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Gestational exposure to $PM_{2.5}$, NO_2, and sex steroid hormones

Identifying critical windows of exposure in the Rochester UPSIDE Cohort

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Background: Sex steroid hormones are critical for maintaining pregnancy and optimal fetal development. Air pollutants are potential endocrine disruptors that may disturb sex steroidogenesis during pregnancy, potentially leading to adverse health outcomes.
Methods: In the Environmental influences on Child Health Outcomes Understanding Pregnancy Signals and Infant Development pregnancy cohort (Rochester, NY), sex steroid concentrations were collected at study visits in early-, mid-, and late-pregnancy in 299 participants. Since these visits varied by the gestational age at blood draw, values were imputed at 14, 22, and 30 weeks gestation. Daily NO_2 and $PM_{2.5}$ concentrations were estimated using random forest models, with daily concentrations from each 1-km^2 grid containing the subject's residence. Associations between gestational week mean NO_2 and $PM_{2.5}$ concentrations and sex steroid concentrations were examined utilizing distributed lag nonlinear models.
Results: Each interquartile range (IQR = 9 ppb) increase in NO_2 during weeks 0–5 was associated with higher early-pregnancy total testosterone levels (cumulative β = 0.45 ln[ng/dl]; 95% CI = 0.07, 0.83), while each IQR increase in NO_2 during weeks 12–14 was associated with lower early-pregnancy total testosterone levels (cumulative β = –0.27 ln[ng/dl]; 95% CI = –0.53, –0.01). Similar NO_2 increases during gestational weeks 0–14 were associated with higher late-pregnancy estradiol concentrations (cumulative β = 0.29 ln[pg/ml]; 95% CI = 0.10, 0.49), while each IQR increase in NO_2 concentrations during gestational weeks 22–30 was associated with lower late-pregnancy estradiol concentrations (cumulative β = –0.18 ln[pg/ml]; 95% CI = –0.34, –0.02). No associations with $PM_{2.5}$ were observed, except for an IQR increase in $PM_{2.5}$ concentrations (IQR = 4 $\mu g/m^3$) during gestational weeks 5–11 which was associated with lower late-pregnancy estriol levels (cumulative β = –0.16 ln[ng/ml]; 95% CI = –0.31, –0.00).
Conclusions: Residential NO_2 exposure was associated with altered sex steroid hormone concentrations during pregnancy with some indication of potential compensatory mechanisms.

Keywords: Air pollution; NO2; PM2.5; Sex steroid hormones; Pregnancy; Distributed lag model; Cohort

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Introduction

Sex steroids play critical roles in the maintenance of pregnancy, placental function, and fetal development.[1,2] Sex steroid hormones contribute to optimal fetal growth and development by promoting a uterine microenvironment that supports increased uterine size and successful placental development.[1] Throughout gestation, steroidogenesis contributes to vascularization, which promotes intrauterine nutrient transfer and supports the evolving physiological demands of pregnancy.[1,2] Estrogens, including estrone (E_1), estradiol (E_2), and estriol (E_3), are produced primarily by the placenta during pregnancy, with some E_1 and E_2 additionally produced by the ovaries.[3] Testosterone is a potent androgen that is also produced by the placenta during pregnancy and has been suggested to regulate fetal growth processes.[4] Sex steroid concentrations vary across pregnancy with

What this study adds

Few epidemiologic studies have assessed the association between air pollution exposure and maternal sex steroid hormone concentrations during pregnancy. This study observed that NO_2 concentrations during several gestational weeks of pregnancy were associated with maternal sex steroid hormone concentrations using distributed lag nonlinear modeling. The results of this study suggest that future experimental and epidemiologic studies are needed to examine potential compensatory mechanisms underlying the association between air pollution and sex steroid hormone concentrations during pregnancy.

substantial estrogen increases in both early and late pregnancy, whereas androgens gradually increase across pregnancy with a peak in concentrations during late pregnancy.[5,6] Disruption in these temporal estrogen and androgen patterns has previously been associated with altered fetal growth, sex-differentiated brain development, and ultimately, child behavioral changes.[4,7–9] Thus, it is important to understand if external factors predict adverse outcomes.

Gestational air pollution exposure has previously been associated with abnormal fetal growth, low birth weight, preterm birth, and disrupted thyroid hormone homeostasis, suggesting potential endocrine-disrupting properties.[10–14] Endocrine-disrupting chemicals, possibly including particulate matter (PM) and nitric dioxide (NO_2), are thought to induce adverse perinatal outcomes by mimicking the properties of endogenous hormones, disrupting the endocrine system, and inducing systemic inflammation.[15,16] Limited evidence from a few studies suggests that PM exposure during pregnancy can act as both an agonist and antagonist of estrogen and androgen receptors, thereby disrupting endogenous levels of sex steroid hormones.[16] The potential endocrine-disrupting properties of air pollution are further supported by recent epidemiological studies that suggest gestational air pollution exposure is associated with disrupted thyroid hormone homeostasis in mothers and offspring.[12–14] While the existing literature supports that ambient air pollution may have endocrine-disrupting properties, further studies are needed to understand the underlying mechanisms.

Although endocrine-disrupting chemicals have been associated with altered sex steroidogenesis during pregnancy in many studies,[17–20] few studies have examined the association between air pollution exposure and sex steroid hormones during pregnancy.[21–23] Plusquin et al[21] measured ambient air pollution across the gestational period and examined associations with steroid hormones related to the androgenic pathway in umbilical cord blood, reporting that each 7.96 μg/m^3 increase in $PM_{2.5}$ concentrations during the third trimester of pregnancy was associated with 23% higher cord blood levels of 17α-hydroxypregnenolone, a precursor to androgens and estrogens.[21] Further, each 0.58 μg/m^3 increase in black carbon levels during the first trimester of pregnancy was associated with 11% lower cord blood levels of androstenedione, a precursor to testosterone.[21] Colicino et al[22] measured ambient air pollution during the preconception and early prenatal periods (up to 20 weeks gestation) and maternal sex steroid hormone concentrations at an average of 29.5 weeks gestation.[22] They reported that average $PM_{2.5}$ concentrations during the first 20 weeks of pregnancy were positively associated with androgen metabolites during late pregnancy.[22] Experimental evidence from animal models further suggests that $PM_{2.5}$ exposure may reduce testosterone and estrogen levels.[24,25]

Limited evidence suggests that gestational air pollution exposure is associated with altered sex steroid hormones. However, the directions of the association have varied, and few studies have leveraged serial measurements of air pollutants and hormones to more fully understand associations across gestation.[21,22] Building on that limited literature, we examined the associations between gestational week mean $PM_{2.5}$ and NO_2 concentrations, and sex steroid hormones in a prospective pregnancy cohort in Rochester, NY, and used distributed lag nonlinear models to examine critical windows of exposure for sex steroid hormones measured in early-, mid-, and late-pregnancy. Based on limited experimental and observational evidence, we hypothesized that gestational week $PM_{2.5}$ and NO_2 concentrations would be negatively associated with both estrogens (estrone, estradiol, and estriol) and free and total testosterone across pregnancy.

Methods

Study Population

The Environmental influences on Child Health Outcomes Understanding Pregnancy Signals and Infant Development (ECHO-UPSIDE) pregnancy cohort has been described previously.[26] Briefly, pregnant women were recruited between December 2015 and April 2019 from clinics associated with the University of Rochester Medical Center in Rochester, NY. Women were eligible to enroll in the study if they had a singleton pregnancy with a gestational age of less than 14 weeks, there was no history of known substance abuse problems or psychotic illness, and they were able to communicate in English. Women were excluded if they had major endocrine disorders (e.g., polycystic ovary syndrome) or significant obstetric problems likely to lead to pregnancy loss at the time of enrollment. The original ECHO-UPSIDE cohort included 326 women who met these inclusion/exclusion criteria. Of these women, 299 had at least one measurement of sex steroids during gestation and were included in the current analysis (Supplemental Figure 1, http://links.lww.com/EE/A324). All women provided written informed consent, and this study was approved by the University of Rochester Research Subjects Review Board (RSRB#2064).

Exposure measures

The methods used to estimate daily $PM_{2.5}$ concentrations have been previously described,[27] with the same methods used to estimate NO_2 concentrations. Briefly, daily $PM_{2.5}$ and NO_2 concentrations were estimated using random forest models with a spatial resolution of 1 × 1 km. Daily $PM_{2.5}$ and NO_2 concentration data were obtained from regulatory monitoring sites from the US Environmental Protection Agency's Air Quality System (AQS, https://www.epa.gov/aqs) in New York State from 2015 to 2020. Random forest models were developed to fit the associations between AQS measurements and potential contributors to air pollution, such as land use areas, traffic intensity indicators, population statistics, and meteorological variables, with high predictive performance ($PM_{2.5}$: external validation r^2 = 0.65, root mean square error = 2.96 μg/m^3; NO_2: external validation r^2 = 0.043, root mean square error = 4.2 ppb).[27] Each participant's address at recruitment was geocoded and then matched to the 1 km^2 grid containing their residence. Daily $PM_{2.5}$ and NO_2 concentrations from that grid were then used for each day during the subject's pregnancy. Daily concentrations were averaged to provide gestational week average concentrations for each participant in the statistical analyses described below. Gestational age was determined using the best dating as measured by crown-rump length or date of the last menstrual period.

Outcome measures

Sex steroid hormones were measured as previously described.[18,28] Blood samples were collected from pregnant women at study visits during early-, mid-, and late-pregnancy. After processing,

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serum aliquots were stored at −80 °C at the University of Rochester Medical Center. Serum samples were shipped on dry ice to the Lundquist Institute at Harbor-University of California, Los Angeles Medical Center where the steroids were measured using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The calibration standards for testosterone had a linear response (range = 2.0–2000 ng/dL) and an accuracy of 100–113%. Total testosterone had a limit of quantification of 2 ng/dL. Free testosterone was measured using an equilibrium dialysis with labeled testosterone. Estrone (E_1), estradiol (E_2), and estriol (E_3) were measured using a triple quadrupole mass spectrometer in the negative mode using multiple-reaction-monitoring. Estrone and estradiol had linear calibration curves (range = 2–2000 pg/ml) with a lower limit of quantification of 2.0 pg/ml. For estriol, the calibration curve was linear (range = 50–100 pg/ml) with a lower limit of quantification of 50 pg/ml. The accuracy for estrone was between 91.9% and 101.2%, estradiol was between 93.9% and 100.3%, and estriol was between 87.2% and 104.3%.

Covariates

Covariates were selected a priori from available clinical and sociodemographic variables that were thought to be confounders of the association or predictors of sex steroid hormone concentrations, based on the prior literature.[21,22,28] Sociodemographic information, such as maternal age (years), race and ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, and other), any Nutrition Program for Women, Infants, and Children (WIC) use during pregnancy (yes/no), and maternal education (high school degree or less, some college or college graduate, and higher than college) were collected at baseline. Clinical data, including parity (nulliparous or multiparous), earliest pregnancy body mass index (BMI) (kg/m^2), fetal sex, and season of birth were abstracted from medical records. Additional details about the collection of covariates have been described previously.[26] Variables were ultimately adjusted for in the models if they were included in the subset that minimized the cross-validated mean squared error when predicting mid-pregnancy total testosterone or estradiol (Supplemental Tables 1 and 2, http://links.lww.com/EE/A324).

Statistical analysis

Distributions of gestational week $PM_{2.5}$ and NO_2 concentrations in early-, mid-, and late-pregnancy were calculated, followed by estimation of interquartile ranges (IQR), which were used to scale associations as described in greater detail below. Sex steroid hormones were log-transformed due to non-normality, and distributions of each in early-, mid-, and late-pregnancy were calculated. Because postconceptual age at blood serum collection varied by participant within early-, mid-, and late-pregnancy, sex steroid hormone concentrations were imputed at 14-, 22-, and 30-weeks' gestation for analyses of the three pregnancy periods. Imputation used linear interpolation between two measured values from the participant when at least one measurement was within 10 weeks of the target gestational age. When a value was measured only before or after the target gestation age, a value was imputed based on linear extrapolation using data from other participants, as long as the closest measurement was within 4 weeks of the gestational age.

The associations between NO_2 and sex steroid hormones measured in early-, mid-, and late-pregnancy were separately analyzed using distributed lag nonlinear models (DLNM).[29] We used a model that constrains the exposure effect at each gestational week to be linear but allows the lag effect across all gestational weeks included in the model to be nonlinear, termed a distributed lag model.[29,30] The estimation of the DLNM utilized penalized cubic regression splines to allow for nonlinearity in the lag dimension. Smoothness was induced with a penalty defined on the second derivative of the function, where the penalty parameter was selected by the model software.[31] We set the initial degrees of freedom for the spline on the lag dimension to 7. However, the penalty parameter reduces the degrees of freedom based on the data and model fit, so the equivalent degrees of freedom for each model fit could be substantially smaller. For our early-pregnancy analyses, each hormone at 14 weeks gestation was fit in a separate DLNM model against NO_2 concentrations from weeks 0 to week 14. For each hormone, the model provides estimates of the increase or decrease in the hormone associated with each IQR increase (9 ppb) in gestational week NO_2 concentration (e.g., NO_2 in gestational week 0, 1,... 14), where the week-specific estimates are obtained from the penalized DLNM. We adjusted for maternal age (years), race and ethnicity (non-Hispanic Black, non-Hispanic White, Hispanic, Other), fetal sex (male, female), highest level of maternal education (high school or less, some college or college graduate, and higher than college education), WIC use during pregnancy (yes, no), parity (nulliparous, multiparous), season of birth, and prepregnancy BMI (kg/m^2) in all models. For any set of gestational weeks where NO_2 concentrations were significant predictors of the hormone, we then estimated the cumulative difference in that hormone (and its 95% confidence interval [CI]) associated with each IQR increase in NO_2 concentration across those weeks. We also estimated the cumulative increase/decrease in the hormone associated with all the NO_2 gestational weeks across the time period included in the model (and its 95% CI) regardless of statistical significance. We then re-ran models for each combination of pollutant ($PM_{2.5}$, NO_2), sex steroid (free testosterone, total testosterone, estrone, estradiol, and estriol), and pregnancy time point (week 14, 22, and 30) (N = 299). The slopes associated with $PM_{2.5}$ were based on its IQR of 4 μg/m^3.

Additionally, to examine potential sex-specific associations, we re-ran models separately for those pregnancies with a male or female fetus. To examine if our findings were robust to pregnancy complications, we re-ran models excluding individuals with pre-eclampsia, gestational diabetes, preterm birth (gestational age <37 weeks), and miscarriage (n = 258). We then compared these findings to the primary analyses described above. All analyses were performed using R version 4.3.2, and the MGCV and DLNM packages.

Results

Participants were predominantly non-Hispanic white (55%), were on average overweight (prepregnancy BMI = 28.1 kg/m^2) in early pregnancy, with most not reporting smoking (7.4%) or any alcohol consumption (3.7%) during pregnancy (Table 1). Women in the cohort were mostly multiparous (66%), and the majority did not use WIC (56%), public assistance (67%), or Medicaid (53%) during pregnancy. There was a relatively equal distribution of fetal sex in this cohort.

The distributions of $PM_{2.5}$, NO_2, and imputed sex steroid concentrations are summarized in Table 2. Distributions of $PM_{2.5}$ and NO_2 concentrations were similar across gestation but were only weakly correlated with each other at any given week during pregnancy ($r < 0.26$). NO_2 concentrations were highly correlated across gestation ($r > 0.66$), but $PM_{2.5}$ concentrations were not ($r < 0.33$) (Supplemental Table 3, http://links.lww.com/EE/A324). Logarithm-transformed total testosterone levels and free testosterone levels were highly correlated across 14-, 22- and 30-weeks' gestation ($r > 0.73$; Supplemental Table 4, http://links.lww.com/EE/A324). Estrone and estradiol concentrations were also highly correlated across the three gestational ages ($r_{estrone}>0.82$ and $r_{estradiol}>0.76$). Estriol concentrations were at best moderately correlated across gestation ($r < 0.49$).

Patterns of the shape of the association and the identified susceptible windows were similar for total and free testosterone levels across gestation. Generally, NO_2 concentrations were positively related to testosterone levels during early pregnancy and

Table 1.

Characteristics of the study participants with at least one measurement of sex steroid concentrations (n = 299)[a]

Characteristic	N (%) or Mean (SD)
Maternal age (years)	28.9 (4.7)
Maternal prepregnancy BMI (kg/m^2)	28.1 (7.0)
Parity	
Nulliparous	102 (34.1%)
Multiparous	197 (65.9%)
Maternal race/ethnicity	
Non-Hispanic White	166 (55.5%)
Non-Hispanic Black	76 (25.4%)
Hispanic	32 (10.7%)
Other	25 (8.4%)
Maternal education	
High school or less than high school	114 (38.1%)
Some college or college graduate	112 (37.5%)
Higher than college education	71 (23.7%)
Missing	2 (0.7%)
Maternal alcohol use during pregnancy	
Yes	11 (3.7%)
No	246 (82.3%)
Missing	42 (14.0%)
Maternal smoking during pregnancy	
Yes	22 (7.4%)
No	266 (89.0%)
Missing	11 (3.7%)
WIC use during pregnancy	
Yes	109 (36.5%)
No	168 (56.2%)
Missing	22 (7.4%)
Medicaid use during pregnancy	
Yes	141 (47.2%)
No	158 (52.8%)
Poverty income ratio at enrollment	3.74 (3.94)
Public assistance use	
Yes	68 (22.7%)
No	199 (66.6%)
Missing	32 (10.7%)
Fetal Sex	
Female	153 (51.2%)
Male	146 (48.8%)
Postconceptual age at time of blood draw (weeks)	Mean (range)
Visit 1	12.4 (6.14, 14.6)
Visit 2	20.9 (18.1, 29.6)
Visit 3	30.9 (28.1, 39.0)

[a]There were 299 participants with sex steroid concentrations at 14 weeks, 296 participants with sex steroid concentrations at 22 weeks, and 299 participants with sex steroid concentrations at 30 weeks.

negatively associated with testosterone levels later in pregnancy (Table 3 and Figure 1). Each IQR increase in NO_2 concentration during gestational weeks 0 through 5 was associated with higher total testosterone levels at 14 weeks gestation (cumulative β = 0.45 ln[ng/dl]; 95% CI = 0.07, 0.83), while each IQR increase in NO_2 concentration during gestational weeks 12–14 was associated with lower total testosterone levels at 14 weeks gestation (cumulative β = −0.27 ln[ng/dl]; 95% CI = −0.53, −0.01) (Figure 1 and Table 3). Each IQR increase in NO_2 during gestational weeks 1 through 6 was associated with higher total testosterone levels at 22 weeks gestation (cumulative β = 0.29 ln[ng/dl]; 95% CI = 0.04, 0.53) (Figure 1 and Table 1). An interquartile increase in NO_2 during gestational weeks 1–5 and gestational weeks 0–10 was associated with higher 14-week (cumulative β = 0.56 ln[ng/dl]; 95% CI = 0.26, 0.86) and 22-week (cumulative β = 0.53 ln[ng/dl]; 95% CI = 0.16, 0.91) free testosterone levels, respectively (Figure 1 and Table 1). An IQR increase in NO_2 concentrations during gestational weeks 12–14 and gestational weeks 16–22 was associated with lower 14-week (cumulative β = −0.68 ln[ng/dl]; 95% CI = −1.02, −0.34) and 22-week (cumulative β = −0.36 ln[ng/dl]; 95% CI = −0.67, −0.04) free testosterone levels, respectively (Figure 1 and Table 1). An IQR increase in NO_2 concentrations during gestational week 15 was associated with higher levels of free testosterone at 30 weeks (cumulative β = 0.01 ln[ng/dl]; 95% CI = 0.00, 0.01) (Figure 1 and Table 1). Similar patterns of the cumulative cross-basis shape were observed for $PM_{2.5}$ and free and total testosterone levels, although no statistically significant susceptible weeks were observed (Supplemental Figure 2, http://links.lww.com/EE/A324).

The overall patterns of effects for the associations between NO_2 concentrations and estrogens tended to be positive in the earlier weeks of each pregnancy period but negative in the later weeks of the same pregnancy period. Specifically, interquartile increases in NO_2 concentrations in gestational week 6 and during gestational weeks 0–15 were associated with higher estrone concentrations at 14 weeks gestation (cumulative β = 0.02 ln[pg/ml]; 95% CI = 0.00, 0.04) and 30 weeks gestation (cumulative β = 0.49 ln[pg/ml]; 95% CI = 0.13, 0.84) (Figure 2 and Table 1). Each interquartile increase in NO_2 concentration during gestational weeks 0–14 was associated with higher 30-week estradiol concentrations (cumulative β = 0.29 ln[pg/ml]; 95% CI = 0.10, 0.49), while each interquartile increase in NO_2 concentrations during gestational weeks 22–30 was associated with lower 30-week estradiol concentrations (cumulative β = −0.18 ln[pg/ml]; 95% CI = −0.34, −0.02) (Figure 2 and Table 1). No sensitive windows were identified for 22-week estrone, 14- and 22-week estradiol, or estriol levels at any time during pregnancy. However, patterns of association were similar across estrogens and throughout gestation. Each IQR increase in $PM_{2.5}$ concentrations during gestational weeks 5–11 was associated with lower late-pregnancy estriol levels (cumulative β = −0.16 ln[ng/ml]; 95% CI = −0.31, −0.00) (Table 3, Supplemental Figure 2, http://links.lww.com/EE/A324). No other sensitive windows of $PM_{2.5}$ were identified for estrogen levels, although as with NO_2, patterns of the association were similar across estrogens and throughout gestation (Supplemental Figure 3, http://links.lww.com/EE/A324).

In sex-stratified analyses, pregnancies with male and female fetuses followed similar trends and had similar patterns of association across gestation for both NO_2 and $PM_{2.5}$ concentrations (Supplemental Figures 4–11, http://links.lww.com/EE/A324).

After excluding women with pregnancy complications (pre-eclampsia, gestational diabetes, preterm birth, and miscarriage), the overall patterns and functional forms of associations across gestation were similar compared to models including women with pregnancy complications (Supplemental Table 5 and Supplemental Figures 12–15, http://links.lww.com/EE/A324). In some instances, exposure levels during additional gestational weeks become statistically significant after excluding women with pregnancy complications. For example, each interquartile increase in $PM_{2.5}$ concentrations during gestational weeks 18–22 was associated with lower 22-week estriol levels only when women with pregnancy complications were excluded (cumulative β = −0.20 ln[pg/ml]; 95% CI = −0.38, −0.02). At the same time, associations during particular gestational weeks were attenuated after women with complications were excluded from the analysis (Supplemental Table 5 and Supplemental Figures 12–15, http://links.lww.com/EE/A324). One notable exception was each interquartile increase in NO_2 concentration during gestational weeks 0–14 was positively associated with 30-week estradiol concentrations when women with pregnancy complications were excluded (cumulative β = 0.23 ln[pg/ml]; 95% CI = 0.02, 0.44), whereas each interquartile increase in NO_2 concentrations during gestational weeks 0–14 were associated with lower 30-week estradiol concentrations

Table 2.

Distributions of air pollution measures and imputed sex steroid hormones by pregnancy timepoint.

	N	Missing	Mean (SD)	IQR	Minimum	25th Percentile	Median	75th Percentile	Maximum
$PM_{2.5}$ exposure ($\mu g/m^3$)									
Gestational week 0	280	7	6.6 (1.7)	2.4	3.3	5.3	6.3	7.7	13.2
Gestational week 10	280	7	6.8 (1.6)	2.4	2.2	5.4	6.7	7.8	12.0
Gestational week 20	217	27	6.6 (1.9)	2.6	3.5	5.1	6.1	7.7	13.4
Gestational week 30	222	30	6.8 (2.0)	2.7	2.8	5.3	6.6	8.0	13.8
NO_2 exposure (ppb)									
Gestational week 0	280	7	11.6 (5.0)	8.3	1.4	7.0	11.7	15.3	24.6
Gestational week 10	280	7	11.5 (5.1)	7.7	1.1	7.1	11.7	14.7	23.9
Gestational week 20	217	27	10.4 (4.8)	7.9	0.8	6.1	10.7	14.0	22.1
Gestational week 30	222	30	10.0 (5.0)	8.0	0.7	5.4	10.1	13.4	25.1
Total testosterone (ng/dl)									
Early pregnancy	294	5	73.6 (46.5)	58.4	8.4	38.2	65.3	96.6	307.1
Mid pregnancy	282	14	77.9 (55.9)	50.6	4.8	43.1	62.0	93.7	406.9
Late pregnancy	276	23	77.9 (56.1)	61.4	9.6	38.8	64.2	100.2	372.0
Free testosterone (ng/dl)									
Early pregnancy	293	6	0.41	0.36	0.06	0.20	0.34	0.56	2.41
Mid pregnancy	281	15	0.39	0.30	0.03	0.20	0.32	0.50	2.50
Late pregnancy	276	23	0.41	0.34	0.05	0.18	0.30	0.52	2.41
Estrone (E_1) (pg/ml)									
Early pregnancy	294	5	1451 (965)	1081	72	836	1223	1917	5908
Mid pregnancy	282	14	4169 (2650)	2639	431	2388	3741	5027	18133
Late pregnancy	276	23	6336 (4272)	4433	737	3484	5580	7917	28750
Estradiol (E_2) (pg/ml)									
Early pregnancy	294	5	2371 (1105)	1274	446	1636	2191	2910	8568
Mid pregnancy	282	14	6636 (2470)	3118	1398	4972	6379	8090	18734
Late pregnancy	276	23	11035 (4096)	5039	2308	8217	10697	13256	29950
Estriol (E_3) (pg/ml)									
Early pregnancy	263	36	462 (315)	432	25	217	381	649	1625
Mid pregnancy	273	23	3210 (1135)	1434	494	2450	3189	3884	7280
Late pregnancy	274	25	6277 (2158)	2728	1183	4782	6063	7510	15033

Imputed values for steroid hormone concentrations were estimated at postconceptual ages 14 weeks (early pregnancy), 22 weeks (mid pregnancy), and 30 weeks (late pregnancy).
IQR indicates interquartile range.

Table 3.

Cumulative increase or decrease in sex steroid hormones across pregnancy for each interquartile range increase in $PM_{2.5}$ (interquartile range = 4) or NO_2 (interquartile range = 9) concentration in each gestational week

	Weekly $PM_{2.5}$				Weekly NO_2			
	Susceptible weeks			Entire period	Susceptible weeks			Entire period
Variable	N	Gestational weeks[a]	Cumulative beta (95% CI)	Cumulative beta (95% CI)	N	Gestational weeks[a]	Cumulative beta (95% CI)	Cumulative beta (95% CI)
Visit 1 (14 weeks)								
Total testosterone (ng/dl)	246	---	---	0.31 (–0.10, 0.73)	246	0–5 12–14	0.45 (0.07, 0.83) –0.27 (–0.53, –0.01)	0.08 (–0.08, 0.23)
Free testosterone (ng/dl)	245	---	---	–0.06 (–0.53, 0.43)	245	1–5 11–14	0.56 (0.26, 0.86) –0.68 (–1.02, –0.34)	10.06 (–0.10, 0.21)
Estrone (pg/ml)	246	---	---	0.18 (–0.31, 0.67)	246	6	0.02 (0.00, 0.04)	0.19 (0.00, 0.37)
Estradiol (pg/ml)	246	---	---	0.24 (–0.09, 0.56)	246	---	---	0.05 (–0.08, 0.17)
Estriol (pg/ml)	219	---	---	–0.05 (–0.73, 0.63)	219	---	---	0.01 (–0.22, 0.23)
Visit 2 (22 weeks)								
Total testosterone (ng/dl)	238	---	---	0.57 (–0.19, 1.33)	238	1–6	0.29 (0.04, 0.54)	0.14 (–0.03, 0.31)
Free testosterone (ng/dl)	237	---	---	0.35 (–0.46, 1.15)	237	0–10 16–22	0.53 (0.16, 0.91) –0.36 (–0.68, –0.04)	0.14 (–0.05, 0.32)
Estrone (pg/ml)	238	---	---	0.70 (–0.11, 1.51)	238	---	---	0.22 (0.04, 0.40)
Estradiol (pg/ml)	238	---	---	0.32 (–0.13, 0.78)	238	---	---	0.05 (–0.06, 0.15)
Estriol (pg/ml)	230	---	---	–0.06 (–0.57, 0.45)	230	---	---	–0.08 (–0.19, 0.04)
Visit 3 (30 weeks)								
Total testosterone (ng/dl)	235	---	---	0.74 (–0.08, 1.56)	235	---	---	0.18 (0.01, 0.36)
Free testosterone (ng/dl)	235	---	---	0.26 (–0.65, 1.17)	235	15	0.01 (0.00, 0.01)	0.21 (0.02, 0.40)
Estrone (pg/ml)	235	---	---	0.34 (–0.57, 1.24)	235	0–15	0.49 (0.13, 0.84)	0.25 (0.06, 0.43)
Estradiol (pg/ml)	235	---	---	0.15 (–0.33, 0.64)	235	0–14 22–30	0.29 (0.10, 0.49) –0.18 (–0.35, –0.02)	0.09 (–0.02, 0.19)
Estriol (pg/ml)	233	5-11	–0.16 (–0.31, –0.00)	–0.44 (–0.95, 0.07)	233	---	---	–0.05 (–0.16, 0.05)

All statistical models were adjusted for maternal age, race and ethnicity, fetal sex, highest level of maternal education, WIC use during pregnancy, parity, season of birth, and pre-pregnancy BMI. Sex steroid values were natural logarithm-transformed for analyses.
[a]Gestational weeks that were identified as statistically significant susceptible windows.

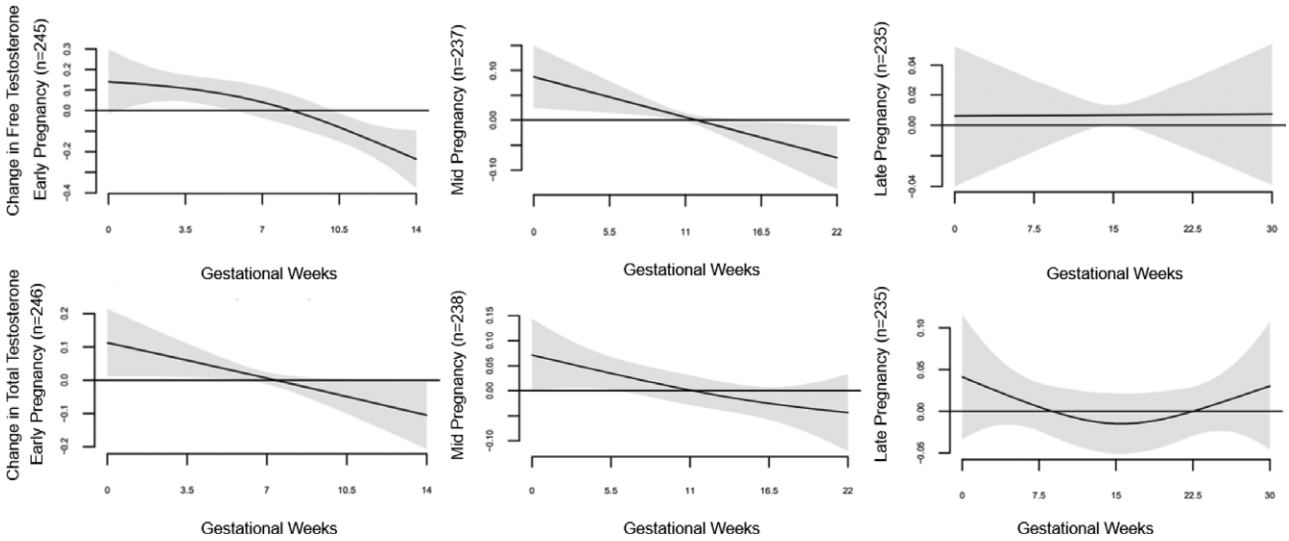

Figure 1. DLNM models estimating the association between gestational NO_2 (IQR = 9 ppb) and natural logarithm-transformed free and total testosterone in early- (14 weeks), mid- (22 weeks), and late-pregnancy (30 weeks). All statistical models were adjusted for maternal age, race and ethnicity, fetal sex, highest level of maternal education, WIC use during pregnancy, parity, season of birth, and pre-pregnancy BMI.

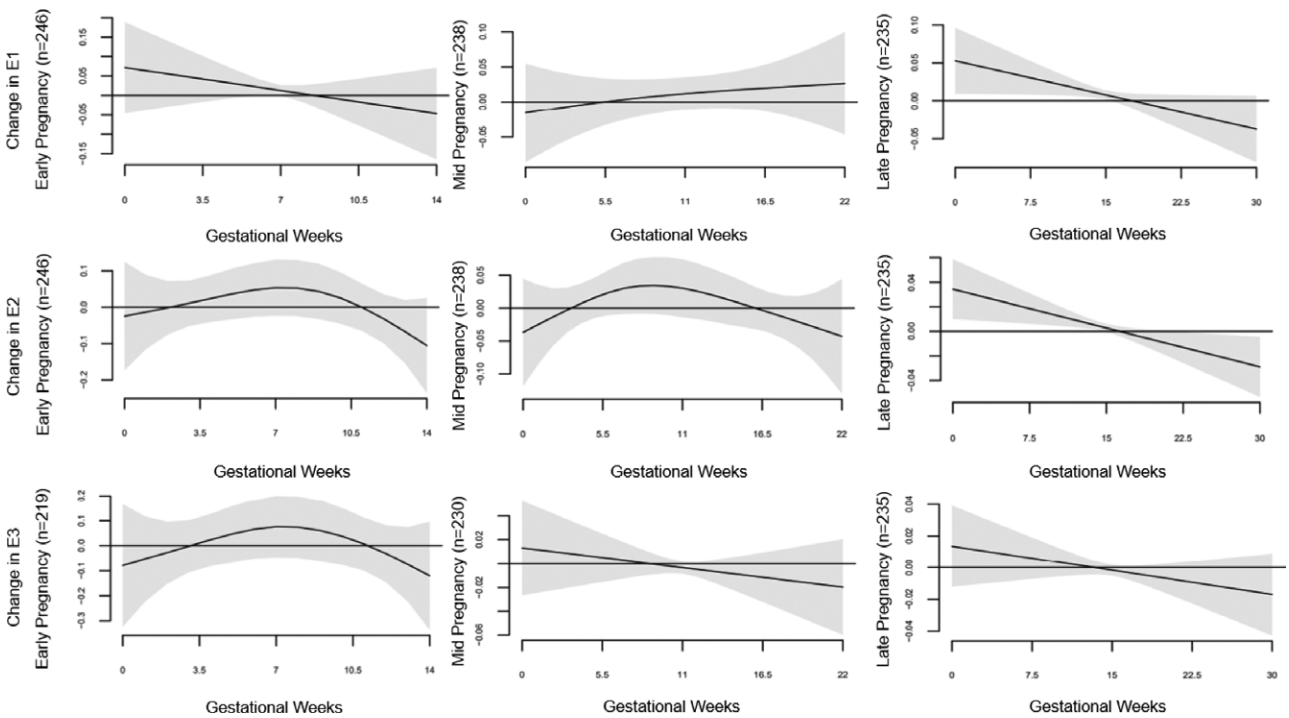

Figure 2. DLNM models estimating the association between gestational NO_2 (IQR = 9 ppb) and natural logarithm-transformed estrogens in early- (14 weeks), mid- (22 weeks), and late-pregnancy (30 weeks). All statistical models were adjusted for maternal age, race and ethnicity, fetal sex, highest level of maternal education, WIC use during pregnancy, parity, season of birth, and pre-pregnancy BMI.

when women with pregnancy complications were included (cumulative β = −0.18 ln[pg/ml]; 95% CI = −0.34, −0.02).

Discussion

In this cohort of pregnant people from Rochester, NY, we observed several sensitive windows of association between NO_2 and sex steroid concentrations during early-, mid-, and late-pregnancy. The observed patterns of cumulative associations between NO_2 and sex steroid concentrations were non-monotonic and appeared to vary in direction across gestation. Specifically, we observed that each 9 ppb increase in NO_2 concentrations during gestational weeks 1–5 was associated with a 0.56 ln(ng/dl) increase in early pregnancy free testosterone concentration, approximately equivalent to a 1.75% higher free testosterone concentration. Further, each 9 ppb increase in NO_2 concentration during gestational weeks 0–5 was associated with a 0.45 ln(ng/dl) increase in total testosterone, which is an ~1.57% higher total testosterone concentration. During the same pregnancy period, 9 ppb increases in NO_2 concentration during gestational weeks 11–14 and during gestational weeks 12–14 were associated with 0.68 ln(ng/dl) decreases in early-pregnancy free testosterone (~1.97% lower free testosterone concentration) and a 0.27 ln(ng/dl) decrease in total testosterone

concentration (~1.13% lower total testosterone concentration), respectively. We speculate that there is a possible compensatory mechanism as a similar pattern has been observed in the ECHO-UPSIDE cohort in the association between NO_2 and placental growth (under review), although further evidence is needed to confirm this. There was little evidence that gestational $PM_{2.5}$ exposure was associated with gestational sex steroid concentrations. Overall, the observed patterns of association were similar between androgens and estrogens across gestation with both NO_2 and $PM_{2.5}$.

Few epidemiologic studies have examined the association between gestational exposure to ambient air pollution and sex steroid concentrations.[21–23] A major finding from this study was that gestational NO_2 exposure was associated with androgens measured in early-, mid-, and late-pregnancy. These results are inconsistent with those of a previous study that reported no association between daily gestational NO_2 exposure (mean [IQR] = 18.5 μg/m^3 [3.30]) and cord blood sex steroid concentrations in 397 Belgian mother-child pairs.[21] The differences in results may be due to the differences in the timing and matrix of outcome measurement, i.e., gestational versus cord blood steroid hormone concentrations, with cord blood concentrations likely more directly reflecting fetal exposure than maternal blood concentrations. The PRogramming of Intergenerational Stress Mechanisms (PRISM) cohort, consisting of 361 mother-fetal pairs sampled from Boston and New York City, evaluated associations with $PM_{2.5}$, but not NO_2.[22] No associations were observed between preconception or early-pregnancy $PM_{2.5}$ exposure and gestational estriol levels in PRISM. Similarly, we observed null associations between $PM_{2.5}$ and estrogen concentrations in this study, except for an association between $PM_{2.5}$ concentrations during weeks 5 through 11 and lower late pregnancy estriol concentrations. At the same time, PRISM also observed positive associations between $PM_{2.5}$ and late-pregnancy maternal androgens,[22] whereas we observed no associations between $PM_{2.5}$ concentrations and androgens at 14-, 22-, or 30-week gestation. The discrepancy in results may be partially due to the higher levels of $PM_{2.5}$ observed in PRISM (mean = 8.89–9.2 μg/m^3) compared to ECHO-UPSIDE (mean = 6.6–6.8 μg/m^3).[22] The observed associations between NO_2 and sex steroid concentrations, but not $PM_{2.5}$ concentrations, may also suggest that traffic-related air pollution may affect sex steroids more so than the wider range of exposure sources estimated by $PM_{2.5}$ levels.

Slight differences in the overall patterns of association were observed when women with pregnancy complications were removed. In some instances, there were more statistically significant gestational weeks of exposure, whereas others had fewer statistically significant gestational weeks. This suggests that differences were likely due to decreases in sample size, increasing random error. It is also possible that pregnancy complications induce physiologic stress that may make pregnant women more susceptible to endocrine disruption. Although this reasoning is speculative, a previous observational study reported that a mixture of phthalates was associated with placental corticotropin-releasing hormone only among women with gestational diabetes.[32] Additionally, total testosterone, free testosterone, and estrone were positively associated with gestational diabetes in the ECHO-UPSIDE cohort.[33] These preliminary findings should be further explored in experimental studies to elucidate the mechanism underlying the potential relationship between endocrine-disrupting exposures, sex steroid hormones, and pregnancy complications.

There is limited experimental evidence to support the underlying mechanism of the association between gestational exposure to ambient air pollution and sex steroid hormones.[24,25] However, observational studies offer some support for the hypothesis that NO_2 has endocrine-disrupting properties. For example, NO_2 exposure during the male programming window and mini-puberty has been associated with decreased penile width at age 1 year, suggesting a possible reduction in early androgen activity.[34] Additionally, a negative association between prenatal NO_2 exposure and pubertal staging in 11-year-old males was observed in a Hong Kong birth cohort.[35] Even within the clinically normal range of hormones, associations with birth outcomes, such as fetal growth restriction, preterm birth, and low birthweight have been observed emphasizing the clinical importance of endocrine disruption during pregnancy.[36–39] Given that, on a population level, the small changes in hormone concentrations observed in this cohort during pregnancy may have a large impact on public health.

There is also evidence that $PM_{2.5}$ may act as an endocrine disruptor,[15,16,24,34] although we observed limited evidence of that in this cohort. Specifically, murine models suggest gestational $PM_{2.5}$ exposure may inhibit estrogen synthesis by the ovaries via epigenetic alteration of the estrogen receptor alpha in uterine tissue.[15] This further supports our finding between $PM_{2.5}$ and estriol, but not androgens. Estriol is solely produced by the placenta, whereas estrone and estradiol are produced by the mother, fetus, and placenta, all of which contribute to maternal serum concentrations of estrogens.[3] The observed positive association between exposure to $PM_{2.5}$ and estriol but not the other estrogens may therefore be due to the differing sources of estrogen production. Due to the paucity of published studies in this research area, the findings of this study should be supplemented by further experimental and observational research.

This study had several strengths. First, we utilized a longitudinal cohort design that measured both estrogens and androgens at three time points in pregnancy. Second, sensitive and specific standard assays were used to measure sex steroid concentrations, which is particularly important for hormones present in low concentrations in pregnant people, such as total and free testosterone. Last, the DLNM framework allowed for the assessment of specific gestational windows when ambient $PM_{2.5}$ and NO_2 exposure may be associated with sex steroid levels.

However, this study also had limitations that should be considered. There may have been misclassification of $PM_{2.5}$ and NO_2 given that daily concentrations were estimated in 1 square-kilometer grid in the study area, and all individuals within a grid used the same daily concentrations no matter where they lived in that 1 square-kilometer grid. However, we believe this misclassification to be minimal given the small area specified and the reduced bias of exposure estimated from spatiotemporal modeling compared with exposure estimated from central monitoring centers. Indoor air quality is not accounted for by this method and may differ by household. Additional exposure misclassification could have arisen from individuals changing addresses during the gestational period. We believe this exposure misclassification would be nondifferential and would lead to a bias toward the null hypothesis. Blood serum samples were collected at varying time points during each trimester of pregnancy, so hormone values were imputed to standardize gestational week for analyses. This approach may have induced outcome misclassification, resulting in reduced statistical power.

The use of *P*-values to determine susceptible exposure windows is not optimal due to a reliance on post hoc analysis and multiple comparisons due to examining many weeks of gestation. Limited a priori information was available to allow us to test specific gestational weeks in relation to sex steroids in pregnancy. Although estimated associations across gestational weeks are not tested independently, due to the smooth nature of the curve fit from the DLNM, the resulting model *P*-values do not fully control type I errors. The use of *P* values to determine susceptible weeks from DLNMs is current practice when a priori information is limited.[40–42] Nonetheless, our results should be considered exploratory. This study did not include lifestyle factors, such as diet and physical activity, that may be predictive of sex steroid concentrations.[43–47] However, lifestyle factors are unlikely to be important predictors of NO_2 and

$PM_{2.5}$ concentrations within the 1 square-kilometer grid, thus we believe that lifestyle factors would not induce any meaningful residual confounding. This study also did not consider time-dependent covariates in the analysis. We believe residual confounding by most time-variable covariates, such as weight gain throughout pregnancy, to be highly correlated with time-fixed variables, such as prepregnancy BMI, thereby minimizing any potential confounding. Further, the ambient temperature was not accounted for in this study, although we did adjust for the season of birth. If in fact temperature is thought to be a significant predictor of sex steroid hormones, although Colicino et al[22] found no association between ambient temperature and androgens and estrogens, there may be residual confounding by time-dependent temperature. The sample size of this study was relatively small (N = 299) and subjects lived in a geographic region with relatively low air pollution concentrations, resulting in reduced statistical power to detect associations with sex steroid hormones.

Conclusions

In this cohort of pregnant women sampled from Rochester, NY, NO_2 exposures throughout pregnancy, most often including weeks 0–15 and weeks 22–30, were associated with disruption of sex steroid hormones during early-, mid-, and late-pregnancy. Few associations with $PM_{2.5}$ were observed. Results from distributed lag models suggested that NO_2 concentrations may be associated with varying directions of association at different weeks of NO_2 exposure with positive associations during the peri-conception period followed by negative associations in later weeks of early pregnancy, suggesting there may be a possible compensatory mechanism that should be further explored in future studies.

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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