Nitrogen dioxide exposure in school classrooms of inner-city children with asthma

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Background: Ambient and home exposure to nitrogen dioxide (NO_2) causes asthma symptoms and decreased lung function in children with asthma. Little is known about the health effects of school classroom pollution exposure.

Objective: We aimed to determine the effect of indoor classroom NO_2 on lung function and symptoms in inner-city school children with asthma.

Methods: Children enrolled in the School Inner-City Asthma Study were followed for 1 academic year. Subjects performed spirometry and had fraction of exhaled nitric oxide values measured twice during the school year at school. Classroom NO_2 was collected by means of passive sampling for 1-week periods twice per year, coinciding with lung function testing. Generalized estimating equation models assessed lung function and symptom relationships with the temporally nearest classroom NO_2 level.

Results: The mean NO_2 value was 11.1 ppb (range, 4.3-29.7 ppb). In total, exposure data were available for 296 subjects, 188 of whom had complete spirometric data. At greater than a threshold of 8 ppb of NO_2 and after adjusting for race and season (spirometry standardized by age, height, and sex), NO_2 levels were associated highly with airflow obstruction, such that each 10-ppb increase in NO_2 level was associated with a 5% decrease in FEV_1/forced vital capacity ratio ($\beta = -0.05$; 95% CI, −0.08 to −0.02; $P = .01$). Percent predicted forced expiratory flow between the 25th and 75th percentile of forced vital capacity was also inversely associated with higher NO_2 exposure ($\beta = -22.8$; 95% CI, −36.0 to −9.7; $P = .01$). There was no significant association of NO_2 levels with percent predicted FEV_1, fraction of exhaled nitric oxide, or asthma symptoms. Additionally, there was no effect modification of atopy on lung function or symptom outcomes.

Conclusion: In children with asthma, indoor classroom NO_2 levels can be associated with increased airflow obstruction. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Asthma, indoor air pollution, obstructive lung disease, nitrogen dioxide, spirometry, exhaled nitric oxide*

Exposure to ambient air pollutants has been associated with asthma development, asthma exacerbations, and reduction in lung function.[1-8] Moreover, home-based measurements of nitrogen dioxide (NO_2) and other pollutants with indoor sources have been associated with asthma symptom severity[9] and lower lung function[2,10,11] in children, even at modest exposure levels.[10]

NO_2, a gaseous pollutant generated from fossil fuel combustion, has emerged as one of the most notable pollutants associated with health effects. In urban environments NO_2 is generated by traffic-related combustion, home heating and cooking with fossil fuels (gas, oil, and coal), and tobacco smoke.[12,13] It is a prevalent indoor pollutant in homes, where heating and cooking are common activities, and during these exposures, asthma symptoms worsen.[11,14] However, little is known about the effect of NO_2 in indoor environments aside from the home.

Urban schools represent a unique and important microenvironment for indoor pollution. In most schools there is no cooking, tobacco smoke is prohibited, and the centralized furnace

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Abbreviations used
FEF_{25-75}: Forced expiratory flow between the 25th and 75th percentile of forced vital capacity
FENO: Fraction of exhaled nitric oxide
FVC: Forced vital capacity
NO_2: Nitrogen dioxide

system minimizes the combustion exposure to any individual classroom. However, exposure to combustion-related pollutants from outside sources can enter through traditional ventilation and intrusion through doors, windows, and structural imperfections of the school building. The school classroom represents the occupational setting for children (ie, the environment in which they spend 6 to 10 hours per day). Therefore exposures encountered in this environment can have a substantial health effect.

Several studies have cataloged indoor air quality in schools[15-19] and associations with respiratory[19-21] and neurodevelopmental measures.[22] However, variation in the source and type of pollutants is significant based on geographic region,[16] and few studies have focused on US inner-city schools.[23] Furthermore, few studies have specifically evaluated lung function in relation to school-based exposure.[24] In this study we examine the symptomatic effects of NO_2 and objective assessment of lung function in inner-city children with asthma.

We hypothesized that exposure to NO_2 in schools would be associated with lung function deficits and higher rates of asthma symptoms in children with asthma.

METHODS

Study population

The School Inner-City Asthma Study is a single-center epidemiologic study of the effect of school classroom environmental exposures on asthma morbidity in inner-city schoolchildren with asthma, with methods that have been published previously.[25] Briefly, children with asthma were recruited from inner-city school classrooms from 2008 to 2013 for participation. Screening surveys were distributed schoolwide to participating schools the spring before the study year. Children with a physician's diagnosis of asthma or with a report of signs and symptoms consistent with persistent asthma and at least 1 asthma symptom within the past year were invited to participate. This study was approved by the Boston Children's Hospital Institutional Review Board. Written informed consent was obtained from the subject's guardian, and assent was obtained from the subjects before enrollment.

Study procedures

Fig 1 shows the study schema. Baseline characterization of study subjects was performed at a formal research clinic visit during the summer before the academic year, during which sociodemographic information, medical history, and baseline symptom profiles were assessed by means of questionnaire. Subjects performed spirometry with a Koko spirometer (Ferraris Respiratory, Louisville, Colo) using American Thoracic Society guidelines,[26] fraction of exhaled nitric oxide (FENO) measurement with the NIOX MINO device (Aerocrine, Solna, Sweden), and aeroallergen sensitization testing by means of allergy skin testing (MultiTest device, Lincoln Diagnostics, Decatur, Ill) and/or serum specific IgE measurement (ImmunoCAP; Phadia AB, Uppsala, Sweden). Sensitization was defined by a wheal 3 mm or larger than that elicited by the negative saline control on skin prick tests or a specific IgE level of 0.35 kU/L or greater. The tested allergens included tree pollen, grass, ragweed, dust mites, cat, dog, mouse, rat, cockroach, and molds (Greer Laboratories, Lenoir, NC).

Subsequently, questionnaire-based symptom assessments were performed up to 4 times throughout the academic school year by telephone interviews at 3, 6, 9, and 12 months. Spirometric and FENO values were assessed at 2 in-school visits that coincided with school environmental assessments approximately 6 months apart. Testing occurred throughout the day, with 90% of tests occurring after 10 AM and the majority occurring between 10 AM and 3 PM.

Exposure assessment

Classrooms of participating students were sampled twice during the academic year while school was in session approximately 6 months apart. NO_2 was collected by means of passive monitoring with Ogawa samplers[27] for 1-week periods. NO_2 analysis was performed with ion chromatography. Average NO_2 levels per assessment period were determined and used for analyses.

Outcome measures

The FEV_1/forced vital capacity (FVC) ratio was chosen as the primary spirometric outcome of interest because it is the most sensitive marker of airflow obstruction in children with asthma.[28,29] FEV_1 percent predicted, FVC percent predicted, and forced expiratory flow between the 25th and 75th percentile of forced vital capacity (FEF_{25-75}), a measure of medium- and small-caliber airways, were also assessed. All spirometric measures were assessed for acceptability and repeatability by study physicians per American Thoracic Society guidelines.[26,30] Reference values were derived from the National Health and Nutrition Examination Survey III[31] reference equations, which account for age, race, and sex. FENO was measured per standardized methodology. Both spirometric and FENO measurements were performed in the school during the same season (fall or spring) of exposure measurement.

Symptom outcomes were measured as maximum symptom days, as used in prior urban home-based[32,33] and school[34,35] studies. To define this outcome, 3 variables of symptoms in the 2 weeks before each survey were evaluated: (1) number of days with wheezing, chest tightness, or cough; (2) number of days on which the child had to slow down or discontinue play activities because of wheezing, chest tightness, or cough; or (3) number of nights with wheezing, chest tightness, or cough leading to disturbed sleep. The greatest result of these 3 variables was used as the asthma symptom days outcome. As such, this outcome was a score of from 0 to 14 days.

Statistical analysis

Characteristics of the cohort are expressed with descriptive statistics. Variability of NO_2 levels between schools and between classrooms within schools was determined with random-effects linear regression. All clinical outcomes were linked to the temporally closest measured exposure during the academic school year. Only outcome measures obtained during the academic school year were used for analysis. The relationship between NO_2 levels and lung function test results was evaluated with locally weighted regression (Lowess) to examine possible nonlinear relationships. On the basis of these smoothers, we then fit a linear spline of NO_2 with a single knot at 8 ppb to be used in all subsequent models. Relationships between NO_2 levels and lung function outcomes are presented as the effect of a 10-ppb change in NO_2 levels of greater than the threshold of 8 ppb. The exposure-outcome relationship was evaluated by using generalized estimating equations with an exchangeable correlation structure and robust variance estimates, with clustering defined at the participant level. We considered clustering at the school level in addition to the participant level within a multilevel random-effects model containing both subject and school random effects, but this was deemed unnecessary because there was little to no between-school variability in all outcomes (intraclass correlations between 0.00 and 0.04). All models included linear and quadratic terms for the number of days since school started to address the time variation of asthma activity across the study period. Symptom outcomes were adjusted for age, race, and sex because of *a priori* assumptions that these might be important

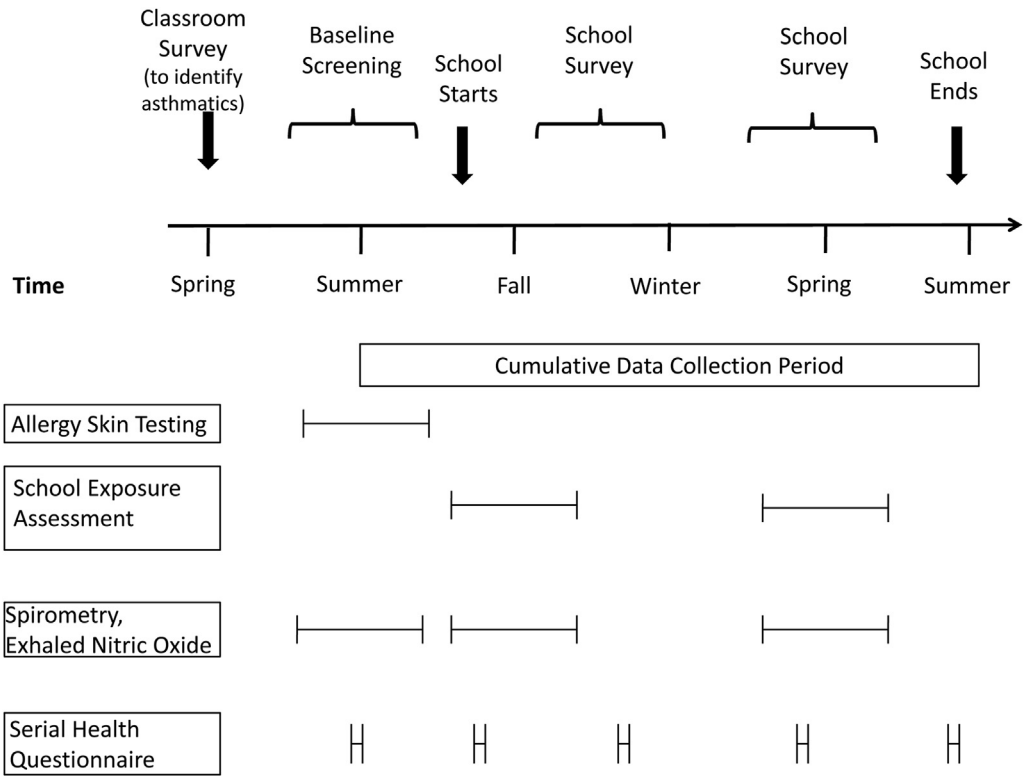

FIG 1. Schema of assessments in the School Inner-City Asthma Study.

confounders. Age and sex were part of the National Health and Nutrition Examination Survey III reference equations and therefore were not used as further adjustment for spirometric outcomes. Binomial family generalized estimating equations with a logit link and an overdispersion parameter were used for 2-week outcomes (ie, 2-week outcomes were modeled as the sum of 14 binomial "successes"). Spirometric and FENO values were modeled by using Gaussian family and identity link. Potential confounders that were not included in models because of a lack of association with NO_2 levels ($P > .1$) included vacuumed dust mouse allergen and endotoxin from the classrooms, income, environmental tobacco smoke exposure, body mass index, time (hour of the day) of lung function testing, and use of asthma controller medication at baseline visit.

A term for "any sensitization" was created to indicate subjects with 1 or more sensitizations determined by skin prick test responses or specific IgE levels of greater than 0.35 kU/L at baseline assessment. Based on prior literature, any sensitization was examined as a potential moderator of the effects of NO_2 on asthma morbidity. *Post hoc* analysis stratified by sensitization status was performed to further evaluate main effects by group. Statistical computations were performed with STATA software (version 13.1; StataCorp, College Station, Tex). All tests were 2-tailed, and a P value of less than .05 was considered significant.

RESULTS

In total, 296 participants had assessments of classroom NO_2 levels and were included in the analysis. Subjects were predominantly black or Hispanic, and 49% were from impoverished households (Table I). Baseline lung function was normal and nonobstructed.

NO_2 levels were measured in 218 classrooms across 37 schools. The mean NO_2 level was 11.1 ppb, the median level was 10.4 ppb, and the range was 4.3 to 29.7 ppb. Fig 2 shows the distribution of NO_2 levels by school for fall and spring measurements, demonstrating the variability between classrooms within and between schools. School-to-school variability accounted for 75% of the variance in NO_2 measures, leaving 25% of the variability attributable to the classroom level.

One hundred eighty-eight participants had complete data for NO_2 levels and acceptable spirometry for analysis. In adjusted analyses NO_2 exposure of greater than 8 ppb was significantly associated with airflow obstruction, as measured based on FEV_1/FVC ratio and FEF_{25-75}, a measure of small-airways dysfunction. For each 10-ppb increase in NO_2 level, there was a 5% decrease in FEV_1/FVC ratio, with ratios crossing the clinically relevant normal value for a FEV_1/FVC ratio of 0.85[36] at approximately 16 ppb of NO_2 (Table II, unadjusted correlations can be found in Table E1 in this article's Online Repository at www.jacionline.org). Fig 3 depicts the relationship between NO_2 level and FEV_1/FVC ratio within the range of our data. Allergic sensitization did not modify the effect of this association ($P = .55$ for the interaction). However, in a *post hoc* stratified analysis, nonatopic children demonstrated decreased FEV_1 percent predicted in association with NO_2 exposure, whereas atopic subjects did not (see Table E2 in this article's Online Repository at www.jacionline.org). There was a 22.8% decrease in FEF_{25-75} for each 10-ppb increase in NO_2 level. Although FEV_1 and FVC percent predicted were negatively associated with NO_2 exposure, associations were not significant at a P value of less than .05. There was no significant association of NO_2 levels with FENO, a measure of airway inflammation, levels of which were also measured at the time of exposure assessment.

There was no significant association of NO_2 exposure with maximum symptom days, the main symptom-based outcome (Table II). Additionally, allergic sensitization did not modify

TABLE I. Characteristics of the study population

Characteristic	No. (%)
Demographics	
Age (y), median (range)	8 (4-13)
Female sex	143 (48)
Race or ethnic group	
White	13 (4)
Black	102 (34)
Hispanic	107 (36)
Mixed race	52 (18)
Other	22 (7)
Annual income <$25,000	120 (49)
Pulmonary function testing*	
FVC (% predicted), mean (SD)	98 (15.5)
FEV_1 (% predicted), mean (SD)	100 (17.9)
FEV_1/FVC, mean (SD)	0.87 (0.08)
FEF_{25-75} (% predicted), mean (SD)	118 (103.2)
F_{ENO} (ppb), mean (SD); n = 73	19.6 (20.9)
Allergy sensitization ≥1 allergen	197 (69)
Maximum symptom days, mean (SD)†	3.0 (4.2)
Controller medication over prior 12 mo	167 (56%)
Environmental tobacco smoke exposure	97 (33%)

*N = 188 for pulmonary function testing.
†Maximum symptom days = the greatest result of the following 3 variables in the 2 weeks before each follow-up survey: (1) number of days with wheezing, chest tightness, or cough; (2) number of days on which the child had to slow down or discontinue play activities because of wheezing, chest tightness, or cough; and (3) number of nights with wheezing, chest tightness, or cough leading to disturbed sleep.

FIG 2. Distribution of NO_2 concentrations by school and season. The *x-axis* represents each school, and the number of subjects attending each school is shown in parentheses. *Box and whiskers plots* represent the distribution of NO_2 across multiple classrooms within each school. *Box parameters* are the interquartile range (IQR), the *hash mark* is the median, and *whiskers* extend to 1.5 times the IQR above the 75th and below the 25th percentiles.

the relationship between NO_2 levels and asthma symptoms ($P = .59$ for the interaction).

DISCUSSION

In this study we demonstrate a temporally distinct association of NO_2 levels measured in school classrooms with airflow obstruction in inner-city schoolchildren with asthma. Because children spend the majority of their day in the school environment, this microenvironment for potential respiratory insults is equivalent to an occupational exposure in adults.

There are several important findings highlighted by these analyses. First, NO_2 levels detected in classrooms were relatively low compared with US Environmental Protection Agency national ambient air quality standards for NO_2, which are currently set at a 1-hour maximum level of 100 ppb and annual average level of 53 ppb.[37] Despite overall low levels, there was a clear signal of lung function impairment and a trend toward more symptoms associated with higher NO_2 exposures in this vulnerable pediatric population. This finding complements work by Belanger et al,[10] who found respiratory health effects at relatively low home NO_2 levels, and Pilotto et al,[15] who found health effects of NO_2 in Australian school classrooms with unflued gas heaters, although the exposure in our school classrooms was far less. In summary, this suggests that there is a concentration-response relationship of NO_2 that adversely affects health at levels less than existing standards, especially in vulnerable populations.

Furthermore, our data indicate a threshold level at which physiologic effects of NO_2 can occur in children with asthma. To our knowledge, this has not been previously demonstrated in other studies, which might be a reflection of our unique study design measuring levels in schools of asthmatic children, where there is no cooking, smoking, or other immediate sources of NO_2 emissions, so that the range of our data was able to elicit this level of detail. Interventional exposure studies typically use high concentrations of NO_2 for short periods of time, which might not elicit the same responses as prolonged exposure to lower levels.[38] This might also be due to differences in statistical methodology used to evaluate nonlinear associations between NO_2 levels and respiratory outcomes.[39]

Second, we did not find any interaction between NO_2 exposure and atopy, as measured by specific sensitization to a battery of common aeroallergens, in relation to asthma outcomes. Furthermore, there was no association between NO_2 exposure and levels of F_{ENO}, a marker of allergic airway inflammation. Although some prior studies have found that air pollution differentially affects allergen-sensitized children with asthma,[40-43] others have found that nonatopic children are more affected.[11] Although our stratified analysis found a significant association between NO_2 levels and FEV_1 percentages in nonsensitized subject, this does not reflect a significant difference between the atopic and nonatopic groups in response to the exposure, which is reflected by the lack of a significant interaction term. Our finding that the relationship of NO_2 and airflow obstruction is not modified by allergic sensitization suggests that it can influence lung function through a direct effect on respiratory epithelium and smooth muscle through induction of oxidative stress and nonallergic inflammation. Previous literature on the biologic effects of NO_2 supports the stimulation of innate immune responses rather than the T_H2-driven inflammation more characteristic of asthma.[44,45] Human exposure studies demonstrate bronchial washings enriched for IL-6, IL-8, neutrophilic infiltration, and acute-phase reactions within 24 hours of NO_2 inhalant exposure.[45] Simultaneously, oxidative stress induction, as evidenced by increased *HMOX1* gene expression after NO_2 exposure to human bronchial epithelial cells, is also likely to play a significant role.[44,46] Summation of these study results with the current epidemiologic findings of our study suggest that respiratory effects caused by inhalation of NO_2 are not

TABLE II. Effect of an NO_2 level of greater than 8 ppb on spirometric results and asthma outcomes in school-aged children with asthma

	Univariate model		Multivariate model†	
	Odds ratio	95% CI	Odds ratio	95% CI
Maximum symptom days*	1.31	0.90 to 1.90	1.15	0.80 to 1.64
	β	95% CI	β	95% CI
FEV_1/FVC ratio	−0.049‡	−0.077 to −0.021	−0.049‡	−0.078 to −0.021
FEV_1 (%)	−5.5	−12.0 to 0.9	−5.5	−11.7 to 0.8
FVC (%)	−0.7	−5.8 to 4.4	−0.5	−5.5 to 4.5
FEF_{25-75} (%)	−22.8‡	−36.0 to −9.7	−22.8‡	−36.0 to −9.7
FENO	3.5	−6.9 to 13.9	−0.5	−12.0 to 11.0

*Maximum symptom days = the greatest result of the following 3 variables in the 2 weeks before each follow-up survey: (1) number of days with wheezing, chest tightness, or cough; (2) number of days on which the child had to slow down or discontinue play activities because wheezing, chest tightness, or cough; and (3) number of nights with wheezing, chest tightness, or cough leading to disturbed sleep.
†Multivariate model: maximum symptom days adjusted for age, race, sex, and season; spirometric and FENO values were adjusted for race and time. Results are scaled to each 10-ppb increment of NO_2 of greater than 8 ppb.
‡$P = .001$.

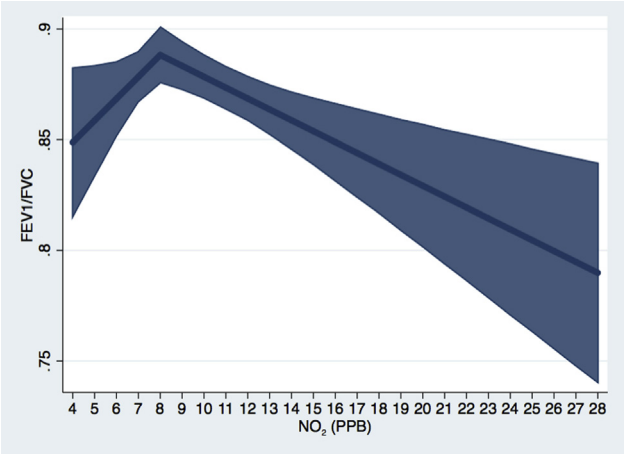

FIG 3. Effect of classroom NO_2 on FEV_1/FVC ratio. The association of NO_2 levels and FEV_1/FVC ratios is shown by using piecewise linear regression with a breakpoint at an NO_2 level of 8 ppb. The *shaded area* represents 95% CIs.

mediated by the T_H2 inflammatory paradigm that is primarily implicated in patients with pediatric asthma.

Third, we found significant variability in NO_2 levels between schools that were not seasonally dependent. The school microenvironment, particularly the school classroom, is unique in that there are few indoor sources of NO_2. Primary sources of indoor combustion leading to increased levels of NO_2 in homes include home heating and cooking and cigarette smoking. Among the schools included in this study, there was only one with a kitchen that cooked food for lunches, and all prohibited smoking on school property. Similarly, the effect of a central furnace heating multiple classrooms is unlikely to account for significant classroom-to-classroom variation in NO_2 exposure. In this case differences in NO_2 levels between classrooms and between schools likely represent variable penetration and ventilation of outdoor-generated ambient gases through the school classroom envelope along with local differences in traffic-related emissions near each school. Similar associations of inner-city school measures of NO_2 were reported by Rivas et al[17] in the BREATHE study of indoor pollutants in Barcelona, Spain. These are potentially modifiable school classroom characteristics that might be amenable to remediation of structural imperfections, ventilation systems, or alterations in local traffic patterns.

The association of classroom NO_2 levels with asthma symptoms was suggestive of a positive relationship but did not reach statistical significance. The lack of precision of the effect estimates can in part be due to exposure misclassification. Through the nature of the study design, lung function testing was carried out at the time exposure measurement devices were deployed in the schools twice per year; however, symptom outcomes were collected by telephone on a quarterly basis and not necessarily in close temporal relation to the exposure measure. As such, temporal variability significantly limits the ability to find acute health effects on asthma symptoms related to the exposure. A larger sample size might have elicited a significant long-term relationship between exposure and outcome that was not found here.

It is also possible that NO_2 found in classrooms is a marker for other unmeasured pollutants produced by the same processes or for other pollutants chemically related to NO_2, such as ozone or particulate matter. Although this is possible, NO_2 is known to be associated with biologically plausible mechanisms to induce airway inflammation,[44] hyperresponsiveness, and airflow obstruction[2] in its own right. Our data are limited in the ability to tease apart NO_2 from other copollutants that might also be present.

Additionally, unmeasured confounding factors, such as viral upper respiratory tract infections or specific characteristics influencing susceptibility to the exposure might have influenced our results. However, we attempted to address any seasonal variation in asthma morbidity, such as viral seasons, by including a variable for time in each analytic model, and known factors related to asthma morbidity, such as low socioeconomic status and environmental tobacco smoke, among others, were evaluated as potential confounders. Notably, time was not associated significantly with lung function or asthma symptoms within our models.

Finally, our exposure measure is an average of NO_2 levels collected over a 1-week timeframe, which limits our ability to determine the potential effect of peak levels and our ability to specify the personal exposure during school hours only.

As such, this runs the risk of some element of exposure misclassification, which might have biased our findings toward the null. Despite this, we found compelling evidence linking exposure to decreases in lung function.

Additional evidence to support the association between NO_2 levels and health effects exists in the form of few interventional studies in schools with high pollution levels because of poor venting of furnaces.[20] In population-based studies ambient NO_2 levels have been associated with the development of childhood asthma[47] and asthma exacerbations requiring emergency services,[48] as well as abnormal lung function test results in asthmatic cohorts.[6] Modeled assessments of effects and benefits of reducing NO_2 levels near primary schools in London indicate that a significant improvement in the number of childhood asthma exacerbations, costs to schools, and costs to parents would be achieved by decreasing exposure.[49]

In conclusion, we demonstrate that exposure to NO_2 in the school classroom microenvironment is significantly related to airflow limitation in children with asthma through a pathway that is not dependent on allergy or production of allergic inflammation. Intervention studies are needed to determine whether reducing inhaled pollutants in the school environment can produce health benefits for vulnerable populations of children.

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Clinical implications: NO_2 in the urban school environment is associated with airflow obstruction in children with asthma. Environmental interventions in schools might improve the health of children with asthma.

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TABLE E1. Raw correlations for NO_2 levels of greater than 8 ppb on spirometry and asthma outcomes in school-aged children with asthma

Variable	Pearson correlation coefficient
FEV_1/FVC ratio	−0.22
FEV_1 (%)	−0.15
FVC (%)	−0.03
FEF_{25-75} (%)	−0.20
F_{ENO}	0.05

TABLE E2. Effect of NO_2 levels of greater than 8 ppb on spirometry and asthma outcomes in school-aged children with asthma stratified by atopy*

	Nonatopic		Atopic	
	Odds ratio	95% CI	Odds ratio	95% CI
Maximum symptom days†	1.32	0.70 to 2.51	1.01	0.67 to 1.54
	β	95% CI	β	95% CI
FEV_1/FVC ratio	−0.052‡	−0.080 to −0.025	−0.051‖	−0.095 to −0.007
FEV_1 (%)	−10.1§	−16.8 to −3.4	−4.2	−13.8 to 5.3
FVC (%)	−2.5	−7.8 to 2.7	−0.2	−7.3 to 7.7
FEF_{25-75} (%)	−30.2‡	−47.2 to −13.3	−23.4‖	−41.9 to −4.9
FENO	−3.8	−12.9 to 5.3	6.1	−7.8 to 20.0

*Maximum symptom days adjusted for age, race, sex, and season; spirometric and FENO values are adjusted for race and time. Results are scaled to each 10-ppb increment of NO_2.

†Maximum symptom days = the greatest result of the following 3 variables in the 2 weeks before each follow-up survey: (1) number of days with wheezing, chest tightness, or cough; (2) number of days on which the child had to slow down or discontinue play activities because of wheezing, chest tightness, or cough; and (3) number of nights with wheezing, chest tightness, or cough leading to disturbed sleep.

‡$P < .001$.

§$P < .001$.

‖$P < .01$.