Indoor Pollutant Exposures Modify the Effect of Airborne Endotoxin on Asthma in Urban Children

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***Rationale*: The effect of endotoxin on asthma morbidity in urban populations is unclear.**

***Objectives*: To determine if indoor pollutant exposure modifies the relationships between indoor airborne endotoxin and asthma health and morbidity.**

***Methods*: One hundred forty-six children and adolescents with persistent asthma underwent repeated clinical assessments at 0, 3, 6, 9, and 12 months. Home visits were conducted at the same time points for assessment of airborne nicotine, endotoxin, and nitrogen dioxide (NO_2) concentrations. The effect of concomitant pollutant exposure on relationships between endotoxin and asthma outcomes were examined in stratified analyses and statistical models with interaction terms.**

***Measurements and Main Results*: Both air nicotine and NO_2 concentrations modified the relationships between airborne endotoxin and asthma outcomes. Among children living in homes with no detectable air nicotine, higher endotoxin was inversely associated with acute visits and oral corticosteroid bursts, whereas among those in homes with detectable air nicotine, endotoxin was positively associated with these outcomes (interaction *P* value = 0.004 and 0.07, respectively). Among children living in homes with lower NO_2 concentrations (<20 ppb), higher endotoxin was positively associated with acute visits, whereas among those living in homes with higher NO_2 concentrations, endotoxin was negatively associated with acute visit (interaction *P* value = 0.05). NO_2 also modified the effect of endotoxin on asthma symptom outcomes in a similar manner.**

***Conclusions*: The effects of household airborne endotoxin exposure on asthma are modified by coexposure to air nicotine and NO_2, and these pollutants have opposite effects on the relationships between endotoxin and asthma-related outcomes.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Endotoxin exposure is known to have anti-Th2 and proinflammatory effects in animal model experiments and in human studies that examine its effects on the development of allergic diseases. However, very little is known about how endotoxin might affect asthma health and morbidity among people in community settings with established disease. Furthermore, animal model and human airway epithelial cell experiments suggest that coexposure to both endotoxin and certain pollutants can modify the effects of endotoxin on markers of airway inflammation, but whether pollutant exposure modifies the effect of endotoxin on asthma symptoms and/or morbidity, particularly in "real world" settings where people are known to be exposed to multiple biologically active substances, is unknown.

What This Study Adds to the Field

This study demonstrates that the respiratory effects of household endotoxin exposure are modified by coexposure to common indoor pollutants, air nicotine, and nitrogen dioxide (NO_2), but not particulate matter. Specifically, airborne endotoxin tends to be protective against markers of asthma morbidity in the setting of very low or absent air nicotine exposure, whereas it is associated with worse asthma in the setting of high air nicotine exposure. In contrast, airborne endotoxin is positively associated with asthma morbidity in the setting of low NO_2 exposure, but tends to be protective in the setting of high NO_2 exposure. These findings highlight the importance of interpreting the effects of endotoxin on asthma in the context of coexposure to cigarette smoke and NO_2. For example, smoking cessation or a home smoking ban might mitigate the deleterious effects of endotoxin on asthma, whereas the benefits of NO_2 reduction could be attenuated if endotoxin concentrations are high.

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Previous work has linked exposure to pest allergens (1, 2) and indoor pollutants, including second-hand smoke (SHS) (3), particulate matter (PM) (4), and nitrogen dioxide (NO_2) (5–7), to asthma symptoms, exacerbations, and acute care use in urban children with asthma. In contrast, endotoxin, an immunologically active component of gram-negative bacteria, has received less attention than allergens or pollutants despite the fact that housing characteristics common in urban communities, such as

lack of air conditioning and pest infestation, are associated with higher endotoxin concentrations. A few studies in urban birth cohorts found that endotoxin exposure was associated with a lower risk of eczema but a higher risk of wheeze (8, 9), a finding that is consistent with both the anti-Th2 effects observed in other cohorts (10–12) and the proinflammatory effects observed in occupational settings (13–15). However, whether endotoxin exposure affects asthma morbidity among urban children with established asthma is unclear, and understanding its potential effects is important for optimizing current approaches to environmental control.

In addition, recent animal model data suggest that the effects of endotoxin on lung disease may be modified by other coexposures, so that in some contexts endotoxin may be beneficial, and in other contexts it may be harmful (16, 17). For example, cigarette smoke extract potentiates the effects of endotoxin by activating the TLR4 receptor. Whether cigarette smoke or other pollutants potentiate or mitigate the effects of endotoxin on asthma in humans is unknown. Therefore, our *a priori* hypothesis was that endotoxin exposure would be associated with worse asthma, but that its effects would be modified by coexposure to indoor pollutants. We tested our hypothesis in a prospective cohort study of Baltimore City children and adolescents with persistent asthma who were followed for 1 year and underwent repeated clinical and environmental exposure assessments.

METHODS

Study Design and Population

Relationships between markers of asthma control, endotoxin, and indoor pollution were examined in an Institutional Review Board–approved prospective cohort study of 150 Baltimore City children and adolescents followed for 1 year. Written consent was obtained from parents or guardians of participants and assent was obtained from participants. Study visits occurred at baseline, 3, 6, 9, and 12 months and participants with at least one valid airborne endotoxin measurement were included, resulting in a sample size of 146 children. Further details can be found in the online supplement.

Study Visit Procedures

Skin prick testing was performed to 14 allergens at the baseline visit using the MultiTest II device (Lincoln Diagnostics, Decatur, IL). Total IgE was measured using the ImmunoCap system (ThermoFisher, Kalamazoo, MI). Spirometry and fractional exhaled nitric oxide measurement were performed at all study visits according to American Thoracic Society standards. Questionnaires were administered to parents or guardians at all study visits by study staff that captured demographic information, pulmonary and allergic history, medications, symptoms, and asthma-related health care use.

Home Environment Assessment

Airborne PM monitoring for PM less than or equal to 10 μm (PM_{10}) and less than or equal to 2.5 μm ($PM_{2.5}$) was conducted in the child's bedroom using integrated sampling methods for a 5- to 7-day period within 2 weeks of the clinic visit. Two PM_{10} samples were collected, one for extraction and analysis of airborne Mus m 1 content by ELISA, and one for extraction and analysis of endotoxin content. Endotoxin was measured with the PyroGene Recombinant Factor C Endotoxin Detection Assay (Lonza, Walkersville, MD). Concentrations were calculated from the standard curve, using a linear fit. Five hundred forty-two samples out of 577 (94%) had values above the level of the blank. Those below the level of the blank were excluded from analyses; the 31 samples that were above the blank but less than the lowest point on the standard curve were assigned half its value (i.e., 0.0008 EU/ml). NO_2 was measured with passive sampling badges (Ogawa and Co. Inc., Pompano Beach, FL) and analyzed spectrophotometrically according to standard methods. Nicotine was collected on a passive sampler and analyzed using gas chromatography with a nitrogen-phosphate detector (18).

Statistical Analyses

Relationships between airborne endotoxin concentrations and asthma-related outcome variables were first examined in bivariate analyses. For stratified analyses, NO_2 was dichotomized at 20 ppb because it was the median concentration and air nicotine was stratified at the 25th percentile, a level that is also approximately the limit of detection of the assay. Interaction terms were used in final models to formally test for interactions between endotoxin and pollutant exposure. Age, sex, lot of the endotoxin assay, airborne mouse allergen, total IgE, and controller medication were included in final statistical models. Measures of socioeconomic status, season, and PM did not confound the relationships between endotoxin and the outcomes of interest so were not included in the final statistical models. Analyses were performed with STATA 11.0/SE (StataCorp, College Station, TX). A P value less than 0.05 was considered statistically significant for main effects and a P value less than 0.10 was considered statistically significant for interactions as recommended by Selvin (19) for modestly sized study populations. Additional details can be found in the online supplement.

RESULTS

Study Population and Exposure Characteristics

The study population ranged in age from 5–17 years and was largely African American and of low socioeconomic status (Table 1). The clinical characteristics were consistent with a highly atopic population with persistent asthma. More than 50% reported having a smoker in the home, and 75% had detectable levels of air nicotine in the child's bedroom. PM and NO_2 concentrations were similar to concentrations observed in other urban populations (4, 5, 7), with median PM_{10}, $PM_{2.5}$, and NO_2 concentrations of 38.8 μg/m^3, 20.6 μg/m^3, and 20 ppb, respectively. The median endotoxin concentration was 0.009 EU/m^3, and airborne endotoxin was not correlated with either settled dust allergen levels or pollutant levels. However, airborne endotoxin was correlated with airborne mouse allergen concentrations ($r = 0.28$; $P = 0.003$) (*see* Table E1 in the online supplement).

Endotoxin and Air Nicotine

The association of endotoxin on asthma outcomes depended on the concentration of air nicotine and NO_2 in the home; thus, the effects of endotoxin are most accurately described in the context of these exposures. The air nicotine level modified the association of endotoxin on exacerbation-related outcomes, acute visit, and oral corticosteroid use (Table 2, Figure 1A). Specifically, among children living in homes with no detectable air nicotine, higher endotoxin was inversely associated with exacerbation-related outcomes (acute visit: odds ratio [OR], 0.38; 95% confidence interval [CI], 0.14–1.03) (oral corticosteroid bursts: OR, 0.85; 95% CI, 0.26–2.76), whereas among children living in homes with detectable air nicotine, endotoxin was positively associated with these outcomes (acute visit: OR, 1.15; 95% CI, 0.82–1.59) (oral corticosteroid burst: OR, 1.53; 95% CI, 1.04–2.25) (interaction P value = 0.004 and 0.07, respectively). Although a similar pattern was seen with bronchodilator reversibility, this interaction was not statistically significant (low air nicotine: OR, 0.44; 95% CI, 0.16–1.19) (high air nicotine: OR, 1.49; 95% CI, 0.16–1.19) (interaction P value = 0.16). There was no interaction between endotoxin and air nicotine on fractional exhaled nitric oxide, lung function, or symptoms or β-agonist use outcomes. Results of analyses of effect modification by report of smoker in the home and $PM_{2.5}$ were consistent with the findings with air nicotine, but interactions were not statistically significant (*see* Table E2). PM_{10} had no effect on relationships between endotoxin and asthma outcomes (data not shown).

TABLE 1. STUDY POPULATION CHARACTERISTICS (N = 146)

Demographic characteristics	
Female	63 (43.2)
Age, yr, median (range)	11 (5–17)
African American	133 (91.1)
Annual household income (n = 127)	
<$30,000	87 (68.5)
Median (IQR), $	22,500 (7,500–37,500)
Public health insurance	124 (84.9)
Caregiver less than high school education	43 (29.5)
Clinical characteristics	
Health care and medication use	
Hospitalization, previous 12 mo	28 (19.2)
Emergency department visit, previous 12 mo	119 (81.5)
Controller medication use*	105 (71.9)
β-Agonist use,† d/2 wk, mean (SD)	4.1 (5.0)
Lung function	
FEV_1/FVC%, mean (SD) (n = 130)	80.7 (9.6)
Bronchodilator reversibility (n = 128)	34 (26.6)
Allergic sensitization (n = 145)	
⩾1 positive skin prick test	131 (90.3)
Cat	93 (64.1)
Cockroach	88 (60.7)
Dust mite	81 (55.9)
Dog	25 (17.2)
Mouse	74 (51.0)
Total IgE, kU/L, median, IQR (n = 143)	178 (47–458)
Environmental exposures,‡ median (IQR)	
Airborne endotoxin, EU/m^3	0.009 (0.003–0.019)
Air nicotine, μg/m^3	0.12 (0.01–0.61)
NO_2, ppb	20.8 (14.0–31.0)
$PM_{2.5}$, μg/m^3	20.6 (13.2–30.6)
PM_{10}, μg/m^3	38.8 (26.1–51.9)
Smoker in the home, n (%)	80 (54.8)

Definition of abbreviations: IQR = interquartile range; NO_2 = nitrogen dioxide; PM_{10} = particulate matter less than or equal to 10 μm; $PM_{2.5}$ = particulate matter less than or equal to 2.5 μm.

Data are given as n (%) unless otherwise noted.

* Inhaled corticosteroid or leukotriene modifier.

† Short-acting β-agonist.

Air endotoxin, n = 124; air nicotine, n = 119; NO_2, n = 135; $PM_{2.5}$ and PM_{10}, n = 125.

Endotoxin and NO_2

NO_2 concentrations also modified the effect of endotoxin on health outcomes, but in the opposite direction of the air nicotine interaction (Table 3, Figure 1B). Specifically, among children living in homes with lower NO_2 concentrations (<20 ppb), higher endotoxin was positively associated with acute visits (OR, 1.27; 95% CI, 0.79–2.02), whereas among children living in homes with higher NO_2 concentrations, endotoxin was negatively associated with acute visits (OR, 0.80; 95% CI, 0.56–1.14; interaction P value = 0.05). The statistically significant interaction term indicates that the relationships between endotoxin and outcomes vary by NO_2 concentration, with generally positive associations with outcomes when NO_2 concentrations are lower and negative associations when NO_2 concentrations are higher. A similar pattern was observed with oral corticosteroid burst (low NO_2: OR, 2.09; 95% CI, 1.12–3.92) (high NO_2: OR, 1.05; 95% CI, 0.70–1.57) (interaction P value = 0.06).

The NO_2 concentration also modified the effect of endotoxin on symptoms outcomes. In homes with low NO_2 concentrations, higher endotoxin was not associated with most symptom outcomes, whereas among homes with high NO_2 concentrations, endotoxin was inversely associated with all symptoms outcomes, with statistically significant associations for all symptoms outcomes except nocturnal symptoms (Table 3, Figure 1B).

Combined Effects of Endotoxin, Nicotine, and NO_2

In models including main effect and interaction terms for endotoxin, nicotine, and NO_2, the interactions between endotoxin and nicotine and endotoxin and NO_2 persisted (Table 4, *see* Table E3). Predicted probabilities of an acute visit were calculated for given NO_2, nicotine, and endotoxin concentrations and are depicted in Table 4. There is a lower probability of having an acute visit with high than low endotoxin concentrations when air nicotine is low and NO_2 is high (12% vs. 76%, respectively). However, there is a higher probability of having an acute visit with high endotoxin than low endotoxin exposure when air nicotine is high and NO_2 is low (59% vs. 15%, respectively). There was no evidence of an interaction between NO_2 and air nicotine (data not shown).

DISCUSSION

This is the first study, to our knowledge, that has examined the effects of airborne endotoxin on established asthma in an urban population. Our findings demonstrate that the respiratory effects of household endotoxin exposure are modified by coexposure to common indoor pollutants, air nicotine, and NO_2. Specifically, airborne endotoxin tends to be protective against markers of asthma morbidity in the setting of very low or absent air nicotine exposure, whereas it is associated with worse asthma in the setting of high air nicotine exposure. In contrast, airborne endotoxin is positively associated with asthma morbidity in the setting of low NO_2 exposure, but tends to be protective in the setting of high NO_2 exposure. These findings highlight the importance of interpreting the effects of endotoxin on asthma in the context of coexposure to cigarette smoke and NO_2. Although prior literature has linked indoor NO_2 exposure to asthma symptoms and suggests that NO_2 reduction may improve asthma (5–7), NO_2 reduction may also modify the effects of concomitant endotoxin exposure on asthma. A more immediate public health implication is that reducing SHS exposure may not only reduce the direct effects of SHS on asthma, but may also reduce susceptibility to the adverse pulmonary effects of endotoxin, so could provide greater benefit than expected.

Interestingly, both protective and adverse effects of endotoxin were observed, and these effects depended on the concentrations of NO_2 and air nicotine, but not PM (either size fraction), in the home. The interactions between these pollutants and endotoxin have not been described in human populations previously, but *in vitro* studies and animal models support the biologic plausibility of these interactions. The fact that air nicotine, rather than PM, influenced the effects of endotoxin suggests that the underlying mechanism is specific to SHS exposure, and possibly specific to the nonparticle components of SHS. In addition to PM from tobacco smoke, nicotine is a marker for many irritant gases in SHS (e.g., aldehydes) (18). Using a less specific marker of tobacco smoke, such as PM, would result in nondifferential exposure misclassification and attenuate the interaction with endotoxin, as was observed in this study.

The endotoxin × nicotine interaction observed in this study is supported by data from mouse models of asthma and human bronchial epithelial cell experiments showing that cigarette smoke exposure enhances TLR4 expression and activity, thereby increasing sensitivity to endotoxin (17, 20–22). Although there is no evidence to suggest that NO_2 directly affects the TLR4 receptor, NO_2 does seem to activate the TLR2 receptor (23), and TLR2 activation can dampen TLR4 signaling through a microRNA-dependent process (24). In addition, NO_2 has been shown to attenuate endotoxin-induced cytokine production in human alveolar macrophages (25). Thus, enhancement of TLR4 expression and/or signaling by cigarette smoke

TABLE 2. EFFECT OF AIR NICOTINE ON ASSOCIATIONS BETWEEN AIRBORNE ENDOTOXIN AND ASTHMA OUTCOMES

	Low Air Nicotine (<0.01 μg/m³)		High Air Nicotine (≥0.01 μg/m³)		
	Crude OR/β (95% CI)	Adjusted* OR/β (95% CI)	Crude OR/β (95% CI)	Adjusted* OR/β (95% CI)	Interaction *P* Value†
Acute visit	**0.42 (0.21 to 0.83)**	0.38 (0.14 to 1.03)	1.21 (0.90 to 1.62)	1.15 (0.82 to 1.59)	**0.004**
Oral corticosteroid burst	0.69 (0.34 to 1.41)	0.85 (0.26 to 2.76)	**1.49 (1.06 to 2.08)**	**1.53 (1.04 to 2.25)**	**0.07**
Reversibility	0.58 (0.27 to 1.27)	0.44 (0.16 to 1.19)	**1.46 (1.04 to 2.04)**	**1.49 (1.02 to 2.18)**	0.16
FEV_1/FVC % (β, 95% CI)	0.50 (−1.91 to 2.91)	0.09 (−2.27 to 2.46)	−0.35 (−1.34 to 0.64)	−0.29 (−1.32 to 0.74)	0.97
$F_{E_{NO}}$ (β, 95% CI)	0.02 (−0.06 to 0.10)	0.03 (−0.07 to 0.12)	−0.01 (−0.06 to 0.03)	−0.02 (−0.06 to 0.02)	0.17
β-Agonist use	0.79 (0.50 to 1.26)	1.03 (0.63 to 1.69)	0.87 (0.72 to 1.05)	**0.78 (0.62 to 0.98)**	0.52
Max symptoms, d‡	0.73 (0.47 to 1.14)	0.80 (0.43 to 1.47)	0.94 (0.77 to 1.15)	0.92 (0.74 to 1.14)	0.50
Wheeze, cough, chest tight	0.86 (0.53 to 1.41)	1.06 (0.58 to 1.96)	1.05 (0.83 to 1.34)	0.99 (0.75 to 1.31)	0.66
Exercise-related symptoms	0.61 (0.37 to 1.00)	0.53 (0.19 to 1.51)	0.75 (0.56 to 1.02)	0.77 (0.56 to 1.05)	0.31
Nocturnal symptoms	0.80 (0.34 to 1.85)	1.23 (0.45 to 3.37)	0.88 (0.66 to 1.17)	0.87 (0.63 to 1.18)	0.66
Slowed activity	0.64 (0.36 to 1.14)	0.64 (0.30 to 1.36)	0.91 (0.71 to 1.16)	0.91 (0.70 to 1.18)	0.57

Definition of abbreviations: CI = confidence interval; $F_{E_{NO}}$ = fractional exhaled nitric oxide; OR = odds ratio.
Bold text indicates statistically significant results.
*Adjusted for age, sex, lot of recombinant factor C reagent, air mouse allergen concentration, total IgE, and controller medication.
† Derived from adjusted models including main effects for air nicotine and endotoxin and a linear interaction term [log(nicotine) × log(endotoxin)].
‡ Highest number of days of slowed activity, nocturnal symptoms, or exercise-related symptoms.

exposure could potentiate the adverse effects of endotoxin, and down-regulation of TLR4 signaling by NO_2 activation of the TLR2 receptor could attenuate the adverse the effects of endotoxin.

Home predictors of endotoxin concentrations have been examined in quite a few studies, but only a handful have examined predictors of airborne endotoxin (26, 27), and none, to our knowledge, have examined predictors of endotoxin measured using the recombinant factor C (rFC) assay. In our study, we did not find any association between settled dust allergen and airborne endotoxin concentrations. However, airborne mouse allergen was weakly, but significantly, correlated with airborne endotoxin, suggesting that mouse infestation could contribute to endotoxin concentrations. An association between mouse infestation and settled dust endotoxin concentration has also been reported in another urban population (8).

In this study, we also used a newer assay for quantifying endotoxin, the rFC, which has implications for our findings. First, the rFC assay has the benefit of being highly specific for endotoxin, whereas the limulus amebocyte lysate (LAL) assay detects fungal glucans in addition to endotoxin (28). It is not surprising, then, that the home airborne endotoxin concentrations measured in this study are lower than those measured in studies using the LAL assay (27, 28). Although we and others have not methodologically compared LAL- with rFC-measured airborne endotoxin levels from community homes, a methods comparative study of

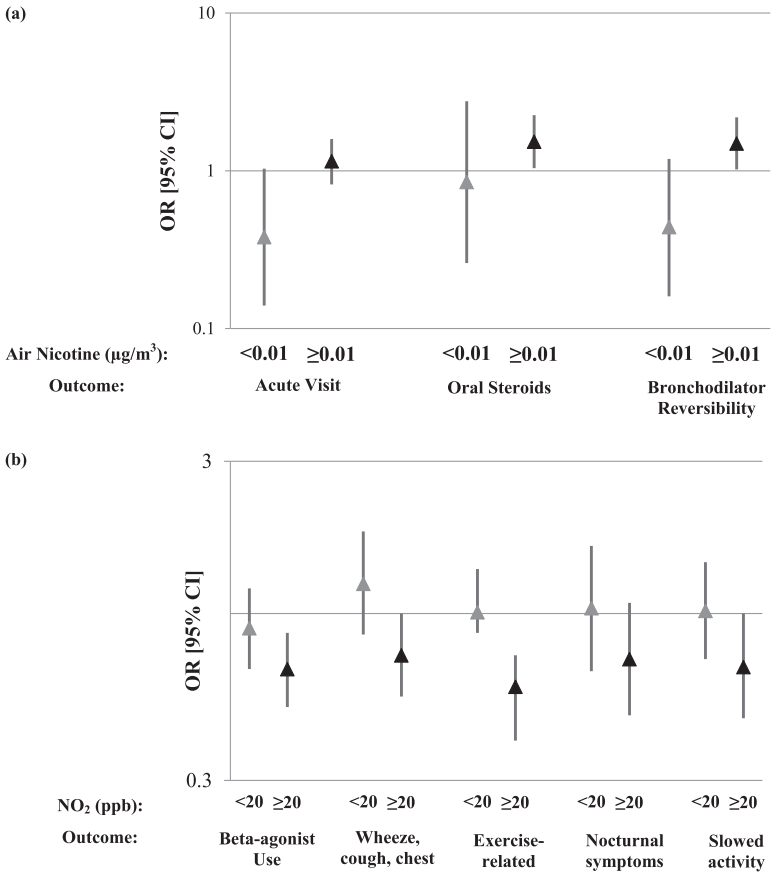

Figure 1. Associations between airborne endotoxin and asthma outcomes by (*A*) air nicotine and (*B*) nitrogen dioxide (NO_2) concentrations. CI = confidence interval; OR = odds ratio.

TABLE 3. EFFECT OF NO_2 ON ASSOCIATIONS BETWEEN AIRBORNE ENDOTOXIN AND ASTHMA OUTCOMES

	Low NO_2 (<20 ppb)		High NO_2 (⩾20 ppb)		
	Crude OR/β (95% CI)	Adjusted* OR/β (95% CI)	Crude OR/β (95% CI)	Adjusted* OR/β (95% CI)	Interaction *P* Value†
Acute visit	1.44 (0.96 to 2.16)	1.27 (0.79 to 2.02)	0.92 (0.68 to 1.26)	0.80 (0.56 to 1.14)	**0.05**
Oral corticosteroid burst	**2.09 (1.20 to 3.62)**	**2.09 (1.12 to 3.92)**	1.09 (0.77 to 1.54)	1.05 (0.70 to 1.57)	**0.06**
Reversibility	1.06 (0.69 to 1.62)	1.07 (0.62 to 1.83)	1.28 (0.91 to 1.79)	1.26 (0.86 to 1.84)	0.46
FEV_1/FVC (β, 95% CI)	−0.22 (−1.65 to 1.21)	−0.07 (−1.66 to 1.53)	0.19 (−1.04 to 1.41)	−0.18 (−1.46 to 1.09)	0.84
$F_{E_{NO}}$ (β, 95% CI)	−0.02 (−0.08 to 0.04)	0.004 (−0.06 to 0.06)	0.008 (−0.04 to 0.05)	−0.005 (−0.05 to 0.04)	0.57
β-Agonist use	1.02 (0.79 to 1.31)	0.90 (0.67 to 1.20)	**0.75 (0.58 to 0.95)**	**0.67 (0.51 to 0.87)**	0.15
Max symptoms, d‡	1.12 (0.85 to 1.46)	1.07 (0.78 to 1.45)	0.77 (0.59 to 1.01)	**0.72 (0.52 to 1.00)**	**0.08**
Wheeze, cough, chest tight	1.26 (0.93 to 1.69)	1.25 (0.86 to 1.81)	0.82 (0.63 to 1.07)	**0.74 (0.55 to 1.00)**	**0.02**
Exercise-related symptoms	1.00 (0.77 to 1.29)	1.01 (0.74 to 1.38)	**0.64 (0.47 to 0.89)**	**0.59 (0.40 to 0.87)**	**0.01**
Nocturnal symptoms	1.02 (0.71 to 1.46)	1.04 (0.66 to 1.63)	0.74 (0.53 to 1.04)	0.72 (0.48 to 1.08)	0.18
Slowed activity	1.09 (0.80 to 1.51)	1.02 (0.72 to 1.45)	**0.73 (0.53 to 0.99)**	**0.68 (0.47 to 1.00)**	0.27

Definition of abbreviations: CI = confidence interval; $F_{E_{NO}}$ = fractional exhaled nitric oxide; NO_2 = nitrogen dioxide; OR = odds ratio.

Bold text indicates statistically significant results.

* Adjusted for age, sex, lot of rFC reagent, air mouse allergen concentration, total IgE, and controller medication.

† Derived from adjusted models including main effects for NO_2 (dichotomized at 20 ppb); endotoxin; and their interaction term [NO_2 (dichotomized) × log (endotoxin)].

‡ Highest number of days of either slowed activity, nocturnal symptoms, or exercise-related symptoms.

house dust endotoxin concentrations found the LAL measures to be nearly double the rFC measures (29). Lower endotoxin levels in our study might be attributable to the greater specificity of the rFC assay for endotoxin, and differences in sampling locales (bedroom vs. family room; Baltimore vs. Boston) and well-established differences in endotoxin levels measured in same samples by different laboratories (30, 31). Although one study comparing rFC with LAL reported that the two assays provided similar results, this study was performed in a laboratory setting and in livestock facilities, where there may be less fungi relative to endotoxin than in homes, which would increase the concordance of the measurements obtained from rFC and LAL assays (32). Although rFC has been criticized for being less sensitive than LAL, in our study, the lower limit of detection (LLOD) was 0.0016 EU/ml, which translates to an LLOD for airborne concentration of 0.0003 EU/m^3 for a sample collected at 4 L per minute for 5 days. The greater specificity of the rFC assay for endotoxin also may have reduced exposure misclassification in this study, thereby increasing the likelihood of observing an endotoxin effect, which may have been missed by using a less specific assay, such as LAL. Although rFC has been criticized for being less sensitive than LAL, in our study the LLOD was 0.0016 EU/ml, which translates to an LLOD for airborne concentration of 0.0003 EU/m^3 for a sample collected at 4 L per minute for 5 days.

One limitation of our study is the fact that the population is drawn from a single site and is predominantly African American and low income so the findings may not be generalizeable; however, Baltimore children with asthma are similar to those living in other urban centers both with respect to sociodemographic characteristics and pollutant exposures, including SHS exposure and NO_2. It is also possible that there were unmeasured exposures that are associated with airborne endotoxin and also activate TLR4, and that the interactions we observed are confounded by these other exposures. Dust mite allergen, which is one such potential confounder, was absent in a substantial proportion of the homes and when detected, present in very low concentrations, so is unlikely to explain the endotoxin × pollutant interactions that we observed. Although less than 30% of the study population demonstrated bronchodilator reversibility, this finding is not surprising because reversibility is known have low sensitivity for asthma in pediatric populations. Another potential limitation is the lack of settled dust endotoxin data; however, others have suggested that measurement of airborne endotoxin is a better approach to exposure assessment for respiratory outcomes (26, 27). We also did not observe statistically significant interactions for all outcomes, so there is potential for type 1 error, which underscores the need to test these hypotheses in future studies. Although the prospective design of the study is a strength, ultimately these interactions should be examined in the context of an environmental intervention trial, which would provide stronger evidence for a causal relationship than an observational study.

TABLE 4. PREDICTED PROBABILITY OF AN ACUTE VISIT UNDER DIFFERENT EXPOSURE LEVELS OF NO_2, AIR NICOTINE, AND AIR ENDOTOXIN*

NO_2†	Air Nicotine‡	Endotoxin§	Predicted Probability of Acute Visit, %	95% Confidence Interval
Low NO_2	Low nicotine	Low endotoxin	52	27–78
	Low nicotine	High endotoxin	22	7–37
	High nicotine	Low endotoxin	15	4–26
	High nicotine	High endotoxin	59	43–76
High NO_2	Low nicotine	Low endotoxin	76	55–97
	Low nicotine	High endotoxin	12	2–21
	High nicotine	Low endotoxin	34	21–47
	High nicotine	High endotoxin	41	27–55

Definition of abbreviations: LLOD = lower limit of detection; NO_2 = nitrogen dioxide.

* Predicted probabilities derived from logistic regression models with generalized estimating equations adjusted for age, sex, lot of recombinant factor C reagent, air mouse allergen concentration, total IgE, and controller medication.

† Low NO_2, less than 20 ppb; high NO_2, greater than or equal to 20 ppb.

‡ Low and high nicotine were defined as the 10th and 90th percentiles, which were less than LLOD and 1.3 μg/m^3, respectively. Less than LLOD was assigned a value one-half of that of the LLOD.

§ Low and high endotoxin were defined as the 10th and 90th percentiles, which were 0.0006 and 0.041 EU/m^3, respectively.

Our findings suggest that the combination of high prevalence of SHS exposure and concomitant indoor endotoxin exposure may explain, in part, the disproportionate asthma morbidity observed in urban populations. These findings also highlight the importance of understanding the complexity of indoor exposures and their effects on asthma to develop effective approaches to environmental control. For example, smoking cessation or a home smoking ban might mitigate the deleterious effects of endotoxin on asthma, whereas the benefits of NO_2 reduction could be attenuated if endotoxin concentrations are high. Because these environment-environment interactions could have important patient care and public health implications, further studies that attempt to replicate these novel interactions should be performed in other settings.

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

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