Prenatal Nitrate Exposure and Childhood Asthma

Influence of Maternal Prenatal Stress and Fetal Sex

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Abstract

Rationale: Impact of ambient pollution upon children's asthma may differ by sex, and exposure dose and timing. Psychosocial stress can also modify pollutant effects. These associations have not been examined for *in utero* ambient nitrate exposure.

Objectives: We implemented Bayesian-distributed lag interaction models to identify sensitive prenatal windows for the influence of nitrate (NO_3^-) on child asthma, accounting for effect modification by sex and stress.

Methods: Analyses included 752 mother-child dyads. Daily ambient NO_3^- exposure during pregnancy was derived using a hybrid chemical transport (Geos-Chem)/land-use regression model and natural log transformed. Prenatal maternal stress was indexed by a negative life events score (high [>2] vs. low [≤ 2]). The outcome was clinician-diagnosed asthma by age 6 years.

Measurements and Main Results: Most mothers were Hispanic (54%) or black (29%), had a high school education or less (66%), never smoked (80%), and reported low prenatal stress (58%); 15% of children developed asthma. BDILMs adjusted for maternal age, race, education, prepregnancy obesity, atopy, and smoking status identified two sensitive windows (7–19 and 33–40 wk gestation), during which increased NO_3^- was associated with greater odds of asthma, specifically among boys born to mothers reporting high prenatal stress. Cumulative effects of NO_3^- across pregnancy were also significant in this subgroup (odds ratio = 2.64, 95% confidence interval = 1.27–5.39; per interquartile range increase in $\ln \text{NO}_3^-$).

Conclusions: Prenatal NO_3^- exposure during distinct sensitive windows was associated with incident asthma in boys concurrently exposed to high prenatal stress.

Keywords: nitrate; air pollution; sensitive window; prenatal; asthma

Evidence links prenatal ambient air pollution and childhood respiratory outcomes (1, 2). Epidemiologic studies have largely considered associations between

prenatal exposures to particulate matter (PM) air pollution with an aerodynamic diameter of 2.5 μm or less ($\text{PM}_{2.5}$) and childhood wheeze (3, 4), asthma (5–7), and

lung function (8, 9). The knowledge that effects of PM can vary among its specific components, and that spatial and temporal variations in chemical composition of

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At a Glance Commentary

Scientific Knowledge on the

Subject: Adverse effects of ambient particulate air pollution upon children's respiratory health, such as the development of asthma, begin *in utero*. Because the development of fetal lung structure and function occurs in stages, these effects may be dependent on timing of exposure, as well as differ by the specific composition of particulate pollution. These effects may also be sex specific and/or influenced by maternal stress. These complex interactions remain poorly understood in human studies.

What This Study Adds to the

Field: This is the first prospective study to demonstrate synergistic associations among prenatal ambient nitrate (NO_3^-) exposure, fetal sex, and maternal stress on the development of childhood asthma. Leveraging weekly gestational NO_3^- exposure estimates, combined with novel, data-driven Bayesian statistical methods, we identified two sensitive windows (7–19 wk and 33–40 wk gestation) during which increasing NO_3^- pollution was associated with asthma development by age 6 years, specifically in boys concurrently exposed to higher maternal prenatal stress. A more definitive understanding of the timing of such sensitive windows to toxins and interactions with other maternal and fetal factors is not only essential to characterize the mechanisms underlying the development of asthma, but allows for the identification of subgroups most vulnerable to pollution.

particulate pollutants may contribute to differential health outcomes, highlights the need to consider specific components of ambient particulate pollution (10–13).

Ambient nitrates (NO_3^-) are a significant component of airborne particulate pollution, and originate from the atmospheric oxidation of nitrogen oxides (NO_x ; nitrogen dioxide [NO_2] and nitrous oxide [NO]) gas emissions generated mainly from combusive stationary (e.g., power plants) as well as

mobile sources (e.g., motor vehicle traffic) (14). Although early-life exposure to ambient gases, such as NO_x , have been associated with increased asthma prevalence (15–17), there are few data on the respiratory effects of their particulate products, which can travel long distances once formed and contribute to air quality in wider geographic distributions than their gaseous precursors.

Because fetal development occurs through a complex orchestration of sequential biologic events, toxins that disrupt these processes can have variable impact, depending on the nature of the pollutant, as well as the timing and/or dose of exposure (18, 19). Despite this understanding, most published studies consider exposures averaged over the entire pregnancy or within clinically defined trimesters (6, 7, 20–22), which can lead to missed or biased findings (23). These studies provide little insight into sensitive windows that may or may not coincide with stages of lung growth and other timed *in utero* processes involved in respiratory system development. A few studies have used traditional constrained distributed lag models (DLMs) to estimate associations between maternal exposure to $\text{PM}_{2.5}$ and children's health outcomes (24–28), including asthma (5), and to begin to better identify sensitive windows. For example, our group previously reported that higher prenatal $\text{PM}_{2.5}$ exposure specifically during Weeks 16–25 of gestation was associated with increased risk of asthma in boys (5).

In addition to air pollution, many studies link prenatal stress with increased childhood asthma risk (29, 30). Others show that higher prenatal stress modifies the impact of ambient pollutants, including PM and its black carbon component, on repeated wheeze in children (31). Islam and colleagues (32) demonstrated that associations between NO_x over childhood and lung function among school-aged children were stronger in high-stress households compared with lower-stress households. Furthermore, human and animal data show that psychosocial stress and chemical toxicants can elicit sex-specific effects on offspring outcomes (33, 34). For example, in sex-stratified analyses, we recently showed that prenatal $\text{PM}_{2.5}$ exposure between 16 and 25 weeks gestation was associated with asthma in boys, but not girls, suggesting that the male fetus may be more vulnerable to inhaled

toxic insults *in utero* (5). Whether similar sex-specific sensitive windows exist for the effects of other pollutants, such as nitrate, remains unknown. Moreover, no study has examined the interaction between prenatal maternal stress and nitrate exposures in relation to childhood asthma development.

We leveraged daily prenatal NO_3^- exposure data and extended the traditional constrained DLM to identify sensitive windows in relation to childhood asthma onset by age 6 years, accounting for modifying effects of prenatal stress and fetal sex using a novel Bayesian-distributed lag interaction modeling (BDLIM) approach. Results were in part reported in abstract form (35).

Methods

Participants

Participants were from the Asthma Coalition on Community, Environment, and Social Stress (ACCESS) project, a pregnancy cohort designed to examine the effects of perinatal exposure to physical toxins and psychosocial stress on childhood respiratory health (36). In brief, English- or Spanish-speaking pregnant women (≥ 18 yr old) receiving care at Brigham and Women's Hospital (Boston, MA), Boston Medical Center (Boston, MA), and affiliated community health centers were enrolled at 28.4 (± 7.9) weeks gestation between August 2002 and July 2009. Among pregnant women approached who were eligible, 989 (78.1%) agreed to enroll; 955 gave birth to a singleton live-born infant and continued follow-up. Based on screening data, mothers who declined versus enrolled were slightly less likely to be ethnic minorities (78.9 and 81.5% Hispanic or African American, respectively) or to have a high school education or less (57.7 vs. 60.6%, respectively), and slightly more likely to report an income level less than \$20,000 annually (37.7 vs. 35.2%, respectively); there were no significant differences between groups on these covariates. Procedures were approved by human studies committees at Brigham and Women's Hospital and Boston Medical Center; written consent was obtained in the subject's primary language.

Ambient Nitrate Exposure

We used a validated hybrid model of a chemical transport model (GEOS-Chem) and land-use regression term, and calibrated

it with ground monitoring data to make predictions at 1 km × 1 km grid cells of daily ambient nitrate particle exposure during pregnancy based on mother's residence during pregnancy (i.e., at enrollment and updated if they moved) (37). Weekly averages of NO₃⁻ were used in the analysis to estimate exposure to reduce potential noise caused by day-to-day variation.

Maternal Prenatal Stress

Prenatal maternal stress was ascertained within 2 weeks of enrollment using the Crisis in Family Systems-Revised (CRISYS-R) survey to assess life events across 11 domains (e.g., financial, relationships, violence, housing, discrimination, and prejudice) (38). Mothers rated their relevant experiences over the past 6 months as positive, negative, or neutral. Continuous negative life event (NLE) scores were determined by summing the number of domains with one or more negative events with higher scores indicating greater stress. Participants in this study reported experiencing events in none to a maximum of nine domains. Low (NLE scores ≤ 2) and high stress (NLE scores > 2) groups were based on the

median split. This approach has been validated as a measure of chronic stress that, in turn, is related to disruption of underlying stress-response systems in pregnant women (immune, autonomic, neuroendocrine, and oxidative stress) that can enhance vulnerability to other toxins (e.g., pollutants) (39, 40). In addition, we have shown a significant main effect of increased prenatal NLE scores using this measure on asthma development in these children (29).

Asthma Outcome

Maternal-reported clinician-diagnosed asthma was ascertained from birth up to age 6 years through telephone and face-to-face interviews at approximately 3-month intervals for the first 24 months, then annually thereafter. Mothers were asked, "Has a doctor or nurse ever said that your child had asthma?" Most of these children were given a diagnosis of asthma after age 3 years (78.6%) (29).

Other Covariates

Mothers reported race/ethnicity, educational status, age at enrollment, prepregnancy height and weight, and child's sex. Child's

gestational age, date of birth, and birth weight were collected from the medical record. Women's prepregnancy body mass index (kg/m²) was calculated from height and prepregnancy weight reported at enrollment, with obesity defined as a body mass index of 30 kg/m² or greater. An internal validation study comparing self-report and measured height and weight showed good agreement across all levels of height and weight (41). Prenatal smoking status was determined by response to questions regarding smoking either at enrollment or in the third trimester; postnatal smoking was based on postpartum interviews asking whether the mother or others in the home smoked. Mothers were considered atopic if they self-reported ever having asthma, hay fever, or eczema. Length of gestation was calculated using last menstrual period, and sex-specific birth weight for gestational age z scores (BWGA) were derived (42).

Statistical Analysis

Research links prenatal air pollution to preterm birth (43) and, in turn, prematurity predicts childhood asthma (44). Thus, we restricted the sample to infants born at

Table 1. Participant Characteristics (n = 752)

	All Children (n = 752)	Boys (n = 382)	Girls (n = 370)	P Value*
Ever had asthma up to 6 yr old, n, %				
No	639 (85.0)	312 (81.7)	327 (88.4)	0.01
Yes	113 (15.0)	70 (18.3)	43 (11.6)	
Race/ethnicity, n, %				
Black	220 (29.3)	119 (31.2)	101 (27.3)	0.32
Hispanic	404 (53.7)	195 (51.1)	209 (56.5)	
White/other	128 (17.0)	68 (17.8)	60 (16.2)	
Maternal education, n, %				
>12 yr	255 (33.9)	130 (34.0)	125 (33.8)	0.94
≤12 yr	497 (66.1)	252 (66.0)	245 (66.2)	
Maternal smoking status, n, %				
Never smoked	601 (79.9)	309 (80.9)	292 (78.9)	0.77
Smoked prenatally but not postnatally	38 (5.1)	20 (5.2)	18 (4.9)	
Did not smoke prenatally but smoked postnatally	44 (5.9)	20 (5.2)	24 (6.5)	
Smoked both pre- and postnatally	69 (9.2)	33 (8.6)	36 (9.7)	
Maternal atopy, n, % [†]	269 (35.8)	134 (35.1)	135 (36.5)	0.57
Maternal obese, n, % [‡]	208 (27.7)	101 (26.4)	107 (28.9)	0.61
Prenatal NLE score, n, % [§]				
Low (NLEs ≤ 2)	438 (58.2)	213 (55.8)	225 (60.8)	0.16
High (NLEs > 2)	314 (41.8)	169 (44.2)	145 (39.2)	
Maternal age at enrollment, yr, median (IQR)	25.6 (22.3–30.9)	25.6 (22.4–31.5)	25.6 (22.2–30.4)	0.55
Birth weight for gestational age z score, mean (SD)	-0.15 (1.06)	-0.07 (1.10)	-0.22 (1.02)	0.06
Averaged prenatal NO ₃ ⁻ level, µg/m ³ , median (IQR)	1.30 (1.11–1.47)	1.30 (1.13–1.47)	1.30 (1.09–1.48)	0.90

Definition of abbreviations: CRISYS-R = Crisis in Family Systems-Revised; IQR = interquartile range; NLEs = negative life events.

*P values comparing boys and girls.

[†]Ever self-reported doctor-diagnosed asthma, eczema, and/or hay fever.

[‡]Prepregnancy obesity: ≥30 kg/m².

[§]Assessed using the CRISYS-R survey; this multi-item survey is summarized into a continuous score.

37 weeks gestation or later to minimize overadjustment for a pathway variable. Analyses included 752 mothers and singleton children with estimated daily prenatal NO_3^- levels and stress data. Nitrate concentrations were natural log transformed due to skewness, and odds ratios (ORs) were estimated per interquartile range (IQR) increase in $\ln \text{NO}_3^-$ in all analyses. For infants ($n = 176$) born between 37 and 40 weeks gestation, exposure estimates for the remaining weeks were based on postnatal nitrate estimates corresponding to this time. Results from a sensitivity analysis using estimates based on extrapolation of the observed prenatal exposure assessments to this time period were similar (see Figure E1 in the online supplement).

To identify sensitive windows for the effects of prenatal NO_3^- , we estimated the time-varying association of each participant's weekly-averaged exposures throughout the gestational period. To examine associations between prenatal NO_3^- and childhood asthma and how that relationship is modified by child sex and prenatal stress, we used BDLIM, which extends the traditional DLM framework that identifies sensitive windows (45) and additionally accounts for effect modifications (46). We first conducted BDLIM in the overall sample, then examined effect modification by fetal sex and prenatal stress. In addition to sensitive windows, BDLIM estimates the cumulative effect of NO_3^- exposure over pregnancy for each sex–stress combination, accounting for sensitive windows and within-window effects. BDLIM partitions the distributed lag function into two components: (1) the weights that identify sensitive windows of susceptibility; and (2) the coefficients that identify the magnitude of the within-window effects. Each sex–stress combination can have either the same, or different, sensitive windows (weights) and within-window effects (effects) (46). This allows situations where subgroups have the same sensitive window, but different within-window effects or *vice versa*, which are not allowed in the standard DLM framework. When the weights are constant over time, the BDLIM is equivalent to a model with mean exposure interacted with child sex and prenatal stress group. When the weights vary by time, the model identifies time periods with greater weight (i.e., sensitive windows) that will graphically appear as a bump, during which exposure is significantly

associated with asthma. This approach determines whether the weights and the coefficients are the same or different for each group. The model quantifies the likelihood of each pattern of heterogeneity and estimates the association between exposure and outcome under the effect modification pattern that is best supported by the data. All models were adjusted for maternal age, race, education level, prepregnancy obesity, maternal atopy, and perinatal smoking status.

In addition, linear regression models were constructed to estimate the interactions between nitrate and prenatal maternal stress in boys, accounting for the average concentrations present during sensitive windows identified by the BDLIM. Finally, we conducted sensitivity analyses additionally adjusted for BWGA, a potential pathway variable. All analyses were implemented in R statistical software, v3.3.1 (Vienna, Austria).

Results

Most mothers were Hispanic (54%) or black (29%), did not smoke pre- or postnatally (80%), had 12 years or less of education

(66%), and reported low stress (58%); these covariates were evenly distributed by infant sex (Table 1). A total of 113 children (15%) had physician-diagnosed asthma, with more boys than girls developing asthma (18 vs. 12%, $P = 0.01$). Girls had lower BWGAs compared with boys (Wilcoxon rank sum test $P = 0.02$). Average (median [IQR]) ambient prenatal NO_3^- concentration was 1.30 (1.11–1.47) $\mu\text{g}/\text{m}^3$ and was similar for both sexes.

Overall Association between Prenatal NO_3^- and Childhood Asthma

Adjusted BDLIMs were used to estimate the overall associations between weekly NO_3^- during gestation and incident childhood asthma (Figure 1). No significant sensitive window was identified for overall NO_3^- exposure on asthma onset by 6 years. The estimated cumulative effect over the entire gestational period demonstrated an increased odds of childhood asthma in relation to nitrate exposure, although this did not quite reach statistical significance (OR [95% confidence interval (CI)] = 1.20 [0.93–1.70]), corresponding to per IQR increase in $\ln \text{NO}_3^-$.

Figure 1. Association between weekly ambient nitrate over gestation and childhood asthma. Bayesian-distributed lag interaction models were adjusted for maternal age, race, education level, prepregnancy obesity, maternal atopy, and pre- and postnatal smoking status. The x-axis demarcates the gestational age in weeks. The y-axis represents the odds ratio (OR) of developing asthma by age 6 years per an interquartile range increase in $\ln \text{NO}_3^-$ (week-specific effects). The dark gray line represents the predicted OR, and the gray area indicates the 95% confidence interval (CI). A sensitive window is identified when the estimated pointwise 95% CI does not include 1.

Effect Modification by Fetal Sex and Prenatal Stress

Figure 2 demonstrates results from a BDLIM accounting for the modification of the association between NO_3^- and asthma by child sex and maternal stress (high vs. low). We found two distinct sensitive windows, one at 7–19 weeks and the other at 33–40 weeks gestation, only in boys born to mothers with high stress during pregnancy. The estimated cumulative effect of NO_3^- across pregnancy, accounting for these two sensitive windows and within-window effects, was also significant for this group (OR [95% CI] = 2.64 [1.27–5.39]; per IQR increase in $\ln \text{NO}_3^-$; Figure 3). When examining interactions due to stress alone, independent of fetal sex, we estimated the cumulative effect over pregnancy for the high-stress group to be significantly increased (OR [95% CI] = 1.95 [1.11–3.46]). No sensitive window was found between

prenatal nitrate exposure and incident asthma among girls, regardless of maternal stress level. The BDLIM analysis suggested that effect modification by sex and stress was attributable to a difference in the magnitude/strength of the within-window association, whereas the window was not different among the sex–stress combinations (the normalized posterior density was 0.99, interpreted as the probability that this was the best fitting pattern of effect modification).

Linear regression models estimating the interactions between nitrate and prenatal maternal stress in boys during the identified sensitive windows (i.e., averaged across 7–19 and 33–40 wk), demonstrated a significant interaction ($P = 0.04$). Finally, sensitivity analyses for models that included adjustment for BWGA did not yield substantive changes in our findings (data not shown).

Discussion

This is the first prospective study to examine synergistic relationships among ambient NO_3^- exposure, fetal sex, and maternal prenatal stress on the development of childhood asthma. Higher prenatal nitrate pollution exposure was associated with increased asthma risk, particularly among boys who were concurrently exposed to higher prenatal stress. Using novel Bayesian statistical methods, we identified two distinct sensitive windows during gestation: one at 7–19 weeks and one later in pregnancy at 33–40 weeks.

Prior research demonstrates variability in downstream health effects, depending on the trimester during which exposure occurs, thereby emphasizing the specificity of timing of prenatal environmental exposures (7, 21, 47). However, these studies employed exposure estimates averaged over clinically defined periods, which may in fact underestimate significant pollutant–disease associations occurring at the time when specific embryologic processes are disrupted in the presence of toxin. In addition, using trimester demarcations may introduce bias if external factors, such as season, covary with these fixed time periods. Moreover, predefining time boundaries in this way limits the ability to objectively reveal sensitive windows of gestation that overlap with developmentally specific events that are most susceptible to the toxic effects (23). Finally, the appreciation that subgroups within a population (e.g., by fetal sex and/or maternal stress level) might be heterogeneous in their response to environmental stimuli is better accounted for by the BDILM methods used here, where the effect size and the timing components of exposure can be examined separately to identify differential effects of each between subgroups. This allows for a more robust estimation of effect heterogeneity across subgroups (46).

A more highly resolved temporal understanding of the perturbation of developmental processes during gestation is essential to elucidating the underlying mechanisms of these toxic effects. The early sensitive window identified in our analysis coincides with the pseudo-glandular stage of fetal lung development (Weeks 7–17) responsible for further structural growth of the conducting airways, including that of smooth muscle, mucosal glands, and vascular formation, and one can hypothesize that toxic exposures in this early

Figure 2. Association between weekly ambient nitrate over gestation and childhood asthma: Bayesian-distributed lag interaction model (BDLIM) examining the interactions by sex and prenatal maternal stress level. The figure demonstrates the association between NO_3^- exposure over gestation and asthma onset by age 6 years using BDLIMs, assuming week-specific effects, taking into account sex- and prenatal stress level-specific windows and within-window effects. Models were adjusted for maternal age, race, education level, prepregnancy obesity, maternal atopy, and pre- and postnatal smoking status. The x-axis demarcates the gestational age in weeks. The y-axis represents the odds ratio (OR) of developing asthma by age 6 years per an interquartile range increase in $\ln \text{NO}_3^-$. The dark gray line represents the predicted OR, and the gray area indicates the 95% confidence interval (CI). A sensitive window is identified when the estimated pointwise 95% CI does not include 1.

Figure 3. Cumulative effect (odds ratio [OR]) of NO_3^- across pregnancy on childhood asthma: Bayesian-distributed lag interaction model (BDLIM). Cumulative effect of NO_3^- across pregnancy on childhood asthma onset estimated by BDLIMs, accounting for both sensitive windows and within-window effects. The model was adjusted for maternal age, race, education level, prepregnancy obesity, maternal atopy, and pre- and postnatal smoking status. IQR = interquartile range.

window of pregnancy may interfere with the global transcriptional program of progenitor cells awaiting differentiation into such structural components (48). In contrast, the later sensitive window identified coincides with the late portion of the terminal saccular period (Weeks 28–36) and early alveolar stage, during which alveolar formation increases airways surface area in preparation for gas exchange (49). Consequently, pollution-mediated effects in the terminal portion of pregnancy might operate through the disruption of alveolar structural development required for adequate lung function. Notably, reduced fetal growth in late gestation has been linked to persistent postnatal changes in alveolar number, as well as a reduction in internal surface area of the lung relative to lung volume (50), which may have implications for the postnatal development of respiratory disorders, such as asthma. Nevertheless, there remains a wide gap in our understanding of the time-sensitive molecular mechanisms linking prenatal pollutant exposure to adverse developmental effects in children (51).

Our finding that males were more vulnerable is consistent with our knowledge of sex differences in human lung maturation and responses to other toxins *in utero* (52). Lung development in males lags behind females and, based on the biochemical composition of amniotic fluid, can be up to 2.5 weeks later in fetal maturity (53). These lags may contribute to greater vulnerability in the male fetus. Indeed, androgen-

mediated delays in pulmonary surfactant synthesis in males are hypothesized to lead to higher morbidity and mortality from neonatal respiratory distress syndrome in males compared with females (54). Animal models also reveal sex-specific effects on lung development (55), including sex-specific responses to noxious agents, such as tobacco smoke, resulting in higher bronchial hyperreactivity and changes in airway compliance to bronchoprovocation among male pups (56), and hyperoxia, whereby male neonatal lungs show greater airway inflammation and morphologic evidence of decreased alveolarization and angiogenic features in rodents (57). Our findings provide evidence for sex-specific vulnerabilities to *in utero* exposure to airborne toxins in these urban children.

Moreover, boys born to mothers with increased *in utero* NO_3^- exposure as well as higher stress were at greatest risk of developing asthma. These results buttress the emerging evidence not only for the independent effect of maternal stress on child respiratory health (3, 29, 58), but also for the role of maternal stress in modifying early environmental health effects. As both prenatal stress and environmental pollutants may have common immunologic and oxidative mechanistic pathways, it is plausible that coincident exposures within the milieu of an immature lung could have synergistic effects on a variety of outcomes. For example, in a cohort of 1,399 Southern Californian school-age children, although no

significant health effects from ambient NO_x were found for children living with low parental psychological stress, increasing NO_x exposure was linked to deficits in lung function in children living in high-stress households (deficit in FEV_1 [CI] = 4.5% [−6.5 to −2.4] for each 21.8 ppb increase in home NO_x) (31). Similarly, in a Boston birth cohort, among children with high individual exposure to violence in the community—an example of neighborhood-level stress—higher NO_2 exposure was significantly associated with asthma development (59). Our work extends these findings by demonstrating similar potentiating effects of stress on the adverse respiratory effects of nitrate pollution, originating in pregnancy.

Animal studies also identify pregnancy as an especially vulnerable period to the adverse effects of prenatal airborne particulate exposure. Challenge studies of both airborne pollutant and inert particles were found to induce greater airway inflammation and hyperresponsiveness in the lungs of pregnant mice compared with minimal pathologic changes in nonpregnant controls (60). This, in turn, may enhance maternal systemic oxidative stress and proinflammatory cytokine production, resulting in placental and endothelial dysfunction, and increased fetal oxidative stress, with consequent effects on fetal immune and lung development. Others have shown that neonatal mouse pups exposed perinatally to urban $\text{PM}_{2.5}$ have lungs with smaller surface-to-volume ratios, reflective of decreased alveolarization, as well as reduced lung volumes, compared with nonexposed animals (61). Such urban particles appear to alter the fetal immune system, with evidence of the infiltration of a variety of inflammatory cells and production of cytokines within the airway already present at birth, especially in male pups (62). In humans, mechanisms underlying epidemiological relationships between prenatal $\text{PM}_{2.5}$ exposure and childhood asthma have been proposed to involve oxidative stress mechanisms. Studies have demonstrated associations between gestational $\text{PM}_{2.5}$ exposure and decreased placental mitochondrial DNA content (63)—a marker for *in utero* oxidative damage. Furthermore, gestational $\text{PM}_{2.5}$ exposure has been linked to epigenetic changes in antioxidant genes important for mitochondrial function (64). These findings, coupled with evidence to

support an increased susceptibility of the male fetus to maternal oxidative stress (65), may contribute to the observed greater risk in males.

Our study has several strengths. First, analyses included an ethnically diverse sample of children born to mothers with potentially higher risk for both pollutant and stress exposures. Second, our environmental assessment methods were robust due to the availability of daily ambient nitrate concentrations derived from a validated hybrid prediction model that had high (1 km × 1 km) spatial resolution. These exposure estimates were then incorporated into a novel advanced statistical model that allowed us to more objectively identify susceptibility windows within pregnancy. Finally, this is the first study to consider three-way interactions involving fetal sex and maternal stress and their modifying effects upon the relationship between prenatal nitrate pollution exposure and development of childhood asthma.

Some limitations are also worth noting. Although we adjust for a variety of factors believed to be significant in asthma development, other environmental influences that covary with nitrate pollution and/or stress may be unaccounted for in our analyses. In addition, although this study isolated the effects of particulate pollution represented by nitrates alone, multipollutant effects, including other airborne toxins, were not explored. Future work to investigate interactions between multiple pollutants that coexist in an urban environment may further advance our understanding of ambient pollution effects on asthma programming. Second, childhood asthma was reported by mothers, though, notably, the majority of these children were given a diagnosis of asthma after the age of 3 years (79%), reducing the likelihood that cases represented wheezing respiratory illnesses other than asthma (e.g., early transient wheeze). As we follow this cohort, it will be informative to see if similar associations hold for more objective

measures, including respiratory function (e.g., spirometry, airway reactivity). Finally, although we focused on a higher-risk sample, our results may not be generalizable to the overall U.S. population.

In summary, increased exposure to ambient NO₃⁻ during distinct *in utero* vulnerable windows was associated with incident asthma, particularly among boys born to mothers who also experienced greater prenatal stress. A more definitive characterization of vulnerable windows may provide insight into underlying mechanisms when coupled with our understanding of lung growth, airway structural and functional development, and asthma pathophysiology. Furthermore, consideration of these complex interactions may more fully identify vulnerable subgroups to airborne toxins. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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