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Identifying Sensitive Windows of Exposure to NO_2 and Fetal Growth Trajectories in a Spanish Birth Cohort

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Background: We previously identified associations between trimester-specific NO_2 exposures and reduced fetal growth in the Spanish INfancia y Medio Ambiente (INMA) project. Here, we use temporally refined exposure estimates to explore the impact of narrow (weekly) windows of exposure on fetal growth.

Methods: We included 1,685 women from INMA with serial ultrasounds at 12, 20, and 34 gestational weeks. We measured biparietal diameter (BPD), femur length, and abdominal circumference (AC) and from them calculated estimated fetal weight (EFW). We calculated z-scores describing trajectories of each parameter during early (0–12 weeks), mid (12–20 weeks), and late (20–34 weeks) pregnancy, based on longitudinal growth curves from mixed-effects models. We estimated weekly NO_2 exposures at each woman's residence using land-use regression models. We applied distributed lag non-linear models to identify sensitive windows of exposure. We present effect estimates as the percentage change in fetal growth per 10 μg/m^3 increase in NO_2 exposure, and we calculated cumulative effect estimates by aggregating estimates across adjacent lags.

Results: We identified weeks 5–12 as a sensitive window for NO_2 exposure on late EFW (cumulative $\beta = -3.0\%$; 95% CI = −4.1%, −1.9%). We identified weeks 6–19 as a sensitive window for late growth in BPD (cumulative $\beta = -2.0\%$; 95% CI = −2.7%, −1.4%) and weeks 8–13 for AC (cumulative $\beta = -0.68\%$; 95% CI = −0.97%, −0.40%). We found suggestive evidence that third trimester NO_2 exposure is associated with increased AC, BPD, and EFW growth in late pregnancy.

Conclusions: Our findings are consistent with the hypothesis that NO_2 exposure is associated with alterations in growth of EFW, BPD, and AC dependent on the specific timing of exposure during gestation.

Keywords: Air pollution; Nitrogen dioxide; Fetal growth; Ultrasonography; Sensitive window; Distributed lag non-linear models

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Air pollution, including traffic-related pollutants such as nitrogen dioxide (NO_2), is ubiquitous and poses a substantial threat to public health, particularly for pregnant women and children who are especially vulnerable.[1] Overall, the epidemiologic literature supports associations between increased

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The authors report no conflicts of interest.

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air pollution exposures and adverse outcomes related to fetal growth such as low birth weight (i.e., birth weight less than 2,500 grams) and small-for-gestational-age (SGA; i.e., birth weight below the 10th percentile for gestational age).[2,3] Although fetal growth is a dynamic process, it is often assessed as a static phenotype based on readily available anthropometric measures (e.g., birth weight and gestational age). However, many studies have assessed the association between air pollution exposures and birth anthropometry, these measures do not adequately capture growth during different gestational periods and cannot inform sensitive windows of exposure to environmental contaminants during pregnancy.[4]

Given that exposure to air pollution may disrupt critical events during fetal development, identifying sensitive windows of exposure may inform biologic mechanisms.[4,5] However, despite observations of more frequent negative associations between exposures to air pollutants and birth anthropometry during the first and third trimesters,[6,7] the identification of sensitive windows of exposure for air pollution on fetal growth remains equivocal,[7,8] perhaps limited by the constraints of both the definitions of fetal growth and windows of exposure (usually trimesters) interrogated in existing studies. Also, because the maturation of individual fetal body segments may be selectively affected by the timing or intensity of exposure, associations between exposure and fetal growth may be missed by defining outcomes by attained growth (i.e., birth weight). Further, delays in specific fetal growth parameters may have specific health consequences[9]; for example, in one study, slowed growth in fetal head circumference at 14 gestational weeks was associated with reasoning ability in early childhood.[10] Thus, a more clear assessment of windows of vulnerability for specific body segments may also inform the causal pathway between air pollution exposure on subsequent childhood health outcomes.

A systematic review and meta-analysis published in 2019 that included original articles published through July 2017 identified only seven studies that utilized fetal biometry in their outcome measures and not all of the studies measured growth.[2] While this meta-analysis provided suggestive evidence of associations between traffic-related air pollutants, including NO_2, and changes in fetal parameters, they ultimately concluded that there were too few studies to adequately assess critical windows of exposure to air pollution on fetal growth.[2] Moreover, all studies included in that review assessed exposure aggregated by trimester or by periods approximating trimesters (e.g., early, mid-, and late-pregnancy). Although six additional studies[11–16] have been published since July 2017 (the date before which studies included in the Fu et al.[2] review was published), only three to our knowledge have since examined NO_2 exposure.[14–16] While these three studies each provide further evidence of associations between NO_2 exposures and altered fetal growth, they have also relied on relatively large exposure windows (e.g., trimesters) hindering their ability to evaluate sensitive windows of exposure.

Our group previously identified associations between averaged maternal NO_2 exposures in the first trimester and reduced fetal growth in the Spanish INfancia y Medio Ambiente (INMA, Childhood and Environment) project.[17] Based on gaps identified in the literature, the goal of the current analysis was to build upon our previous study and reanalyze these data utilizing weekly NO_2 exposure estimates and apply distributed lag nonlinear models (DLNMs)[18,19] to identify more precise windows in which fetal growth trajectories may be particularly sensitive to NO_2 exposures.

METHODS

This study was conducted among women recruited as part of the INMA project, a population-based, prospective birth cohort study.[20] The present analysis includes data for pregnant women from several Spanish regions: Valencia, Sabadell, and Gipuzkoa who were recruited from November 2003 to February 2008. The study was approved by the Ethics Committee at the reference hospitals, and all women gave written informed consent before enrollment. Serial ultrasound scans were conducted by specialized obstetricians at 12, 20, and 34 weeks of gestation for participants as part of this study. Many women additionally underwent additional ultrasounds as part of their routine prenatal care and, thus, data were available for additional ultrasounds for many women. We recorded measurements (mm) of biparietal diameter (BPD), femur length (FL), and abdominal circumference (AC) from each ultrasound. We also calculated estimated fetal weight (EFW, g), derived according to Hadlock et al.,[21] at each time point. We estimated gestational age based on the last menstrual period unless it differed from gestational age based on the first ultrasound by ≥7 days, in which case we used early ultrasound data on crown-rump length.

As previously described,[17] we applied mixed-effects models to fetal biometry data from each INMA region separately to obtain longitudinal growth curves for each fetal size parameter (i.e., BPD, AC, FL, EFW). These mixed-effects models were adjusted for the following constitutional factors known to affect fetal growth: maternal age, pre-pregnancy weight, height, parity, country of birth, paternal height, and fetal sex. Additionally, gestational age was included in the models as a nonlinear random effect using the best fitting second-degree transformation function of gestational age from a set of fractional polynomials as in Royston[22]; see Iñiguez et al.[17] for further details. These fetal growth curves provide predictions of fetal size in each body segment at weeks 12, 20, and 34 weeks that we used to calculate conditional z-scores describing the growth trajectory of the fetus during each time interval (i.e., up to week 12, from gestational week 12 to week 20, and from gestational week 20 to week 34). We calculated conditional z-scores as the conditional expectation of the size of the fetus at a given time point given the size of the fetus at the previous time point. For example, we calculated the conditional z-score for BPD at 20 weeks by conditioning the size

of BPD at 20 weeks on the size of BPD at 12 weeks; this z-score thus represents the growth trajectory of BPD from 12 to 20 weeks. The use of conditional z-scores allows for a comparison among fetuses with comparable patterns of earlier growth. The focus of our investigation was on the unconditional z-scores at 12 weeks (which by default represent fetal growth during the first 12 weeks of gestation) and the conditional z-scores at 20 and 34 weeks that allowed us to explore the impact of NO_2 exposures on altered fetal growth during early, mid-, and late-pregnancy, respectively.

We briefly describe details of the exposure assessment that have been previously published:[23,24] within each cohort, 7-day sampling campaigns during the years 2004–2007 were undertaken using passive samplers located across each study area considering exposure gradients, population density, and distribution of women's residences. We conducted two sampling campaigns in Gipuzkoa between February and June 2007, four campaigns in Sabadell between April 2005 and March 2006, and four campaigns in Valencia between April 2004 and February 2005. We combined measured annual mean NO_2 concentrations from these sampling campaigns with land-use, traffic, and altitude variables in region-specific land-use regression models to produce a spatial surface from which we estimated annual mean residential NO_2 exposures for each woman, considering residential mobility. Next, we computed daily NO_2 exposure estimates by temporally adjusting the annual average (spatial) concentrations of NO_2 based on daily records from stationary ambient monitoring networks that operated continuously in each study area during the study period. We temporally adjusted by multiplying the land-use-regression-based annual average NO_2 estimate by the ratio of the daily NO_2 estimate from the stationary monitoring site to the annual average NO_2 estimate from the stationary monitoring site. We then averaged predicted daily NO_2 concentrations at each woman's residence for each week of each woman's pregnancy period.

Statistical Analysis

We applied DLNMs using the r package 'dlnm'[19] in version 4.0.2 (R Core Team 2020). The DLNM framework simultaneously models the exposure- and lag-response relationships using a cross-basis, a bidimensional space of functions obtained from integrating two basis functions: one over the range of exposure that represents the exposure (NO_2)-response relationship and the other over the lag dimension that represents the change of this exposure-response relationship along lags (i.e., gestational weeks). In our models, we assumed a linear exposure (NO_2)-response varying smoothly across gestational weeks; thus, we used a linear term as the basis for the dose-response relationship and a natural cubic spline as the basis for the lag-response relationship to assess nonlinear associations between the week and the weekly NO_2 exposure with each fetal growth parameter. The number of exposure weeks corresponded to one less than the gestational week of the growth trajectory (e.g., we estimated associations up to 11 exposure weeks in analyses of 12-week growth trajectories and up to 19 exposure weeks in analyses of 20-week growth trajectories). We adjusted models for maternal age (years), pre-pregnancy body mass index (BMI; underweight, normal weight, overweight, obese), parity (0, 1, or ≥2 previous pregnancies), cohabitation (living with father vs. not), social class (based on occupation), alcohol use during pregnancy (at least one drink/week vs. fewer than one drink/per week), smoking during pregnancy (yes/no), maternal and paternal education (primary, secondary, or university), and urbanicity of residence (urban, semi-urban, or rural), which were informed by a directed acyclic graph and previous literature. Models were applied separately to each cohort and overall effect estimates were obtained using random-effects meta-analyses[25] using the r package 'mixmeta'. Our sample included 1,685 women with complete exposure, covariate, and outcome data.

The cohort-specific model for each fetal growth parameter is given by:

$$Y_j = \boldsymbol{W}_j \boldsymbol{\eta}_j + \boldsymbol{Z}_j \boldsymbol{\gamma}_j + \epsilon,$$

Where Y_j represents the z-score vector of a fetal growth parameter $(\mathrm{EFW, BPD, AC, or FL})$ during a gestational time period (early-, mid-, or late-pregnancy, ending at $t = 12, 20,$ or 34 weeks, respectively) for the cohort $j = 1,2,\text{or}\,3$ (i.e., j represents Sabadell, Gipuzkoa, or Valencia); $\boldsymbol{W}_j = \boldsymbol{Q}_j \boldsymbol{R}$ is the cross-basis for NO_2 exposure from gestational week 1 to week t-1, where $\boldsymbol{Q}_j = \left[X^{t-\ell}\right]_\ell, \ell = 1,\ldots,t-1$, is the matrix whose (t-1) columns are the vectors of lagged exposure to NO_2 at cohort j, with ℓ representing the lag, and $\boldsymbol{R} = \left[b^k\right]_k, k = 1,\ldots,\nu,$ is the matrix whose ν columns are the basis vectors of the spline over the lag dimension; $\boldsymbol{\eta}_j$ are the associated effects (to estimate) along the cross-basis surface for cohort j, from which the associated effects of each lag, $\boldsymbol{\beta}_j = \left(\beta_1,\ldots,\beta_{t-1}\right)'$, will be obtained by means of: $\beta_j = R\eta_j$. Additionally, $\boldsymbol{Z}_j$ represents the covariates and $\boldsymbol{\gamma}_j$ are their associated effects. The pooled model for each fetal growth parameter is obtained by means of a random effects meta-analysis of $\widehat{\boldsymbol{\eta}_j}$, i.e., by assuming a cohort specific deviation from the overall distribution, $\mathbf{v}_j$, $\boldsymbol{v}_j \sim \boldsymbol{N}_\nu(0, \boldsymbol{\psi})$, such as $\hat{\boldsymbol{\eta}}_j \mid \boldsymbol{v}_j \sim \boldsymbol{N}_\nu\left(\boldsymbol{\eta} + \boldsymbol{v}_j, \boldsymbol{S}_j\right)$, where $\boldsymbol{\eta}$ can be interpreted as the population-average coefficients along the cross-basis; Ψ is the unknown between-study covariance matrix and $\boldsymbol{S}_j$ is the intra-study covariance matrix for cohort *j*.

Because there is no *a priori* knowledge regarding ideal knot placement in the cross-basis matrix for studies of air pollution and fetal growth, we selected the number of knots and their placement in the meta-analyzed model by evaluating the fit of multiple models. For each fetal growth outcome, models were constructed with varying numbers of knots, ranging from at least 1 to a maximum of either 4 or one-quarter the number of exposure weeks (whichever was larger). We allowed the flexible placement of knots such that no two

knots were placed within 3 weeks of one another. The model with the lowest Akaike Information Criterion (AIC) was then selected as the final model.[18] Effect estimates are presented as the percentage change in fetal growth per 10 μg/m^3 increase in NO_2 exposure. The cumulative effect estimates for windows of interest were calculated by aggregating effect estimates across adjacent lags.

RESULTS

Overall demographics of our study population are shown in the Table. Women in the study were, on average, 30.4 years of age (standard deviation [sd] = 4.3 years). Just over one-third (34.7%) of the women had a university education and almost all women (98.6%) reported living with the father of their child. Most women were also classified as in the middle or upper social class (58.9%). While nearly one-third of women reported smoking during pregnancy (32.8%), only 9.3% of women reported drinking one or more alcoholic drinks per week during their pregnancy. The majority (76.4%) of women in this study lived in urban areas. F1,F2

TABLE. Characteristics of 1,685 INMA participants from Valencia, Sabadell, and Gipuzkoa, 2003–2008.

Maternal age (years), mean (SD)	**30.4 (4.3)**
Maternal educational level, %	
Up to primary	25
Secondary	40
University	35
Paternal educational level, %	
Up to primary	37
Secondary	44
University	20
Social class, %	
Low	41
Middle	26
High	33
Cohabitation, %	
Living with father	99
Not living with father	1
Pre-pregnancy body mass index, %	
<18.5 (underweight)	5
18.5–24.9 (normal weight)	70
25.0–29.9 (overweight)	18
≥30.0 (obese)	8
Parity, %	
0	56
1	38
≥2	7
Smoking during pregnancy, %	
No	67
Yes	33
Alcohol use during pregnancy, %	
None	91
≥1 drink per week	9
Urbanicity of residence during 1st trimester, %	
Urban	76
Semi-urban	18
Rural	5

We observed little evidence of sensitive windows of exposure to NO_2 on delayed fetal growth in early or mid-pregnancy (Figures 1 and 2, respectively). We observed suggestive evidence that NO_2 exposure during the first trimester may delay fetal growth late in pregnancy (Figure 3) including a sensitive window of exposure to NO_2 during weeks 5–12 on EFW (cumulative β = −2.98%, 95% confidence interval [CI] = −4.09%, −1.87% per 10 μg/m^3 increase in NO_2). We also identified gestational weeks 6–19 as a sensitive window of exposure to NO_2 for late pregnancy growth in BPD (cumulative β = −2.01%, 95% CI = −2.67%, −1.35% per 10 μg/m^3 increase in NO_2). Last, gestational weeks 8–13 was identified as a sensitive window of exposure to NO_2 on late pregnancy AC growth (Figure 3), though the magnitude of the effect was smaller than observed for EFW or BPD (cumulative β = −0.68%, 95% CI = −0.97%, −0.40% per 10 μg/m^3 increase in NO_2).

In addition, we identified positive associations between exposure to NO_2 during weeks in the second and third trimesters and increased AC growth during both mid- and late-pregnancy (Figures 2 and 3, respectively). Specifically, we found that NO_2 exposure during weeks 13–19 was associated with mid-pregnancy AC growth (cumulative β = 1.99%; 95% CI = 0.84%, 3.13% per 10 μg/m^3 increase in NO_2) while NO_2 exposure during gestational weeks 22–33 was associated with increased AC growth during late pregnancy (cumulative β = 2.10%; 95% CI = 0.97%, 3.24% per 10 μg/m^3 increase in NO_2). We found similar positive associations between NO_2 exposure during weeks 24–33 and late pregnancy BPD growth (Figure 3: cumulative β = 1.38%; 95% CI = 0.27%, 2.49% per 10 μg/m^3 increase in NO_2) and during weeks 20–33 with late pregnancy growth in EFW (Figure 3: cumulative β = 5.76%; 95% CI = 2.28%, 9.24% per 10 μg/m^3 increase in NO_2).

DISCUSSION

We applied distributed lag nonlinear models to investigate weekly critical windows of exposure of NO_2 on fetal growth trajectories in an established Spanish birth cohort. Our study provides evidence that NO_2 exposures during pregnancy are differentially associated with the growth of specific fetal body segments dependent on the timing of exposure. Overall, we identified gestational periods spanning the first and second trimesters when delayed growth in EFW, BPD, and AC in late pregnancy may be especially sensitive to the adverse effects of maternal NO_2 exposure. We also observed positive associations between NO_2 exposures in the second and third trimesters and increased fetal growth in mid- and late-pregnancy. We did not observe evidence of associations between NO_2 exposure and delayed fetal growth in either early (i.e., up to 12 weeks) or mid- (between 12 and 20 weeks) pregnancy.

Previous studies have implicated air pollution exposures in adverse pregnancy outcomes,[2,3] though many have relied

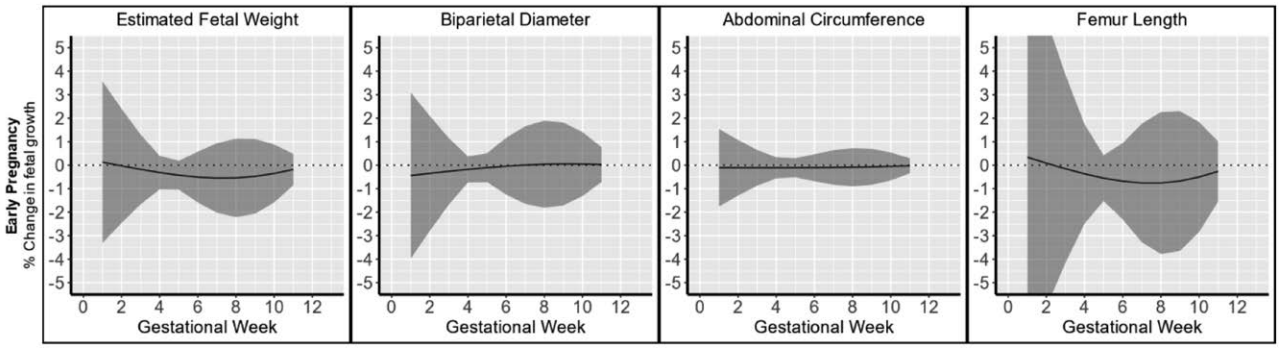

FIGURE 1. Associations between weekly nitrogen dioxide (NO_2) exposure (per 10 μg/m^3) and percentage change in fetal growth of estimated fetal weight, biparietal diameter, abdominal circumference, and femur length during early pregnancy (i.e., first 12 weeks of gestation). The x-axis represents gestational week, and the y-axis represents the percentage change in fetal growth. The solid lines represent the estimated values from the fitted distributed lag nonlinear models and shaded areas represent 95% confidence intervals around the estimate for each lag (i.e., gestational week); the dotted horizontal line represents the null.

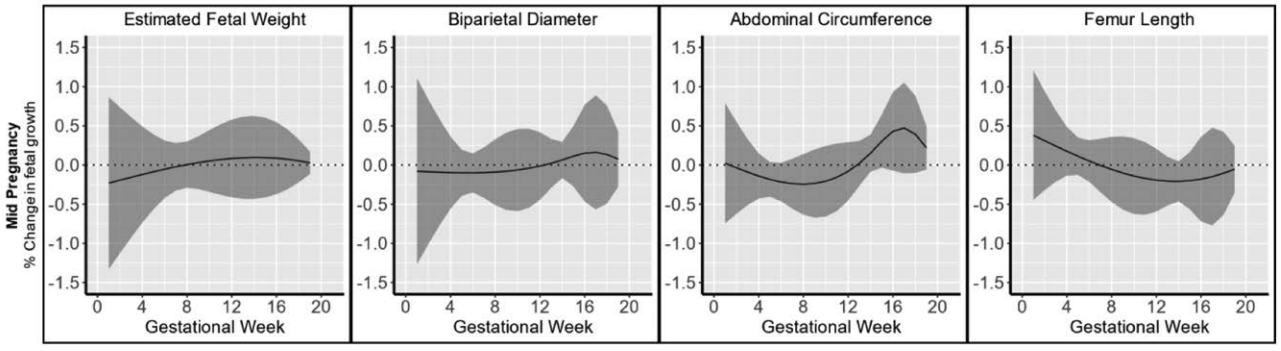

FIGURE 2. Associations between weekly nitrogen dioxide (NO_2) exposure (per 10 μg/m^3) and percentage change in fetal growth of estimated fetal weight, biparietal diameter, abdominal circumference, and femur length during mid pregnancy (i.e., 12–20 weeks of gestation). The x-axis represents gestational week, and the y-axis represents the percentage change in fetal growth. The solid lines represent the estimated values from the fitted distributed lag non-linear models and shaded areas represent 95% confidence intervals around the estimate for each lag (i.e., gestational week); the dotted horizontal line represents the null.

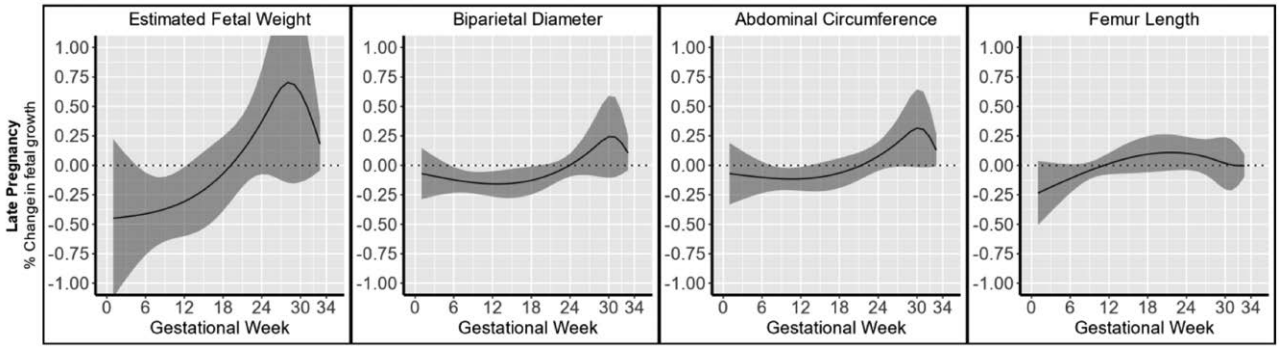

FIGURE 3. Associations between weekly nitrogen dioxide (NO_2) exposure (per 10 μg/m^3) and percentage change in fetal growth of estimated fetal weight, biparietal diameter, abdominal circumference, and femur length during late pregnancy (i.e., 20–34 weeks of gestation). The x-axis represents gestational week, and the y-axis represents the percentage change in fetal growth. The solid lines represent the estimated values from the fitted distributed lag non-linear models and shaded areas represent 95% confidence intervals around the estimate for each lag (i.e., gestational week); the dotted horizontal line represents the null.

on anthropometric measures at birth even though these measures represent the culmination of fetal growth and not growth itself. On the other hand, the use of longitudinal ultrasound measures to define fetal growth in air pollution epidemiology studies supports the understanding of biologic mechanisms driving observed associations and aids in the identification of sensitive windows of exposure.[4] Yet, to date, only a handful of studies have utilized longitudinal fetal biometry to assess associations between maternal NO_2 exposures and fetal growth. Common among these studies are assessments that examine exposures averaged across multiple weeks, months, or trimesters. Wang et al.[16] reported associations between NO_2 exposure aggregated across the first 22 weeks of pregnancy with changes in BPD, AC, and FL from the second and third trimesters. A study of Korean women[15] also revealed reductions in fetal head size and length between the second and third trimesters associated with aggregated NO_2 exposure across the entire pregnancy period. In contrast, a recent article by Shao et al.[14] provided little evidence of an association between trimester-specific NO_2 exposures and fetal growth. While there are several hypothesized mechanisms by which air pollution exposures could influence delayed fetal growth, including oxidative stress and DNA damage,[4] placental insufficiency has also been implicated. It is also possible that early placental insults may lead to delayed effects. For example, Griffin, et al.[26] found changes in umbilical blood flow in the third trimester following maternal infections that occurred before 20 weeks of gestation.

Though most studies of air pollution and fetal growth focus on delayed growth, abnormal fetal growth also includes increased growth, which may lead to macrosomia or large-for-gestational-age due to unbalanced or accelerated growth, which can result in long-term health consequences for the infant.[27,28] Similar to our findings, others have also reported positive associations between prenatal air pollution exposures and increased growth. A Dutch study applied generalized additive models to standard deviation scores calculated from the longitudinally measured fetal size in second and third-trimester ultrasounds; this study revealed evidence of a positive association between the highest quartile of NO_2 exposure during pregnancy and growth in mid-to late-EFW.[29] Similarly, Lamicchane et al. observed evidence of increased fetal head size and length during the last weeks of pregnancy associated with NO_2 exposure aggregated across the pregnancy period.[15] In a large study of Chinese women, investigators reported that high PM10 exposures were associated with over-growth (z-score classified >97thg centile) of fetal BPD.[12] Positive associations have also been reported for increased air pollution exposures and macrosomia.[30–32] While it is possible that these observations are due to chance, air pollution exposures may mediate increased fetal growth (and birthweight) through increased leptin and adiponectin concentrations in late pregnancy. For example, traffic-related air pollution exposures have been shown to influence maternal and cord blood concentrations of leptin and adiponectin,[33,34] and these two adipokines have also been associated with fetal and infant growth-related outcomes.[35,36]

Within a large prospective pregnancy cohort, we were able to construct longitudinal growth curves using multiple ultrasounds per woman to calculate fetal growth trajectories whereas many existing studies utilized fetal biometry data from only two-time points to characterize fetal growth. The calculation of fetal growth z-scores based on multiple observations of size reduces the random error associated with modeling growth. Additionally, we had spatially and temporally resolved exposure estimates for each study participant from land-use-regression models, which allowed us to estimate weekly NO_2 exposures and account for residential mobility. Nonetheless, our exposure and outcome variables may have been estimated with some error which could have introduced uncertainty in the distributed lag nonlinear models. Moreover, we anticipate that the direction of the errors in the land-use-regression models would be nondifferential because these errors are expected to be random. As demonstrated by Ander Wilson et al.,[37] there is a large potential for bias when estimating critical windows of exposure to air pollution in children's environmental health, particularly when evaluating effects of exposure during a given window without controlling for exposure during other windows. Wilson's simulation studies demonstrated that this bias could be eliminated using distributed lag models and, given the ability of these models to evaluate critical windows that do not align with clinically defined pregnancy trimesters, they recommended this model when temporally refined exposure estimates, as in our study, are available.[37] Another advantage of distributed lag models is the structure of the cross-basis that allows for the simultaneous evaluation of the lag–exposure–outcome relationship, thus eliminating the issue of multiple comparisons.[38,39] The model flexibility further allows us to summarize effects over specific time windows for all exposure levels or summarize across specific exposure levels for specific lags of interest.[38,39]

Our study provides evidence consistent with the hypothesized vulnerability of the fetus to the effects of NO_2 exposure and highlights specific periods of heightened vulnerability of the fetus, particularly during early-to-mid pregnancy. Our findings further highlight a scenario in which traditional exposure metrics (i.e., those aggregated to trimesters) may not align with periods of fetal vulnerability and the potential differential impacts of exposure on growth in specific body segments. Our study also adds to a growing body of suggestive evidence of a positive association between air pollution exposures during late pregnancy and accelerated fetal growth. In particular, these associations should be carefully explored to rule out spurious findings. Future work should continue to seek opportunities to apply novel methods to better understand mechanisms underlying adverse health effects of

prenatal air pollution exposure with the goal of improving children's environmental health.

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