Background/Objectives: Prenatal air pollution exposure has been associated with adverse childhood cardiometabolic outcomes. It is unknown whether evidence of metabolic disruption associated with air pollution is identifiable at birth. We examined exposure to prenatal ambient air pollution and cord blood cardiometabolic biomarkers among 812 mother-infant pairs in the Healthy Start study.

Methods: Using inverse-distance-weighted interpolation of ambient concentrations obtained from stationary monitors, we estimated daily particulate matter ≤2.5 micrometers (PM$_{2.5}$) and ozone (O$_3$) concentrations at participant residences. Daily estimates were averaged by trimester, full-pregnancy, and the 7 and 30 days prior to delivery. Associations of air pollution with the following cord blood biomarkers were estimated via multivariable linear regression: glucose, insulin, glucose/insulin ratio (GIR), leptin, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, free fatty acids, and triglycerides.

Results: In this Denver-based cohort, PM$_{2.5}$ concentrations were lower than in many US urban areas, but O$_3$ concentrations regularly exceeded federal air quality standards. Higher O$_3$ concentrations during pregnancy were consistently associated with higher insulin and lower GIR in cord blood. For example, an interquartile range increase in full pregnancy O$_3$ (6.3 parts per billion [ppb]) was associated with 0.13 log-µIU/ml (95% confidence interval [CI] = 0.04, 0.22) higher cord blood insulin, after adjusting for PM$_{2.5}$ and other confounders. We found positive, but generally nonsignificant, associations between pollutants during certain exposure periods and lipids.

Conclusions: In this cohort with moderately high O$_3$ exposure, prenatal concentrations of O$_3$ were positively associated with cord blood insulin. Future studies should examine the implications for offspring long-term health.

Keywords: Air pollution; Lipids; Metabolism; Neonate; Prenatal exposure

Introduction

In the United States, the prevalence of cardiometabolic diseases in childhood, including obesity,$^1$ type 2 diabetes,$^2$ and other cardiovascular disease risk factors,$^3,4$ has risen markedly in recent decades. While dietary factors and increasingly sedentary life-style have played a major role in the rising prevalence of childhood obesity, exposure to higher levels of air pollution early in life has also been posited as a potential explanatory factor.$^5,6$

The prenatal period may represent a critical time for environmental chemical exposures to impact health later in life.$^7,8$ For example, maternal exposure to ambient air pollution during pregnancy has been associated with offspring obesity and adverse cardiometabolic outcomes during childhood and beyond, in both animal$^9,10$ and human studies.$^11-14$

Despite these suggestive findings, understanding of the potential pathophysiological mechanisms of these observed associations between prenatal air pollution and childhood cardiometabolic outcomes remains limited. In utero exposure to air pollutants may adversely affect offspring’s metabolic function.
and promote the development of childhood obesity. Further, disturbances in leptin and other adipokines in cord blood have been associated with cardiometabolic disease markers in infancy and childhood, although there are sparse data describing this relationship with other cardiometabolic biomarkers. Alterations in cord blood cardiometabolic profiles may indicate both short-term, and potentially long-term, consequences of in utero air pollution exposure for offspring metabolic health.

A small number of epidemiologic studies have investigated air pollution-related changes in biomarkers of cardiometabolic function at birth in umbilical cord blood. Both Alderete et al and Lavigne et al found associations between maternal air pollution exposure and higher levels of cord blood leptin and adiponectin, two adipocyte-secreted hormones associated with adiposity at birth and in childhood.18,27 Notably, Lavigne et al observed a significant association between maternal air pollution exposure and cord blood leptin only after adjustment for birthweight z-score, suggesting that for any given birth weight, the relative size of the fat compartment may be altered by exposure. Further, in a cross-sectional study of 150 mother-infant pairs, maternal exposures to particulate matter of aerodynamic diameter <1 µm (PM1), 2.5 µm (PM2.5), and 10 µm (PM10) were associated with lipid biomarkers in cord blood, including triglycerides and cholesterol. Finally, Madhlopa et al reported that higher maternal PM10 and PM2.5 exposures during pregnancy were associated with higher cord blood insulin. However, no study has examined multiple markers of cardiometabolic function concomitantly, nor have previous studies examined the role of ozone (O3), recently linked to diabetes risk in adults.28

The US Environmental Protection Agency (US EPA) sets the National Ambient Air Quality Standards (NAAQS) for PM2.5 and PM10 levels, and 10 µm (PM10) have been published previously and are briefly described below. Average ambient PM2.5 and average 8-hour maximum O3 concentrations outside the maternal residences were estimated for the following periods during pregnancy: first, second, and third trimesters, full pregnancy, and the 30 and 7 days prior to delivery. There were 10 stationary monitors that measured 24-hour average PM2.5 and 19 stationary monitors that reported hourly O3 during the study period that were within 50 km of at least one participant’s residence. The frequency of reporting varied by monitor for PM2.5, ranging from daily to every 6 days, with most reports occurring every 3 days. All O3 monitors reported hourly values. We used all monitors within 50 km of the participant’s residence at enrollment and for which ≥75% of the expected concentrations were available to estimate period-specific averages for each participant. Distance-weighting (1/distance2) was used to calculate the average exposure during the specified period for each participant.

Assessment of outcomes

Umbilical cord blood was collected at delivery, and samples were analyzed by the University of Colorado Clinical and Translational Sciences Institute Core Laboratories. Serum leptin (ng/ml) was measured by ELISA (Alpco, Salem, NH). Insulin (µU/ml) was measured using radioimmunoassay according to manufacturer’s instructions (EMD Millipore Corporation, Billerica, MA). Glucose (mg/dl), total cholesterol (mg/dl), HDL cholesterol (mg/dl), triglycerides (mg/dl), and free fatty acids (µU/ml) were measured using an AU400e Chemistry Analyzer (Olympus America, Center Valley, PA). We calculated non-HDL cholesterol by subtracting HDL cholesterol from total cholesterol in cord blood. Glucose/insulin ratio (GIR) was used as an indirect measure of insulin sensitivity.

Other variables

Maternal age, race, ethnicity, education, and parity were self-reported at the time of study enrollment. Smoking status and exposure to secondhand smoke were self-reported at all three study visits during pregnancy. Maternal prepregnancy body mass index (BMI, kg/m2) was calculated using height and weight information collected at the beginning of pregnancy. Maternal prepregnancy weight was obtained by subtracting 24 weeks of gestation from the pregnancy weight, which was calculated from the gestation weight. Gestational diabetes (GDM) status was determined by a positive oral glucose tolerance test and/or diabetes in the medical record. Mode of delivery, season of birth, and infant sex were collected from medical records. Median income in the Census tract of the mother’s residence was obtained by linkage of the geocoded address reported at enrollment to 2012-2016 American Community Survey data and used an indicator of neighborhood-level socioeconomic status.

Methods

Study sample

Participants included in this analysis were enrolled in the Healthy Start study, an ongoing, prebirth cohort study of ethnically diverse mother-infant pairs. Between 2009 and 2014, pregnant women ≥16 years old who had not yet reached 24 weeks of gestation were recruited from the obstetrics clinics at the University of Colorado Hospital in Aurora, Colorado. Additional inclusion criteria were singleton pregnancy, no previous stillbirth or birth <25 weeks, and no preexisting chronic diseases, including diabetes, cancer, asthma managed with steroids, or serious psychiatric illness. Participants were invited to attend three in-person visits: in early pregnancy (median 17 weeks gestation), in mid-pregnancy (median 27 weeks gestation), and at delivery (median 1 day after birth). All participants provided written informed consent, and all study protocols were approved by the Colorado Multiple Institutional Review Board.

Of the 1,410 women enrolled in the Healthy Start study, participants were excluded from this analysis if they withdrew from the study prior to delivery (n = 11), experienced fetal demise (n = 17), did not have cord blood analyzed for cardiometabolic biomarkers (n = 527), and/or did not reside in the Denver-metropolitan area or had no O3 and/or PM2.5 monitors within 50 km of the residence with sufficient nonmissing data, as described below (n = 43). This led to a sample of 812 eligible mother-infant pairs (Supplementary Figure 1; http://links.lww.com/EAE/A181).
Statistical analysis

Spearman correlation coefficients were calculated for each pair of cardiometabolic markers. Insulin, GIR, leptin, and triglycerides in cord blood had right-skewed distributions and were natural log-transformed prior to analysis to better approximate normality and minimize the influence of outliers.

Multivariable linear regression models were used to investigate the relationships between prenatal PM$_{2.5}$ and O$_3$ and cardiometabolic biomarkers in cord blood. Associations were expressed as beta-coefficients and 95% confidence intervals (CIs) per interquartile range (IQR) increase in air pollutant exposure. Potential confounders were identified via directed acyclical graphs based on published literature, and included maternal age at delivery (years), prepregnancy BMI (kg/m$^2$), race/ethnicity (Hispanic, non-Hispanic White, non-Hispanic Black, all others), maternal smoking during pregnancy (any, none), exposure to secondhand smoke during pregnancy (>1 hour per week at any point during pregnancy), median annual income in the census tract (per $1,000), maternal education completed (<12th grade, some college or Associate's degree, four years of college, graduate degree), mode of delivery (cesarean, vaginal), previous pregnancy (any, none), season of birth (winter, spring, summer, fall), and the corresponding copollutant during the specified pregnancy period (PM$_{2.5}$ or O$_3$). Infant sex (male, female) was included as a precision covariate of the outcome. Birth weight and gestational age at birth were considered potential causal intermediates and accordingly were not included in the models. Because there is some evidence that fetal metabolic response to maternal environmental chemical exposures may differ by sex, potential effect modification by infant sex was assessed by including product interaction terms between each air pollutant exposure and infant sex in fully adjusted models. We hypothesized that the corresponding pollutant (PM$_{2.5}$ or O$_3$) confounded the relationships between the other pollutant and the cardiometabolic biomarkers; therefore, we included both pollutants in all primary models. However, in a sensitivity analysis, we investigated the results of single pollutant models. To evaluate the potential for nonlinear relationships between the air pollutants and the cardiometabolic markers, adjusted generalized additive models with penalized regression splines were run. Finally, because the levels of cardiometabolic biomarkers of infants born to mothers with GDM may differ, we conducted a sensitivity analysis excluding 34 participants with gestational diabetes.

We conducted all analyses in SAS (Version 9.4; The SAS Institute, Cary, NC) and R (Version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

The mean ± SD of maternal age at delivery was 28 ± 6 years, and the mean prepregnancy BMI was 26 ± 7 kg/m$^2$. There were 34 participants (4%) with GDM. The sample was racially and ethnically diverse (Hispanic, 25%; non-Hispanic White, 53%; non-Hispanic Black, 16%; and all others, 7%). Approximately 9% of participants smoked during pregnancy, and 23% of participants were exposed to secondhand smoke (>1 hour per week at any point during pregnancy) (Table 1). There were 432 (53%) male infants. Characteristics of participants in this subsample were comparable to those of the excluded participants and to the full potentially eligible cohort (Supplemental Table 1; http://links.lww.com/EE/A181).

The median full pregnancy average PM$_{2.5}$ exposure was 7.5 µg/m$^3$, the 5th percentile was 6.6 µg/m$^3$, and the 95th percentile was 8.4 µg/m$^3$. Similar median and range values were observed for trimester-average PM$_{2.5}$ exposures and the 30- and 7-day exposure windows. The median full pregnancy average 8-hour maximum O$_3$ concentration was 43.9 parts per billion (ppb), the 5th percentile was 36.6 ppb, and the 95th percentile was 49.9 ppb (Table 1 and Supplemental Table 2; http://links.lww.com/EE/A181).

Glucose, insulin, GIR, and leptin in cord blood were generally weakly correlated with each other, except for a strong inverse correlation between insulin and GIR ($r = -0.92$). Most cord blood lipid biomarkers were weakly to moderately correlated with each other, with the strongest correlation observed between triglycerides and non-HDL cholesterol (r = 0.44). For insulin, higher average 8-hour maximum O$_3$ during trimesters 2 and 3, full pregnancy, and the prior 7 and 30 days to delivery was associated with higher insulin (Table 3). For example, an IQR increase in prior 30-day O$_3$ (21.7 ppb) was associated with a 0.21 log-unit (95% CI = 0.07, 0.35) increase in cord blood insulin, after adjusting for covariates. Additionally, higher O$_3$ during all

| Table 1. Characteristics of 812 eligible mother-infant pairs in the Healthy Start Study |
|---------------------------------|--------------------------|
| Characteristic                  | Mean ± SD, median (25th–75th percentiles), or N (%) |
| **Covariates**                  |                          |
| Maternal age at delivery (years)| 27.5 ±6.2                |
| Maternal smoking during pregnancy (any) | 77 (9)                |
| Maternal smoking during pregnancy (any) | 77 (9)                |
| **Exposures**                   |                          |
| Average PM$_{2.5}$ (µg/m$^3$)    |                          |
| Trimester 1                      | 7.5 (6.9–8.2)           |
| Trimester 2                      | 7.4 (6.8–8.1)           |
| Trimester 3                      | 7.3 (6.9–8.1)           |
| Full pregnancy                   | 7.5 (7.0–7.9)           |
| Prior 30 days to delivery        | 7.2 (6.4–8.3)           |
| Prior 7 days to delivery         | 6.7 (5.6–8.2)           |
| Average 8-hour maximum O$_3$ (ppb) |                          |
| Trimester 1                      | 41.8 (33.0–53.0)        |
| Trimester 2                      | 42.0 (33.9–52.4)        |
| Trimester 3                      | 46.6 (35.5–54.0)        |
| Full pregnancy                   | 43.9 (40.7–47.0)        |
| Prior 30 days to delivery        | 48.0 (34.2–55.9)        |
| Prior 7 days to delivery         | 47.0 (35.5–55.6)        |

*Sample sizes differ for the following variables due to missing data: gestational diabetes, n = 774; average PM$_{2.5}$, trimester 1, n = 749; average PM$_{2.5}$, trimester 2, n = 749; average PM$_{2.5}$, trimester 3, n = 787; average PM$_{2.5}$, full pregnancy, n = 806; average PM$_{2.5}$ prior 30 days to delivery, n = 765; and average PM$_{2.5}$ prior 7 days to delivery, n = 771.

The characteristics of 812 eligible mother-infant pairs in the Healthy Start Study.
exposure windows, except for trimester 1, was associated with lower GIR. For example, an IQR increase in prior 30-day O₃ (21.7 ppb) was associated with a 0.22 log-unit (95% CI = –0.36, –0.09) decrease in cord blood GIR, after adjusting for covariates. Higher full pregnancy PM₂.₅ was associated with higher leptin, and average PM₂.₅ in each trimester was nonsignificantly positively associated with leptin. We did not observe consistent associations between O₃ and leptin, nor between PM₂.₅ or O₃ and glucose (Table 3). Associations in single pollutant models were similar to those observed in copollutant adjusted models (Supplemental Table 3; http://links.lww.com/EE/A181).

We observed significant exposure-by-infant sex interactions in models for associations between trimester 2 PM₂.₅ and insulin (P = 0.04), and trimester 2 PM₂.₅ and GIR (P = 0.02) (Table 3). Sex-specific analyses indicated different directions of association in male and female infants (Supplemental Tables 5 and 6; http://links.lww.com/EE/A181). There was a nonsignificant, positive association between trimester 2 PM₂.₅ and insulin among males

### Table 2.
Levels and pairwise spearman correlations of cardiometabolic biomarkers in cord blood among eligible mother-infant pairs in the Healthy Start Study

| Cardiometabolic biomarker | Median (25th–75th percentiles) | Glucose (mg/dl)a | Insulin (µIU/ml)a | Glucose/insulin ratio* | Leptin (ng/ml)a | Non-HDL cholesterol (mg/dl)a | HDL (mg/dl)a* | Free fatty acids (µIU/ml)a | Triglycerides (mg/dl)a* |
|---------------------------|--------------------------------|-----------------|------------------|------------------------|----------------|----------------------------|--------------|--------------------------|------------------------|
| Glucose (mg/dl)a          | 79 (70–90)                    | 1.00            | 0.19             | 0.17                   | 0.10           | –0.03                      | –0.07       | 0.10                     | 0.08                   |
| Insulin (µIU/ml)a         | 7 (6–11)                      | 1.00            | –0.92            | 0.27                   | –0.09          | –0.02                      | –0.23       | –0.26                    | –0.26                  |
| Glucose/insulin ratio*    | 11 (7–15)                     | 1.00            | –0.23            | 0.07                   | –0.02          | 0.26                       | 0.28        |                          |                        |
| Leptin (ng/ml)a           | 11 (6–21)                     | 1.00            | –0.06            | –0.01                  | 0.09           | 0.02                       | 0.02        |                          |                        |
| Non-HDL cholesterol (mg/dl)a | 33 (26–41)               | 1.00            | 0.40             | 0.13                   | 0.44           |                            |              |                          |                        |
| HDL (mg/dl)a              | 25 (21–30)                    | 1.00            | 0.06             | –0.13                  |                |                            |              |                          |                        |
| Free fatty acids (µIU/ml)a | 260 (178–366)                | 1.00            | 0.36             |                        |                |                            |              |                          |                        |
| Triglycerides (mg/dl)a    | 39 (29–55)                    | 1.00            | 0.02             |                        |                |                            |              |                          |                        |

*aAdjusted for race, maternal age, prepregnancy BMI, smoking status, exposure to secondhand smoke during pregnancy, median income in the census tract, education, mode of delivery, parity, season of birth, infant sex, and the corresponding copollutant during the specified pregnancy period.

### Table 3.
Associations between average PM₂.₅ and 8-hour maximum O₃ at the residential address during pregnancy and glucose, insulin, glucose/insulin ratio, and leptin in cord blood

| Air pollution exposure | Glucose (mg/dl)a | Sex interaction | Log-transformed insulin (log-unit) | Sex interaction | Log-transformed glucose/insulin ratio* | Sex interaction | Log-transformed Leptin (log-unit) | Sex interaction |
|------------------------|-----------------|----------------|-----------------------------|----------------|--------------------------------------|----------------|----------------------------------|----------------|
| Trimester 1            |                 |                |                             |                |                                      |                |                                  |                |
| Average PM₂.₅ (µg/m³)  |                 |                |                             |                |                                      |                |                                  |                |
| Prior 30 days to delivery |                 |                |                             |                |                                      |                |                                  |                |
| Average PM₂.₅ (µg/m³)  |                 |                |                             |                |                                      |                |                                  |                |
| Full pregnancy         |                 |                |                             |                |                                      |                |                                  |                |
| Air pollution exposure |                 |                |                             |                |                                      |                |                                  |                |
| Trimester 2            |                 |                |                             |                |                                      |                |                                  |                |
| Average PM₂.₅ (µg/m³)  |                 |                |                             |                |                                      |                |                                  |                |
| Prior 30 days to delivery |                 |                |                             |                |                                      |                |                                  |                |
| Average PM₂.₅ (µg/m³)  |                 |                |                             |                |                                      |                |                                  |                |
| Full pregnancy         |                 |                |                             |                |                                      |                |                                  |                |
| Air pollution exposure |                 |                |                             |                |                                      |                |                                  |                |
| Trimester 3            |                 |                |                             |                |                                      |                |                                  |                |
| Average PM₂.₅ (µg/m³)  |                 |                |                             |                |                                      |                |                                  |                |
| Prior 30 days to delivery |                 |                |                             |                |                                      |                |                                  |                |
| Average PM₂.₅ (µg/m³)  |                 |                |                             |                |                                      |                |                                  |                |
| Full pregnancy         |                 |                |                             |                |                                      |                |                                  |                |

*Adjusted for race, maternal age, prepregnancy BMI, smoking status, exposure to secondhand smoke during pregnancy, median income in the census tract, education, mode of delivery, parity, season of birth, infant sex, and the corresponding copollutant during the specified pregnancy period.
and an inverse association among females (Supplemental Table 5; http://links.lww.com/EE/A181). There was a nonsignificant inverse association between trimester 2 PM$_{2.5}$ and GIR observed among males and a positive association among females ($\beta = 0.09$ per IQR; 95% CI = 0.02, 0.17) (Supplemental Table 6; http://links.lww.com/EE/A181). We did not observe significant exposure-by-infant sex interactions for glucose, leptin, or lipids (Table 4 and Supplemental Tables 5–8; http://links.lww.com/EE/A181).

Trimester 1, 2 and full-pregnancy average 8-hour maximum O$_3$ were positively associated with non-HDL cholesterol in cord blood, although the 95% CI for trimester 1 included the null. We observed inverse associations of third trimester and full pregnancy average PM$_{2.5}$ with triglycerides in cord blood. Generally, there were nonsignificant, inverse associations between PM$_{2.5}$ and FFA, and nonsignificant, positive associations between O$_3$ during trimesters 2 and 3 and the prior 30 and 7 days to delivery and FFA. We did not observe consistent patterns of associations between either PM$_{2.5}$ or O$_3$ and HDL cholesterol (Table 4). Results from single pollutant models were similar (Supplemental Table 4; http://links.lww.com/EE/A181).

In a sensitivity analysis excluding participants with gestational diabetes (n = 34), results did not change appreciably (Supplemental Tables 9 and 10; http://links.lww.com/EE/A181). Evidence for nonlinearity, defined as lower AIC in the model with spline transformation of the exposure, was observed for some associations; therefore, we ran additional models in which cord blood biomarkers were regressed on quartiles of exposure (Supplemental Tables 11 and 12; http://links.lww.com/EE/A181). We observed a nonsignificant, U-shaped relationship between O$_3$ in the 7 days prior to delivery and cord blood glucose (Supplemental Table 11; http://links.lww.com/EE/A181). Several quartile models showed nonmonotonic responses between both pollutants and non-HDL cholesterol, HDL cholesterol, FFA, and triglycerides in cord blood, but results were generally nonsignificant (Supplemental Table 12; http://links.lww.com/EE/A181).

Discussion

In this prospective birth cohort study, greater average 8-hour maximum O$_3$ during trimesters 2, 3, full-pregnancy and the 30 and 7 days prior to delivery was associated with higher levels of cord blood insulin and lower GIR. Additionally, average PM$_{2.5}$ during certain periods of pregnancy was positively associated with cord blood leptin, and O$_3$ was positively associated with non-HDL cholesterol and inversely associated with triglycerides and FFA. Our findings provide suggestive evidence that prenatal air pollution exposure may be associated with dysregulation of metabolism at birth. Alterations in some metabolic biomarkers in cord blood have been associated with cardiometabolic health outcomes later in infancy and childhood, including weight gain, BMI, and body size and composition.

The Denver-metropolitan area represents a unique exposure profile (Table 4 and Supplemental Tables 5–8; http://links.lww.com/EE/A181). We observed a nonsignificant, U-shaped relationship between both pollutants and non-HDL cholesterol, HDL cholesterol, FFA, and triglycerides in cord blood, but results were generally nonsignificant (Supplemental Table 12; http://links.lww.com/EE/A181).

### Table 4

| Air pollution exposure | Non-HDL Cholesterol (mg/dl) | Sex interaction | P | HDL (mg/dl) | Sex interaction | P | Free fatty acids (µIU/ml) | Sex interaction | P | Log-transformed triglycerides (log-unit) | Sex interaction | P |
|------------------------|-----------------------------|-----------------|---|-------------|-----------------|---|-------------------------|-----------------|---|----------------------------------|-----------------|---|
| Trimester 1            |                             |                 |   |             |                 |   |                         |                 |   |                                  |                 |   |
| Trimester 1            |                             |                 |   |             |                 |   |                         |                 |   | Average PM$_{2.5}$ (µg/m$^3$) |                 |   |
| Trimester 2            |                             |                 |   |             |                 |   |                         |                 |   | Average PM$_{2.5}$ (µg/m$^3$) |                 |   |
| Trimester 3            |                             |                 |   |             |                 |   |                         |                 |   | Average PM$_{2.5}$ (µg/m$^3$) |                 |   |
| Full pregnancy         |                             |                 |   |             |                 |   |                         |                 |   | Average PM$_{2.5}$ (µg/m$^3$) |                 |   |
| Prior 30 days to delivery |                       |                 |   |             |                 |   |                         |                 |   | Average PM$_{2.5}$ (µg/m$^3$) |                 |   |
| Prior 7 days to delivery |                       |                 |   |             |                 |   |                         |                 |   | Average PM$_{2.5}$ (µg/m$^3$) |                 |   |

Adjusted for race, maternal age, prepregnancy BMI, smoking status, exposure to secondhand smoke during pregnancy, median income in the census tract, education, mode of delivery, parity, season of birth, infant sex, and the corresponding copollutant during the specified pregnancy period.

5
In Belgium, cord blood insulin was positively associated with full knowledge, no previous study has examined this relationship. Cord blood leptin is a known marker for neonatal fat mass, how shorter exposure windows and cord blood leptin. In infants, cord gain was observed in both sexes. We also noted sex-specific associations between full pregnancy nonfreeway Nitrogen oxide (NOx) exposure and proximity to a major roadway at the birth address has been associated with insulin resistance in children 10 years old. Importantly, cord blood collection in the Healthy Start study did not discriminate between the umbilical vein (from the mother) and artery (from the fetus) sources, and thus, the origin of insulin in our samples is unknown. However, given that the fetal pancreas produces measurable levels of insulin by mid-gestation, and maternal insulin does not typically cross the placenta (with a few exceptions), we can assume that most of the insulin in our cord blood samples collected at delivery is from fetal production. In our study, we used GIR as a measure of insulin sensitivity; alterations in GIR in cord blood have been identified in infants whose mothers were overweight or obese and had impaired glucose tolerance, suggesting it is a useful marker in cord blood of metabolic homeostasis.

Studies from animal models also support an association between prenatal air pollution exposure and impaired metabolic homeostasis later in life. Among mice exposed prenatally to diesel exhaust and fed a high-fat diet postnatally, males developed hyperinsulinemia and insulin resistance, and excess weight gain was observed in both sexes. We also noted sex-specific effects for some of the PM10 exposure windows and cord blood insulin. Moreover, our finding of an inverse association between O3 and GIR is consistent with animal studies of insulin resistance. There are several proposed mechanisms through which particulate matter and ozone may induce insulin resistance, including oxidative stress and inflammation. We found positive associations between trimester-long and full-pregnancy PM2.5 and cord blood leptin. However, we did not find consistent associations between PM10 during any of the shorter exposure windows and cord blood leptin. In infants, cord blood leptin is a known marker for neonatal fat mass, however, the nature of the relationship between cord blood leptin and adiposity across infancy and childhood is complex. In a Canadian cohort study, authors reported a positive association between full pregnancy PM10 and NO2 and cord blood leptin, when adjusting for birthweight z-score. More recently, results from a California-based birth cohort suggest positive associations between full pregnancy nonfreeway Nitrogen dioxide (NOx) exposure and proximity to major roadways and cord blood leptin. Further, an epigenetic study found PM2.5 exposure during trimester 2 was associated with lower deoxyribonucleic acid methylation of the leptin gene in the placenta. We did not observe associations between exposure to PM2.5 and O3 during pregnancy and glucose in cord blood. To our knowledge, no previous study has examined this relationship in the perinatal period. During gestation, placental transport of maternal glucose serves as the primary source of fuel for the fetus, and it is therefore difficult to compare our findings with existing literature investigating this relationship in childhood. Nonetheless, in one prior study, among overweight and obese African American and Hispanic children in Los Angeles, CA, PM2.5, NO2, and nonfreeway NOx, but not O3, exposure was associated with higher fasting glucose. Our findings on lipid biomarkers showed little consistency across lipids or across periods of pregnancy. We observed positive associations between O3 and non-HDL cholesterol in cord blood during some periods of pregnancy and inverse associations between trimester-long PM2.5 exposure and triglycerides. Although we observed limited evidence of associations between PM2.5 (inverse) and O3 (positive) during some periods of pregnancy and cord blood FFA, patterns were inconsistent and generally nonsignificant. In a cross-sectional analysis of Iranian mother-infant pairs, higher exposure to PM2.5, PM10, and PM1 was associated with higher total cholesterol levels and triglycerides, but not HDL, in cord blood. It is notable that our study had a much smaller range of variability in average full pregnancy PM2.5 (IQR, 0.9 µg/m3) compared to that of the Heydari et al study (IQR, 33.2 µg/m3). A recent investigation in a Mexico city-based birth cohort found that exposure to PM2.5 during trimester 3 of pregnancy was associated with higher levels of low-density lipoprotein (LDL), non-HDL, and total cholesterol levels in childhood (mean 4.8 years), suggesting that these alterations may also be identifiable in childhood. Authors also found that prenatal exposure to late-pregnancy PM2.5 was associated with lower triglyceride levels in children. While we observed only isolated associations between prenatal air pollution exposure and lipid biomarkers in cord blood, the abovementioned studies suggest that prenatal air pollution exposure remains an important potentially modifiable early-life exposure with evidence of enduring disruptions to cardiovascular risk later in life.

There are several strengths of this study. We used data from a prospective, prebirth cohort study, which allowed for the longitudinal assessment of the relationship between in utero air pollution exposure and cardiometabolic biomarker outcomes at delivery. The study population was racially and ethnically diverse, with well-characterized covariate data. We found novel associations between period-specific exposures to PM10 and O3 during pregnancy and some cardiometabolic biomarkers in cord blood.

Our study also has some limitations. Residential address information was only collected at enrollment. Because we did not have information on residential mobility during the study period, there is the possibility for misclassification of air pollution exposure. A previous study in New York showed limited residential mobility during pregnancy. While it is unknown if these findings are generalizable to women living in Colorado, it provides evidence that the degree of exposure misclassification due to local moves may be minimal. Further, we were not able to account for differences in human behavior that may affect personal air pollution exposure, including time spent indoors and in nonresidential settings. We also did not account for potential differences in exposure levels due to small-scale spatial variability, such as proximity to roadways or other concentrated sources of air pollution. The measurement error in air pollution due to the relatively sparse monitoring network is likely nondifferential with respect to the cord blood metabolic markers, and thus, may have resulted in an underestimation of associations. While we included several relevant covariates in our models, there is still the possibility for residual confounding due to unmeasured factors related to air pollution, including green space and traffic noise, or due to the use of imperfect proxies of socioeconomic status. There is the possibility of residual confounding due to the use of self-reported smoking data, as it may have resulted in an underestimation of smoking status, in our study. We performed a large number of statistical analyses and did not adjust for multiple testing, because our emphasis was on the magnitude and precision of the associations rather than their statistical significance. It is possible that some of the findings are statistically significant due to chance. However, the main findings of our study were consistent across several exposure time periods, and thus, are less likely to be spurious results. Independent replication of these results in another cohort would strengthen the interpretation.
Conclusions
In conclusion, we found higher average 8-hour maximum \( O_3 \) concentrations associated with higher cord blood insulin and lower GIR. We also found limited evidence of associations of ambient air pollution with lipids and the adipocyte-derived hormone leptin. These findings highlight the potential for air pollution exposure during pregnancy to disrupt the metabolic and lipid homeostasis of the offspring in the perinatal period. These findings were noted in an urban area with a unique air pollution profile, with levels of PM\(_{2.5}\) below the NAAQS standard, but \( O_3 \) regularly out of attainment. Future studies from Healthy Start and other birth cohorts should examine whether air pollution-associated changes in biomarkers persist into childhood, and therefore whether maternal exposure to air pollutants during pregnancy may contribute to insulin resistance and other adverse child cardiometabolic outcomes.

Conflicts of interest statement
The authors declare that they have no conflicts of interest with regard to the content of this report.

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