ORIGINAL RESEARCH

In utero exposure to gestational diabetes mellitus and cardiovascular risk factors in youth: A longitudinal analysis in the EPOCH cohort

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Summary

Objective: To examine associations of maternal gestational diabetes mellitus (GDM) with offspring cardiovascular biomarkers from late childhood through adolescence.

Methods: We used mixed effects linear regression models to examine associations of maternal GDM (n = 92 cases of 597) with average offspring levels of serum lipids (total cholesterol, high-density lipoprotein [HDL], low-density lipoprotein [LDL], and triglycerides) and systolic blood pressure (SBP) across two research visits spanning approximately 10.6 and 16.9 years of age. In sex-stratified analysis, we evaluated the impact of adjustment for sociodemographic characteristics, pubertal status, physical activity and total energy intake, maternal body mass index (BMI), GDM treatment, and child's BMI.

Results: After adjusting for child's age, pubertal status, race/ethnicity, and maternal education and smoking, GDM exposure was associated with higher total (0.38 [95% CI, 0.16-0.61] mmol/L) and LDL cholesterol (0.34 [95% CI, 0.14-0.53] mmol/L) in girls. These estimates were robust to adjustment for lifestyle characteristics and maternal BMI but were attenuated after accounting for GDM treatment with no appreciable change following further adjustment for current BMI. In boys, maternal GDM corresponded with 4.50 (1.90-7.10) mmHg higher SBP. This association persisted after accounting for sociodemographic/lifestyle characteristics, maternal BMI, and GDM treatment but was attenuated after adjusting for current BMI.

Conclusions: Maternal GDM is related to offspring lipid profile and SBP in a sex-specific manner.

KEYWORDS

adolescence, blood pressure, gestational diabetes mellitus, longitudinal, low-density lipoprotein, total cholesterol

1 | INTRODUCTION

In his famous 1980 lecture, "Of Pregnancy and Progeny," Norbert Freinkel called gestational diabetes mellitus (GDM) a "teratogen" that leads to higher birthweight and congenital defects.[1] A number of

epidemiological studies have since noted detrimental effects of maternal GDM on offspring fat distribution starting at birth[2-6] and on glycaemic regulation (insulin resistance, impaired glucose tolerance, low insulin secretion, and reciprocally altered adipokines[6-9]) by age 6 years. Fewer studies have evaluated associations of in utero exposure to GDM with biomarkers of cardiovascular risk, which are important to consider given the interrelations among cardiovascular and metabolic biomarkers, and the persistent burden of cardiovascular disease worldwide[10]—a nonnegligible proportion of which may be traced back to the gestational metabolic milieu.[11] The majority of publications that have assessed the relation between maternal GDM and cardiovascular health in offspring focused on the adolescent life stage (although one study was conducted in children aged 3 years[12]) and identified associations with higher offspring blood pressure[6,12-16] and an adverse lipid profile.[16] Many of these studies were able to establish associations independent of offspring adiposity,[6,12,14] suggesting a unique influence of maternal GDM on in utero "programming" of cardiovascular risk.

In this prospective study of greater than 500 multi-ethnic youth, we test the hypothesis that in utero exposure to maternal GDM with biomarkers of cardiovascular risk in offspring from late childhood through adolescence in the Exploring Perinatal Outcomes in Children (EPOCH) cohort. The biomarkers of interest included serum lipid profile (total cholesterol, triglycerides, high-density lipoprotein [HDL], and low-density lipoprotein [LDL]) and blood pressure, which track from childhood/adolescence into adulthood and are independent predictors of future cardiovascular and metabolic disease risk.[17-21] This work extends current knowledge in this area of research in two key ways. First, we investigate associations of maternal GDM with biomarkers of cardiovascular risk longitudinally across a period that spans the adolescent transition via a repeated measures approach. Given that multiple measurements of health are more informative than single measurements, our analytical strategy is an improvement upon that of current literature, which has focused on evaluating associations of early life exposures with health outcomes at a single point in time later in life (including an earlier study in EPOCH that focused on data at the first research visit only[6]). Second, in all models, we consider sex-specific associations given documented differences in fetal (as well as later-life[2]) response to in utero exposures[22-24] and sex differences in metabolism during the adolescent life stage.[25]

2 | METHODS

2.1 | Study population

Study participants were from a historical prospective cohort of youth whose mothers were members of the Kaiser Permanente of Colorado (KPCO) Health plan. Details on eligibility and recruitment are published.[3] Of the original 604 participants enrolled, we excluded from the present analysis children of seven women with type 1 diabetes. The analytic sample comprised 597 youth at the first research visit and 410 at the second one with information on at least one cardiovascular

risk factor of interest. The Colorado Multiple Institutional Review Board approved this study.

2.2 | Exposure

All pregnant women at KPCO are routinely screened for GDM at 24 to 28 weeks using the standard two-step protocol.[26] A total of 92 women in the sample had a GDM diagnosis.

2.3 | Outcomes

Using blood collected after an 8-hour fast, total cholesterol, HDL, and triglycerides were assayed on the Olympus AU400 advanced chemistry analyser system. We calculated LDL as (total cholesterol) − (HDL cholesterol) − (triglycerides/5). Research assistants measured participants' blood pressure twice in the sitting position using an oscillometric monitor (Dinamap ProCare V100). We used the average of the two values in the analysis and focused on systolic blood pressure (SBP) rather than diastolic blood pressure because it is more accurately measured in children and is a stronger determinant of future health outcomes.[27]

2.4 | Covariates

2.4.1 | Perinatal and delivery characteristics

We calculated maternal pre-pregnancy body mass index (BMI; kg/m^2) using clinically recorded pre-pregnancy weight from KPCO medical records and measured height at the first research visit. The medical record also provided information on pregnancy complications such as preeclampsia and birthweight (g) as well as delivery characteristics like gestational age at delivery (weeks) for the index birth.

At T1, the women reported on smoking habits during pregnancy, education level at time of birth, and total gestational weight gain (lbs) via a self-administered questionnaire. In the analysis, we categorized maternal education as a three-level variable ("<high school," "high school diploma or equivalent," and ">high school") and smoking as a dichotomous variable (smoked while pregnant with index child, yes vs no). At this visit, the mothers also filled out a questionnaire regarding any treatment they received for GDM. GDM treatment was categorized as diet and/or exercise only, diet and/or exercise with insulin, and insulin only. Because of the small sample size for insulin only treatment (n = 5), we combined this category with diet and/or exercise when using this variable as a covariate in the analysis.

2.4.2 | Participant characteristics

We calculated participants' age as the difference between date of each research visit and delivery date. At both visits, we measured the

participants' weight on a digital scale to the nearest 0.1 kg and measured height (cm) via a calibrated stadiometer to the nearest 0.1 m. We calculated BMI and standardized it according to the age- and sex-specific World Health Organization (WHO) growth reference.[28] Participants self-reported on their race/ethnicity as non-Hispanic White, non-Hispanic Black, Hispanic, and non-Hispanic other. Participants reported their pubertal development based on diagrams of the Tanner stages. We based pubertal status on pubic hair development in boys and breast development in girls. For each characteristic, we classified a child as pre-pubertal (Tanner stage = 1) or pubertal (Tanner stage ≥ 2). We obtained information on the participants' physical activity levels using the 3-Day Physical Activity Recall (3DPAR) Questionnaire.[29] Using 3DPAR, we derived average energy expenditure (metabolic equivalents; METs) and mean number of moderate- to-vigorous 30-minute blocks of physical activity (METs) over the 3-day period. At both visits, participants completed the Block Kid's Food Frequency Questionnaire (FFQ).[30] We used these data in conjunction with the USDA Food Composition Database to estimate total energy intake (kcals/day).

2.5 | Data analysis

First, we assessed bivariate associations of in utero GDM exposure with maternal, perinatal, and offspring characteristics to identify potential confounders. This step, in conjunction with our a priori knowledge of determinants of cardiovascular health in youth, informed our multivariable analysis.

Next, we examined associations of GDM exposure with cardiovascular biomarkers from late childhood through adolescence using a mixed effects linear regression model wherein the outcome of interest was repeated measurements of each biomarker. The explanatory variables included longitudinal assessments of age at each research visit, a random effect for individual ID to account for the repeated outcomes, and an unstructured covariance matrix. We have previously used this approach as a way to characterize growth trajectories from longitudinal assessments of anthropometric data.[31-33] A key advantage of this approach (as opposed to conventional linear regression to examine associations of an exposure with the outcome of interest at separate time points, or with change in the outcome between specific time points) is that it does not require that all participants have the same number of measurements assessed at exactly the same time. In this study, the participants may have a maximum of two measurements for each biomarker, but if a participant has biomarker data at one visit only, their information still contributes to estimation of standard errors. This approach most efficiently leverages our longitudinal data, both from an analytical standpoint and from the perspective of interpreting the biological relevance of results given that multiple assessments of health are likely more meaningful than single evaluations. Using this approach, the estimate of interest (β for GDM exposure) is interpreted as the association of GDM with average levels of a biomarker over the 6 years of follow-up. A key assumption of using mixed effects models to generate population-level estimates is

homogeneity of associations in the face of variation in the age distribution of the population. Thus, we tested for birth cohort effects by including an interaction term between age at first research visit and GDM exposure; the interaction terms provided no evidence of birth cohort effects.

In multivariable analysis, we evaluated the impact of covariate adjustment on the β and 95% confidence interval (CI) for maternal GDM. Covariates for model 1 included longitudinal assessments of the child's age in years and pubertal status (ie, up to two data points for each variable per child, measured at the same time as the cardiovascular outcomes), as well as race/ethnicity, maternal education at the time of birth, and smoking habits during pregnancy. Model 2 further accounted for longitudinal assessments of mean energy expenditure and total energy intake. Model 3 included model 1 covariates plus maternal pre-pregnancy BMI, given that a woman's pre-gravid weight status likely influences offspring health through shared pathways with hyperglycaemia. Model 4 included model 1 covariates plus GDM treatment to account for variability in maternal hyperglycaemia following a diagnosis of GDM. In Model 5, we adjusted for model 4 covariates plus the child's current BMI to assess for an independent association of maternal GDM (while adjusting for treatment) with offspring cardiovascular biomarkers independent of offspring adiposity.

In all models, we tested for interactions with sex (which tests for a difference in association of GDM with cardiovascular outcomes for girls vs boys), pubertal status at the first visit (which tests for a difference in association of GDM with cardiovascular outcomes by pubertal status), and repeated measurements of age across the two research visit (which tests for differences in slopes of biomarker trajectories over time for GDM exposed vs unexposed). We considered P interaction <.05 as the threshold for a need to conduct stratified analysis. As expected, we found evidence of effect modification of association between GDM and cardiovascular risk factors by sex, so we conducted all models separately for boys and girls. We did not observe evidence of effect modification by the other abovementioned variables.

2.5.1 | Sensitivity analyses

In addition to the main analysis, we carried out some sensitivity analyses. First, because height is a strong determinant of BP in children and adolescents[34] and because clinical assessments of paediatric blood pressure are based on age-, sex-, and height-specific values,[35] we reran models for blood pressure with SBP z score as the outcome. Results for SBP and SBP z score were similar, so we focus on interpretation of SBP in mmHg for interpretability. Second, given the co-occurrence of GDM and preeclampsia,[36] we reran all models after excluding 27 mother-offspring pairs affected by preeclampsia. Exclusion of these individuals did not appreciably change our findings; thus, we included these participants in our analysis. Finally, we assessed the impact of adjustment for birthweight and gestational age at delivery, two mediating perinatal characteristics that are determinants of long-term cardiovascular health.[37-39] Adjusting for these variables did not

change the direction, magnitude, or precision of the results, so we did not include them in the final models.

We carried out all analyses using Statistical Analyses System software (version 9.3; SAS Institute Inc., Cary, NC).

3 | RESULTS

Mean ± SD age of participants at the first research visit was 10.4 ± 1.5 years (range: 6.0-13.9 years) and 16.7 ± 1.2 years (range: 12.6-19.6 years) at the second research visit, with an approximate follow-up time of 6 years. Fifty percent of the participants were female, and 48.1% (n = 287) were non-Hispanic White. Approximately 15% (n = 92) of participants were exposed to GDM in utero. Table 1 shows descriptive characteristics for mother-child dyads stratified by GDM exposure, and Table S1 shows the same characteristics stratified by sex. Associations of GDM exposure status with background characteristics were similar across sex, although we noted that a higher proportion of exposed girls were post-pubertal at the first visit (53.5% post-pubertal) then exposed boys (24.5% post-pubertal).

TABLE 1 Bivariate associations of in utero GDM exposure with background characteristics of 597 EPOCH participants

	GDM exposed	Unexposed	
	n = 92	n = 505	P value*
Maternal characteristics			
Maternal pre-pregnancy BMI (kg/m^2)	27.8 ± 6.3	25.4 ± 5.8	.001
Maternal education level			.50
<High school	5.4%	3.4%	
High school or equivalent	15.2%	18.4%	
>High school	79.4%	78.2%	
Mother smoked during pregnancy	14.1%	7.3%	.03
GDM treatment			
Diet and/or exercise	66.3%	–	
Diet and/or exercise with insulin, or insulin only	27.2%	–	
None	6.5%	–	
Preeclampsia			.33
Yes	6.5%	4.2%	
No	93.5%	95.8%	
Child's characteristics			
Female	46.70%	50.90%	.46
Race/ethnicity			.02
Non-Hispanic White	63.0%	45.4%	
Hispanic	28.3%	40.0%	
Non-Hispanic Black	4.4%	8.5%	
Non-Hispanic other	4.4%	6.1%	
Anthropometry and lifestyle at T1			
BMI z score	0.37 ± 1.33	0.23 ± 1.21	.32
Post-pubertal (Tanner stage ≥2)	57.10%	37.40%	.003
Mean energy expenditure (METs)	1.85 ± 0.32	1.90 ± 0.31	.13
Mean moderate-to-vigorous physical activity (METs)	4.15 ± 2.94	4.26 ± 2.80	.74
Total energy intake (kcal/per day)	1744 ± 458	1798 ± 562	.38
Anthropometry and lifestyle at T2			
BMI z score	0.54 ± 1.05	0.38 ± 1.13	.26
Post-pubertal (Tanner stage ≥2)	100.00%	100.00%	–
Mean energy expenditure (METs)	1.85 ± 0.27	1.91 ± 0.32	.12
Mean moderate-to-vigorous physical activity (METs)	4.81 ± 4.48	4.70 ± 3.77	.82
Total energy intake (kcal/d)	1683 ± 767	1653 ± 725	.76

Abbreviations: BMI, body mass index; EPOCH, Exploring Perinatal Outcomes in Children; GDM, gestational diabetes mellitus; MET, metabolic equivalent.
*From an independent t test for continuous variables; from a Pearson chi-squared test for categorical variables.

TABLE 2 Descriptive statistics for cardiovascular risk factors of 597 EPOCH participants at two research visits (ages 6-14 and 12-19 y)

	Visit 1	Visit 2
	n = 300	n = 206
Girls		
Age (years)	10.4 ± 1.4	16.7 ± 1.2
Cholesterol (mmol/L)	4.1 ± 0.7	3.8 ± 0.8
HDL (mmol/L)	1.3 ± 0.3	1.2 ± 0.3
LDL (mmol/L)	2.3 ± 0.6	2.1 ± 0.6
Triglycerides (mmol/L)	1.1 ± 0.5	1.0 ± 0.6
SBP (mmHg)	102.1 ± 9.6	112.0 ± 9.7
Boys	n = 297	n = 204
Age (years)	10.5 ± 1.5	16.6 ± 1.1
Cholesterol (mmol/L)	4.1 ± 0.8	3.6 ± 0.6
HDL (mmol/L)	1.3 ± 0.3	1.1 ± 0.2
LDL (mmol/L)	2.4 ± 0.7	2.0 ± 0.5
Triglycerides (mmol/L)	1.0 ± 0.4	1.0 ± 0.4
SBP (mmHg)	103.8 ± 10.3	119.4 ± 10.6

Abbreviations: EPOCH, Exploring Perinatal Outcomes in Children; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

Table 2 shows mean ± SD for the cardiovascular risk biomarkers of interest at each visit for all children and separately by sex, respectively.

Table 3 displays sex-specific associations of GDM exposure with cardiovascular risk factors over the entire age-range of study participants from both research visits. We interpret these results as the association of in utero GDM exposure on average levels of each cardiovascular risk factor across the two research visits (rather than average trajectories of change per year) as we did not find a significant interaction between GDM exposure and time, which suggests a consistent influence of GDM exposure on these health outcomes. After accounting for key confounders in model 1, girls who were exposed to GDM in utero had 0.38 (95% CI, 0.16-0.61) mmol/L and 0.34 (95% CI, 0.14-0.53) mmol/L higher total cholesterol and LDL, respectively, than their unexposed counterparts. This association was robust to adjustment for physical activity and total energy intake (model 2) and maternal pre-pregnancy BMI (model 3). However, adjustment for GDM treatment in model 4 attenuated both estimates to approximately 2/3 the magnitude, with the lower CI crossing the null (Table 3). Further adjustment for the offspring's current BMI z score in model 5 did not change the estimates.

In boys, GDM exposure corresponded with 4.50 (95% CI, 1.90-7.10) mmHg higher SBP from late childhood through adolescence

TABLE 3 Associations of in utero exposure to GDM with CV traits across in 597 EPOCH participants across T1 (age 6-14 y) and T2 (age 12-19 y)

	Model 1	Model 2	Model 3	Model 4	Model 5
	n = 300	n = 298	n = 207	n = 300	n = 300
Girls (n = 300)					
Cholesterol (mmol/L)	**0.38 (0.16-0.61)**	**0.39 (0.16-0.62)**	**0.34 (0.07-0.61)**	0.24 (−0.08 to 0.57)	0.25 (−0.08 to 0.57)
HDL (mmol/L)	0.01 (−0.06 to 0.08)	0.01 (−0.06 to 0.08)	−0.05 (−0.13 to 0.02)	−0.01 (−0.12 to 0.10)	−0.02 (−0.13 to 0.10)
LDL (mmol/L)	**0.34 (0.14-0.53)**	**0.36 (0.16-0.55)**	**0.33 (0.10-0.55)**	0.21 (−0.10 to 0.51)	0.21 (−0.10 to 0.52)
Triglycerides (mmol/L)	0.11 (−0.07 to 0.29)	0.08 (−0.10 to 0.26)	0.16 (−0.04 to 0.35)	0.10 (−0.12 to 0.31)	0.11 (−0.10 to 0.32)
SBP (mmHg)	0.93 (−1.16 to 3.03)	0.81 (−1.30 to 2.93)	−1.52 (−4.04 to 0.99)	−1.75 (−6.32 to 2.83)	−1.10 (−4.94 to 2.74)
	n = 296	n = 293	n = 230	n = 297	n = 297
Boys (n = 297)					
Cholesterol (mmol/L)	−0.06 (−0.28 to 0.16)	−0.06 (−0.29 to 0.16)	0.03 (−0.22 to 0.27)	0.15 (−0.40 to 0.70)	0.09 (−0.47 to 0.65)
HDL (mmol/L)	−0.06 (−0.14 to 0.02)	−0.06 (−0.14 to 0.02)	**−0.11 (−0.20 to −0.02)**	0.13 (−0.01 to 0.27)	0.14 (−0.01 to 0.28)
LDL (mmol/L)	0.01 (−0.18 to 0.19)	−0.02 (−0.21 to 0.17)	0.10 (−0.11 to 0.30)	0.08 (−0.34 to 0.51)	0.05 (−0.39 to 0.48)
Triglycerides (mmol/L)	0.04 (−0.07 to 0.15)	0.06 (−0.06 to 0.17)	0.09 (−0.03 to 0.21)	−0.12 (−0.54 to 0.30)	−0.18 (−0.59 to 0.22)
SBP (mmHg)	**4.50 (1.90-7.10)**	**4.56 (2.02-7.10)**	**3.11 (0.28-5.94)**	**6.27 (0.18-12.36)**	4.60 (−1.82 to 11.02)

Bold face font indicates statistical significance at α = 0.05.
Notes: Estimates are from a linear mixed effects model where the dependent variable is repeated measurements of the CVD traits across T1 and T2, and the predictors include GDM (yes vs no), covariates, and a random effect for individual intercept.
Model 1: Adjusted for child's age (years), pubertal status (pre vs post-pubertal), race/ethnicity (non-Hispanic White, Hispanic, non-Hispanic Black, and non-Hispanic other), maternal smoking habits during pregnancy (yes vs no) and education level at time of birth (<high school, high school, and >high school).
Model 2: Model 1 + physical activity levels (average energy expenditure; METs) and total energy intake (kcals).
Model 3: Model 1 + maternal pre-pregnancy BMI.
Model 4: Model 1 + GDM treatment (diet/exercise only vs none, diet/exercise with insulin vs none).
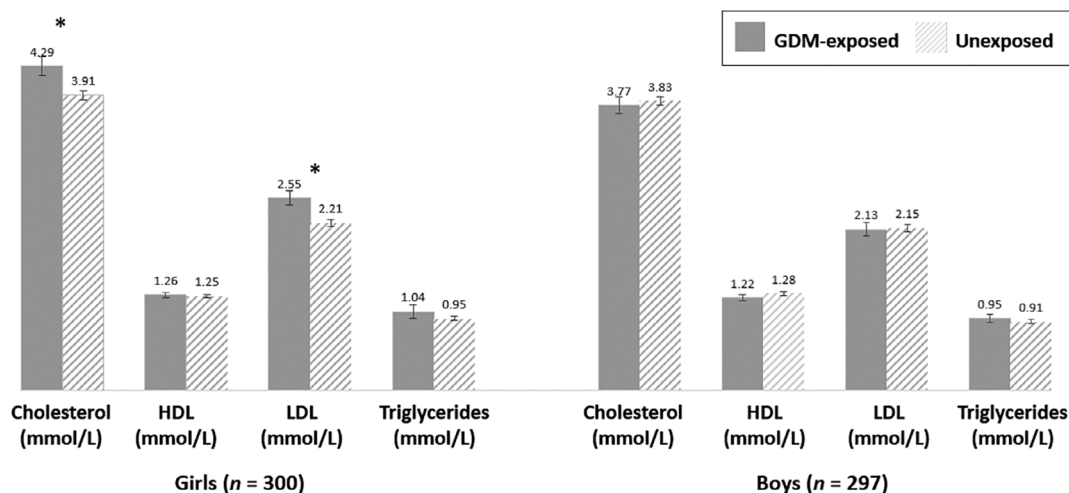Model 5: Model 4 + child's BMI z score.
Abbreviations: CV, cardiovascular; EPOCH, Exploring Perinatal Outcomes in Children; GDM, gestational diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.
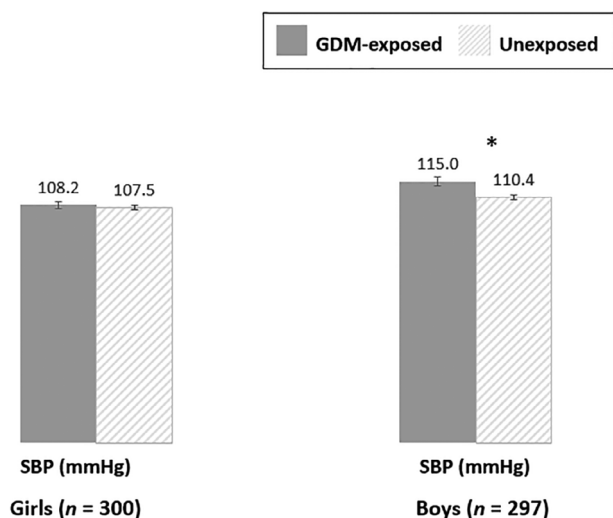
FIGURE 1 Mean ± *SE* for lipid profile components among GDM-exposed vs unexposed EPOCH participants. Estimates are adjusted for child's age (years), pubertal status (pre- vs post-pubertal), race/ethnicity (non-Hispanic White, Hispanic, non-Hispanic Black, and non-Hispanic other), maternal smoking habits during pregnancy (yes vs no), and education level at time of birth (<high school, high school, and >high school). *Statistically significant difference at α = .05. EPOCH, Exploring Perinatal Outcomes in Children; GDM, gestational diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein

FIGURE 2 Mean ± *SE* for SBP among GDM-exposed vs unexposed EPOCH participants. Estimates are adjusted for child's age (years), pubertal status (pre- vs post-pubertal), race/ethnicity (non-Hispanic White, Hispanic, non-Hispanic Black, and non-Hispanic other), maternal smoking habits during pregnancy (yes vs no) and education level at time of birth (<high school, high school, and >high school). *Statistically significant difference at α = .05. EPOCH, Exploring Perinatal Outcomes in Children; GDM, gestational diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure

(Table 3, model 1). Accounting for physical activity and total energy intake in model 2 did not change this estimate, although adjusting for maternal pre-pregnancy BMI in model 3 attenuated the estimate by approximately 30%. In model 4, adjustment for GDM treatment augmented the association to 6.27 (95% CI, 0.18-12.36) mmHg for exposed vs unexposed males. However, additional adjustment for the

offspring's current BMI *z* score attenuated the estimate such that the magnitude was similar to those detected in models 1 and 2, and the lower CI crossed the null (4.60 [95% CI, −1.82 to 11.02] mmHg). We also noted that GDM exposure was associated with 0.11 (95% CI, 0.02-0.20) mmol/L lower HDL in boys, although the 95% CI included the null for all models except Model 3.

Table S2 presents estimates of mean ± *SE* for the cardiovascular risk factors with respect to GDM exposure after confounder adjustment (model 1). We provide graphical depictions of these results in Figure 1 (lipid profile) and Figure 2 (blood pressure).

Table S3 shows the same set of models as in Table 3 for sex-, age-, and height-specific blood pressure *z* scores. The results from analyses of *z* scores are similar to those of blood pressure in mmHg, indicating no concern regarding residual confounding by age or height in our primary analysis.

4 | DISCUSSION

In this prospective study of 597 multi-ethnic youth in Colorado, we found sex-specific associations of in utero exposure to GDM with lipid profile and blood pressure in offspring from late childhood through adolescence. In girls, GDM was associated with higher total cholesterol and LDL. In boys, GDM corresponded with consistently higher SBP. These associations were consistent across the age-range studied and did not change over time.

4.1 | Girls

In utero exposure to GDM corresponded with an average of 0.38 mmol/L (6.8 mg/dL) higher total cholesterol, and 0.34 mmol/L

(6.1 mg/dL) higher LDL cholesterol levels. These findings are comparable with those of an analysis of 96 male and female Polish youth aged 7 to 16 years wherein Wilk et al[40] detected unadjusted differences of 19.0 and 14.2 mg/dL higher total and LDL cholesterol, respectively, among GDM-exposed (n = 50) vs unexposed participants (n = 46). In a recent study of greater than 1000 boys and girls in the Danish National Birth Cohort, Grunnet et al reported higher serum triglycerides (5% [95% CI, 1-10%]) and lower HDL (−0.07 [95% CI, −0.11 to −0.02] mmol/L), as well as marginally higher LDL (0.06 [95% CI, −0.01 to 0.14] mmol/L) at 12 years of age among GDM-exposed youth.[16] However, these associations became null after adjusting for maternal and child BMI.

In the present analysis, the influence of maternal GDM on the above offspring cardiovascular risk factors remained apparent after accounting for offspring characteristics known to impact lipid profile, including pubertal status, physical activity, and energy intake, as well as maternal pre-pregnancy BMI, a proxy for maternal adiposity that is hypothesized to account for a portion of variability in the relationship between maternal hyperglycaemia and offspring health. However, accounting for GDM treatment attenuated the estimates for cholesterol and LDL towards the null, thereby providing support for a specific effect of maternal hyperglycaemia on these cardiovascular traits. In other words, if removing variability in maternal hyperglycaemia during gestation by adjusting for GDM treatment in the analysis resulted in smaller and nonsignificant effect estimates for maternal GDM, then maternal hyperglycaemia is likely a determinant of the offspring health outcomes.

Our finding that exposure to maternal GDM was associated with an adverse lipid profile that is likely related to the occurrence of perturbed maternal lipid metabolism in the context of gestational hyperglycaemia,[41] which in turn, may affect offspring lipid metabolism.[42] Mechanistic pathways are difficult to disentangle in humans, but animal models indicate an impact of maternal hyperglycaemia on hepatic lipid content and metabolism through oxidative stress and inflammatory pathways.[43] Although we adjusted for pubertal status in multivariable analyses, the female-specific nature of the relationship between exposure to maternal GDM and offspring lipid profile may be related to unmeasured confounding by tempo of sexual maturation (which exists on a spectrum that is likely not wholly captured by discrete Tanner staging) given recent findings that GDM-exposed girls undergo puberty earlier than their male counterparts. Earlier puberty may in turn translate to more rapid increases in total cholesterol and LDL.[44]

4.2 | Boys

Boys who were exposed to GDM in utero had approximately 5 mmHg higher SBP than their unexposed counterparts. This relationship remained apparent after accounting for sociodemographic and lifestyle characteristics during follow-up but was modestly attenuated after accounting for maternal pre-pregnancy BMI and current BMI. Interestingly, adjustment for GDM treatment did not attenuate the

estimate, but rather, slightly increased the magnitude of effect of maternal GDM from approximately 5 mmHg (models 1 and 2) to approximately 6 mmHg higher SBP for GDM-exposed vs unexposed boys (model 4). This indicates the existence of mechanisms other than hyperglycaemia linking in utero GDM exposure to offspring blood pressure. Indeed, when we further adjusted for the offspring's current BMI z score (in addition to GDM treatment), the estimate for maternal GDM in relation to SBP was approximately 5 mmHg and attenuated to nonsignificance, suggesting that the relationship between maternal hyperglycaemia and offspring SBP is mediated by the offspring adiposity.

In a previous study conducted in this cohort that examined consequences of maternal GDM on adiposity and cardiometabolic biomarkers at the first visit, West et al[6] detected a marginally significant 2 mmHg higher SBP between GDM-exposed and unexposed participants at 6 to 13 years. At this time, the investigators reported no differences in the relationship between GDM and health outcomes by sex and adjustment for maternal and child BMI attenuated the associations to the null. Similarly, in an investigation of 1238 children in the Project Viva cohort, Wright et al[12] found that GDM exposure corresponded with 3.2 mmHg higher SBP at 3 years of age after adjusting for key covariates including for maternal pre-pregnancy BMI. As with the study of West et al, further adjustment for the child's current BMI markedly attenuated the estimate.

In addition to investigations that focused exclusively on GDM, smaller studies have explored offspring cardiovascular consequences of exposure to maternal diabetes during pregnancy based on a combination of pre-existing diabetes (type 1 or type 2) and GDM with offspring blood pressure. Cho et al[15] noted 8 mmHg higher SBP (P < .001) in a multi-ethnic population of 99 offspring aged 10 to 16 years, as compared with 80 unexposed counterparts after controlling for height, weight, sex, age, and pubertal status. Similarly, Rostand et al[13] reported 8 mmHg (P < .05) higher SBP among Black and White children born to diabetic mothers as compared with those of nondiabetic mothers (n = 10 diabetic vs 252 nondiabetic) after accounting for sex, child's height, and maternal socio-economic factors. Finally, in the Pima Indian Study, Bunt et al[14] investigated associations of maternal diabetes with offspring cardiovascular traits after accounting for % fat mass and sex and detected 11 mmHg higher SBP among 22 Pima Indian children aged 7 to 11 years exposed to diabetes in utero, as compared with 20 offspring of nondiabetic women. Yet again, these studies did not assess the impact of any treatments or interventions to address hyperglycaemia during pregnancy.

Taken together, the current body of evidence suggestions that maternal GDM is associated with higher blood pressure in offspring. While the underlying physiological mechanisms are not known, our data and those of others[12] suggest that this relationship may be mediated by excess adiposity given the established influence of maternal hyperglycaemia on offspring fat mass at birth and beyond.[2-6] Animal studies also suggest the possibility of a direct effect of hyperglycaemia on kidney structure and function,[45] which can affect blood pressure. While this hypothesis has not specifically been tested in humans, a study in the Pima Indians found that exposure to maternal

diabetes corresponded with nearly four times higher urinary albumin excretion in the offspring, pointing towards the capacity of maternal diabetes to affect fetal nephron development.[46] Another possible mechanism linking maternal GDM to an adverse lipid profile involves the co-occurrence of maternal dyslipidaemia, which may influence offspring blood pressure through other mechanisms.[15] The fact that we detected associations of GDM with SBP in boys but not girls may be related to the more rapid increase and greater variability in SBP from late childhood through adolescence in males than females.[25]

4.2.1 | Strengths and limitations

Our study had several strengths. First, we were able to examine long-term consequences of exposure to maternal GDM on offspring cardiovascular risk factors across the adolescent transition, a sensitive period for development of cardiometabolic disease.[47] Second, rich covariate data enabled us to adjust for variables that may confound or mediate associations of interest. Third, our longitudinal modelling strategy appropriately accounted for correlations among repeated assessments of cardiovascular risk factors and efficiently leveraged the outcome data across two study visits spanning approximately 6 years of follow-up.

However, this study is not without limitations. A weakness of this study is the fact that we only had access to medical record data that provided information on GDM diagnosis, thereby precluding our ability to explore associations of the in utero glycaemic milieu at a finer resolution. A second limitation is the slightly decreasing sample sizes in multivariable models because of missing data for covariates, although the subsamples did not differ with respect to sociodemographic characteristics. A third limitation is the potential lack of generalizability because of over-selection of participants exposed to maternal GDM. Finally, we cannot discount the potential for false positive findings given the large number of models. However, our research focus was to describe and assess the direction, magnitude, and precision of the estimates rather than focus on statistical significance, especially in light of the fact that many of the biomarkers are correlated markers of the same biological phenomena.

5 | CONCLUSIONS

In this prospective study of approximately 600 multi-ethnic youth, we extend current literature regarding the relationship between in utero GDM exposure and cardiovascular health in offspring in two key aspects. First, we were able to explore the long-term consequences of maternal GDM on offspring cardiovascular risk factors throughout late childhood and adolescence via a repeated measures technique. This approach is not only more efficient from a statistical standpoint but also lends itself to more robust findings given that the biomarkers of interest—including total cholesterol and LDL, which we found to differ by GDM exposure in females—fluctuate across the age range of our study sample.[44] Second, we considered sex-specific associations to

reflect not only biological differences metabolic and cardiovascular traits as youth progress through the adolescent transition[25] but also potential discrepancies in fetal response to in utero exposures like maternal glycaemic control.[2]

While the effect sizes that we detected are modest (approximately 0.35 mmol/L higher total cholesterol and 0.31 mmol/L higher LDL in girls; approximately 5 mmHg higher SBP in boys for GDM exposed vs unexposed), they may have important long-term ramifications given that cardiometabolic risk factors track from childhood/adolescence into adulthood and are independent predictors of cardiovascular disease risk.[48] For example, in the Bogalusa Heart Study, a 1-mg/dL increment in LDL (equivalent to an increment of 0.026-mmol/L LDL) at 11 years of age corresponded with nearly two times higher odds of metabolic syndrome in young adulthood.[49] Similarly, a 1-mmHg increment in childhood SBP predicted elevations in a cluster of metabolic risk factors in young adulthood including SBP, cholesterol, triglycerides, and fasting glucose among Bogalusa Heart Study participants.[21] Given the increasing prevalence of GDM in developed countries like the United States, our findings underscore the importance of efforts to prevent maternal diabetes in order to improve the health of future generations.

CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

AUTHOR CONTRIBUTIONS

WP and DD conceived research questions. WP conducted the analysis, wrote the initial draft of the paper, and incorporated co-author comments. CWH and KS contributed to the analytical approach. All authors were involved in writing the paper and approved the final version.

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