)] (interaction $p < 0.01$) (Table 2). However, one of the boys of the 7 mothers with GDM who smoked had outlier concentrations of cord blood insulin (93.9 μ U/mL) and C-peptide (7.6 ng/mL) which may have driven the sex-specific associations with insulin and C-peptide. Thus, we reexamined these associations among the 920 pairs without GDM, and found that the association of maternal smoking with insulin and C-peptide among boys was no longer positive [e.g., C-peptide: 0.1 ng/mL (95% CI: -0.0, 0.3)]. Also, although the interaction between prenatal smoking and child sex remained significant for C-peptide ($p=0.03$), there was no longer a significant interaction for insulin ($p=0.16$) in the restricted cohort (Table 3).

Maternal smoking habits and fetal growth

Infants of mothers who smoked during early pregnancy had a birth weight-for-gestational age z-score that was 0.2 units lower (95% CI: -0.4, -0.0) in covariate-adjusted analyses.

When we additionally included IGF-1 in the model, the association between early pregnancy smoking and birth weight-for-gestational age z-score was attenuated [−0.1 units (95% CI: −0.3, 0.1)].

DISCUSSION

In our analysis of a large, prospective cohort, infants of mothers who smoked during early pregnancy had a lower cord blood IGF-1, with the strongest association in girls. We also observed higher cord blood insulin and C-peptide in boys of prenatal smokers, but the association was attenuated after we excluded infants of mothers with GDM, suggesting that it may have been driven by a small number of smokers who developed GDM, including one with particularly high cord blood insulin and C-peptide concentrations. Contrary to our *a priori* hypotheses, we found no association between prenatal smoking and other umbilical cord blood hormones. Infants of mothers who smoked before pregnancy but stopped at least 3 months prior to learning they were pregnant had a cord blood hormone profile similar to infants of mothers who never smoked.

Our findings suggest that smoking during early pregnancy may inhibit the growth-promoting IGF-1 axis *in utero*. A growing body of evidence suggests that fetal hypoxia lowers IGF-1 [31, 32]. Components of cigarette smoke, including nicotine, via vasoconstriction and reduced placental blood flow, and carbon monoxide, via formation of carboxyhemoglobin, induce fetal hypoxia [5], which may, in turn, lead to low IGF-1.

IGF-1 is a primary driver of fetal growth *in utero* [33], and lower cord blood IGF-1 is associated with lower birth size in the general population [7]. We found that the association between early pregnancy smoking and low fetal growth, the strength of which depends on the analytic sample in our cohort [3], was attenuated by cord blood IGF-1. This raises the possibility that lower IGF-1 may be a pathway through which prenatal smoking reduces fetal growth.

Low IGF-1 *in utero* as a result of prenatal smoking could also prime offspring for adverse cardiometabolic health. In humans, mutations in IGF-1 lead to decreased insulin secretion and high risk of type 2 diabetes, in addition to low fetal growth [34]. Consistent with this, in *in vitro* models, low IGF-1 impairs pancreatic beta cell formation, presumably due to structural similarity and cross-talk between IGF-1 and insulin [35]. In one study [14], prenatal smoking was associated with lower IGF-1 and higher proinsulin-to-insulin ratio in the cord blood, raising the possibility that lower intrauterine IGF-1 from smoking may impair beta cell function. To better elucidate these relationships, future studies should consider the full pathway from prenatal smoking, to cord blood hormone profile, to later cardiometabolic health.

Of the insulin-like growth factors (IGFs) that we measured, IGF-1 had the strongest association with prenatal smoking. IGFBP-3 is also important for growth and metabolic outcomes, as it is a major IGF-1 binding protein and extends the half-life of IGF-1 in circulation [33]. In our cohort, the association between prenatal smoking and IGF-1 was attenuated when we accounted for IGFBP-3, and prenatal smoking was associated with

lower IGFBP-3, albeit imprecisely. We found no association between prenatal smoking and IGF-2, which activates the IGF-1 receptor [33] and also regulates fetal and placental growth but is not as closely linked to hypoxia [32] or birth size [7] as IGF-1. Thus, smoking during early pregnancy may have a weak inverse association with IGFBP-3, but we found it to be most strongly associated with IGF-1.

Our results are in line with several other, mostly smaller studies that have demonstrated associations between smoking throughout pregnancy and the IGF axis. Most have shown an association between prenatal smoking and lower IGF-1 [11, 13, 14, 16, 22], consistent with our findings. One study [10] found an association between prenatal smoking and higher IGF-1, although this was in a very small sample (n=62) without control for potential confounding variables. Also somewhat consistent with our results, in prior studies, prenatal smoking was not associated with IGF-2 [14, 22] and was inversely associated with IGFBP-3 [16, 22].

Although some prior studies have considered sex-specific associations between prenatal smoking and birth size, none have considered sex differences in the association between prenatal smoking and cord blood hormones. Our results showed a tendency toward a stronger association between prenatal smoking and low IGF-1 in girls. This is consistent with one study that showed a more pronounced association between prenatal smoking and low birth size in girls versus boys [23], but inconsistent with other studies showing either no sex differences in the association between prenatal smoking and low birth size [e.g. [36]] or a more pronounced association in boys [e.g. [37]]. Our sex-specific results require replication in other cohorts.

In the present study, we did not find an association between prenatal smoking and any of the other cord blood hormones. The association we observed between prenatal smoking and higher insulin and C-peptide in boys became null after we excluded women with GDM. The initial finding could have been by chance. Alternatively, GDM is strongly associated with cord blood insulin and C-peptide in boys in our cohort [25], and one boy born to a smoking mother with GDM had particularly high insulin and C-peptide concentrations. Thus, the initial finding may have been driven by a small number of smokers who developed GDM. Our results suggest the importance of considering women with GDM separately in future studies of prenatal smoking and cord blood hormone profile.

Our finding of no association between early pregnancy smoking and cord blood leptin, adiponectin, insulin, or C-peptide is generally consistent with studies of women who smoked throughout pregnancy. Most prior studies found no difference in cord blood leptin [12, 14, 15, 21] or adiponectin [14] in infants of mothers who smoked versus did not smoke during pregnancy, although a few found lower cord blood leptin [18] and adiponectin [12, 20] in infants of smokers. While prior studies did not evaluate the association between prenatal smoking and C-peptide, thought to be a more stable marker of pancreatic function than insulin due to its longer half-life [38], of two studies that evaluated cord blood insulin, one [19] found lower insulin in smokers, and the other [14] found no difference in smokers versus non-smokers. Thus, while prenatal smoking has been associated with adverse

cardiometabolic health later in life [3, 4], this may not be a result of alterations in leptin, adiponectin, insulin, or C-peptide *in utero*.

Our data are important because low IGF-1 as a result of prenatal smoking has the potential to independently drive changes in both birth size and later cardiometabolic health. Understanding potential mechanisms of long-term programming by prenatal smoke exposure adds biological plausibility to epidemiologic observations and could lead to hormonally-driven therapeutic interventions to ameliorate health effects of maternal prenatal smoking.

Generalizability is a limitation of Project Viva because participants are mostly white and of moderately high socioeconomic status. Also, we assessed smoking by self-report without biochemical validation. This could result in under-reporting of smoking due to social desirability which could bias results toward the null. In addition, we considered women who smoked in the 3 months before learning they were pregnant but reported having quit smoking prior to learning they were pregnant to be early pregnancy smokers. Some of these women may have quit smoking before conception, leading to over-reporting of smoking which could have also biased results toward the null. In the present study, we analyzed IGF-1 adjusted for IGFBP-3 as a proxy for bioactive IGF-1. Future studies would benefit from direct measurement of bioactive IGF-1 and also from measurement of cord blood insulin-like growth factor binding protein 1, which is directly induced by hypoxia [39], binds and sequesters fetal IGF-1 [40], and has a strong, inverse correlation with birth size [31].

Strengths of our study include use of a large prospective cohort with inclusion of multiple potential confounding variables and consideration of several cord blood hormones. Also, in contrast to prior studies of prenatal smoking and cord blood hormones, we additionally considered mothers who were former smokers and examined sex-specific associations.

In conclusion, we observed an association between smoking during early pregnancy and lower cord blood IGF-1. Early pregnancy smoking was not associated with other cord blood hormones. Our results suggest that the intrauterine metabolic environment may be programmed by early pregnancy smoking and provide further reason to support smoking cessation programs in women of reproductive age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMI (body mass index)

GDM	(gestational diabetes mellitus)
IGF-1	(insulin-like growth factor-1)
IGF-2	(insulin-like growth factor 2)
IGFBP-3	(insulin-like growth factor binding protein 3)
SD	(standard deviation)

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Table 1

Participant characteristics in the total analytic dataset (n=978) and by maternal smoking habits

	Total n=978	Maternal smoking habits		
		Early pregnancy n=125	Former n=191	Never n=662
Mean (SD) or %				
Maternal characteristics				
Age (years)	31.8 (5.2)	29.1 (6.2)	33.1 (4.6)	31.9 (5.0)
Pre-pregnancy BMI (kg/m ²)	24.9 (5.5)	26.4 (6.2)	24.7 (4.7)	24.7 (5.5)
Race/ethnicity (%) ^a				
Black	15	14	5	17
Hispanic	7	15	4	6
White	70	65	87	66
Other	9	6	3	11
College graduate (%)	63	28	69	68
Nulliparous (%)	46	45	43	47
GDM (%)	6	6	7	6
Infant characteristics				
Female (%)	48	41	49	48
Cord blood hormones				
IGF-1 (ng/mL)	56.6 (24.3)	50.2 (21.2)	56.8 (25.9)	57.8 (24.2)
IGF-2 (ng/mL)	408.9 (93.3)	405.9 (84.6)	408.6 (101)	409.6 (92.6)
IGFBP-3 (ng/mL)	1084 (319)	1032 (279)	1101 (362)	1089 (312)
Leptin (ng/mL)	9.0 (6.6)	9.0 (6.7)	8.0 (6.3)	9.3 (6.7)
Adiponectin (µg/mL)	28.7 (6.8)	28.9 (7.3)	28.0 (7.1)	28.9 (6.6)
Insulin (µU/mL)	6.5 (7.1)	6.9 (9.5)	6.9 (9.6)	6.3 (5.6)
C-peptide (ng/mL)	1.0 (0.6)	1.1 (0.8)	1.1 (0.8)	1.0 (0.5)

Abbreviations: BMI – body mass index; GDM – gestational diabetes mellitus; IGF – insulin-like growth factor; IGFBP – insulin-like growth factor binding protein

^aPercentages rounded to the nearest whole number.

Table 2

Covariate-adjusted^a associations of maternal smoking habits with cord blood hormone concentrations in the total cohort and stratified by infant sex. Estimates with 95% confidence intervals that do not cross the null are bolded.

Cord blood hormone	Maternal smoking habits	Total cohort ^b β (95% CI)	Girls ^c β (95% CI)	Boys ^d β (95% CI)	Sex*smoking interaction p-value
IGF-1 (ng/mL)	Early pregnancy	-8.0 (-12.6, -3.3)	-10.7 (-18.5, -2.9)	-6.3 (-12.1, -0.5)	0.34
	Former	-2.1 (-5.9, 1.7)	-0.3 (-6.0, 5.3)	-4.0 (-9.2, 1.1)	
	Never	Reference	Reference	Reference	
IGF-1 adjusted for IGFBP-3 (ng/mL)	Early pregnancy	-5.2 (-8.6, -1.7)	-7.3 (-13.0, -1.6)	-4.0 (-8.4, 0.4)	0.49
	Former	-1.8 (-4.6, 1.0)	-1.0 (-5.1, 3.1)	-2.7 (-6.5, 1.2)	
	Never	Reference	Reference	Reference	
IGF-2 (ng/mL)	Early pregnancy	-1.2 (-20.1, 17.6)	5.3 (-24.9, 35.4)	-3.3 (-27.7, 21.1)	0.94
	Former	-3.8 (-19.2, 11.5)	-5.4 (-27.2, 16.5)	-3.3 (-24.9, 18.3)	
	Never	Reference	Reference	Reference	
IGF-2 adjusted for IGFBP-3 (ng/mL)	Early pregnancy	10.4 (-3.2, 24.1)	18.5 (-3.3, 40.3)	7.0 (-10.5, 24.6)	0.39
	Former	-2.5 (-13.6, 8.6)	-8.0 (-23.8, 7.8)	2.8 (-12.8, 18.3)	
	Never	Reference	Reference	Reference	
IGFBP-3 (ng/mL)	Early pregnancy	-56.0 (-119.0, 6.6)	-66.1 (-170.0, 38.0)	-47.3 (-125.0, 30.8)	0.66
	Former	-6.5 (-57.4, 44.5)	13.3 (-62.4, 89.0)	-27.9 (-97.1, 41.3)	
	Never	Reference	Reference	Reference	
Leptin (ng/mL)	Early pregnancy	-0.1 (-1.5, 1.4)	-0.3 (-3.0, 2.3)	-0.2 (-1.8, 1.5)	0.53
	Former	-1.0 (-2.2, 0.1)	-1.5 (-3.3, 0.3)	-0.8 (-2.2, 0.6)	
	Never	Reference	Reference	Reference	
Adiponectin (μ g/mL)	Early pregnancy	-0.4 (-1.9, 1.1)	-0.1 (-2.3, 2.1)	-0.7 (-2.7, 1.4)	0.51
	Former	-1.1 (-2.3, 0.1)	-1.5 (-3.1, 0.0)	-0.7 (-2.5, 1.0)	
	Never	Reference	Reference	Reference	
Insulin (μ U/mL)	Early pregnancy	1.0 (-0.4, 2.5)	-0.8 (-3.2, 1.5)	2.3 (0.5, 4.1)	0.03
	Former	0.9 (-0.2, 2.1)	1.3 (-0.4, 3.0)	0.6 (-1.0, 2.2)	
	Never	Reference	Reference	Reference	

Cord blood hormone	Maternal smoking habits	Total cohort ^b β (95% CI)	Girls ^c β (95% CI)	Boys ^d β (95% CI)	Sex*smoking interaction p-value
	Never	Reference	Reference	Reference	
	Early pregnancy	0.1 (−0.1, 0.2)	−0.2 (−0.4, 0.1)	0.2 (0.1, 0.4)	
C-peptide (ng/mL)	Former	0.1 (−0.0, 0.2)	0.1 (−0.1, 0.2)	0.1 (−0.1, 0.2)	<0.01
	Never	Reference	Reference	Reference	

Abbreviations: IGF – insulin-like growth factor; IGFBP – insulin-like growth factor binding protein

^aAdjusted for maternal pre-pregnancy BMI, education, race/ethnicity, parity. Adjusted for infant sex in models of total cohort.

^bCovariate-adjusted N for total cohort varies based on outcome. N=950 for IGF-1, IGF-2, and IGFBP-3. N=822 for leptin. N=856 for adiponectin. N=949 for insulin and C-peptide.

^cCovariate-adjusted N for analyses of girls only varies based on outcome. N=458 for IGF-1, IGF-2, and IGFBP-3. N=393 for leptin. N=407 for adiponectin. N=458 for insulin and C-peptide.

^dCovariate-adjusted N for analyses of boys only varies based on outcome. N=492 for IGF-1, IGF-2, and IGFBP-3. N=429 for leptin. N=449 for adiponectin. N=491 for insulin and C-peptide.

Table 3

Covariate-adjusted^a associations of maternal smoking habits with cord blood hormone concentrations in the total cohort and stratified by infant sex, restricted to women without gestational diabetes mellitus. Estimates with 95% confidence intervals that do not cross the null are bolded.

Cord blood hormone	Smoking habits	Total cohort ^b β (95% CI)	Girls ^c β (95% CI)	Boys ^d β (95% CI)	Sex*smoking interaction p-value
IGF-1 (ng/mL)	Early pregnancy	-7.1 (-11.9, -2.3)	-9.3 (-17.1, -1.5)	-5.6 (-11.7, 0.4)	0.48
	Former	-3.0 (-6.9, 0.9)	-2.0 (-7.7, 3.7)	-4.1 (-9.5, 1.2)	
	Never	Reference	Reference	Reference	
IGF-1 adjusted for IGFBP-3 (ng/mL)	Early pregnancy	-4.5 (-8.1, -0.9)	-6.1 (-11.9, -0.3)	-3.5 (-8.1, 1.1)	0.62
	Former	-2.1 (-5.0, 0.8)	-1.7 (-6.0, 2.5)	-2.5 (-6.6, 1.5)	
	Never	Reference	Reference	Reference	
IGF-2 (ng/mL)	Early pregnancy	-3.5 (-23.0, 16.0)	2.5 (-28.3, 33.2)	-6.4 (-31.9, 19.2)	0.85
	Former	-8.9 (-24.7, 7.0)	-12.8 (-35.3, 9.7)	-6.5 (-28.9, 16.0)	
	Never	Reference	Reference	Reference	
IGF-2 adjusted for IGFBP-3 (ng/mL)	Early pregnancy	7.4 (-6.8, 21.7)	15.2 (-7.4, 37.8)	3.2 (-15.3, 21.6)	0.38
	Former	-5.2 (-16.8, 6.4)	-11.7 (-28.2, 4.8)	0.6 (-15.7, 16.8)	
	Never	Reference	Reference	Reference	
IGFBP-3 (ng/mL)	Early pregnancy	-52.4 (-116.0, 11.6)	-64.3 (-169.0, 40.7)	-43.5 (-124.0, 37.4)	0.76
	Former	-17.7 (-69.7, 34.3)	-5.4 (-82.3, 71.4)	-32.2 (-103.0, 38.8)	
	Never	Reference	Reference	Reference	
Leptin (ng/mL)	Early pregnancy	-0.4 (-1.9, 1.1)	-0.3 (-3.0, 2.3)	-0.9 (-2.5, 0.8)	0.48
	Former	-1.1 (-2.3, 0.0)	-1.6 (-3.4, 0.3)	-1.0 (-2.3, 0.4)	
	Never	Reference	Reference	Reference	
Adiponectin (μ g/mL)	Early pregnancy	-0.3 (-1.8, 1.3)	-0.3 (-2.5, 2.0)	-0.4 (-2.6, 1.8)	0.62
	Former	-0.9 (-2.2, 0.3)	-1.3 (-2.9, 0.3)	-0.6 (-2.4, 1.3)	
	Never	Reference	Reference	Reference	
Insulin (μ U/mL)	Early pregnancy	0.2 (-1.2, 1.6)	-0.7 (-3.2, 1.8)	0.9 (-0.6, 2.4)	0.16
	Former	0.8 (-0.3, 1.9)	1.4 (-0.4, 3.2)	0.3 (-1.1, 1.6)	

Cord blood hormone	Smoking habits	Total cohort ^b β (95% CI)	Girls ^c β (95% CI)	Boys ^d β (95% CI)	Sex*smoking interaction p-value
	Never	Reference	Reference	Reference	
	Early pregnancy	0.0 (−0.1, 0.1)	−0.2 (−0.4, 0.1)	0.1 (−0.0, 0.3)	
C-peptide (ng/mL)	Former	0.1 (−0.0, 0.2)	0.1 (−0.1, 0.3)	0.0 (−0.1, 0.2)	0.03
	Never	Reference	Reference	Reference	

Abbreviations: IGF – insulin-like growth factor; IGFBP – insulin-like growth factor-binding protein

^aAdjusted for maternal pre-pregnancy BMI, education, race/ethnicity, parity. Adjusted for infant sex in models of total cohort.

^bCovariate-adjusted N for total cohort varies based on outcome. N=893 for IGF-1, IGF-2, and IGFBP-3. N=776 for leptin. N=809 for adiponectin. N=892 for insulin and C-peptide.

^cCovariate-adjusted N for analyses of girls only varies based on outcome. N=434 for IGF-1, IGF-2, and IGFBP-3. N=376 for leptin. N= 389 for adiponectin. N=434 for insulin and C-peptide.

^dCovariate-adjusted N for analyses of boys only varies based on outcome. N= 459 for IGF-1, IGF-2, and IGFBP-3. N=400 for leptin. N=420 for adiponectin. N=458 for insulin and C-peptide.