



Contents lists available at ScienceDirect



Indoor endotoxin, proximity to a major roadway, and severe asthma exacerbations among children in Puerto Rico

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ARTICLE INFO

Article history:

Received for publication June 25, 2020.

Received in revised form August 31, 2020.

Accepted for publication September 2, 2020.

ABSTRACT

Background: Few studies have examined concurrent exposure to household endotoxin and traffic-related air pollution in relation to childhood asthma, yet both factors are associated with asthma outcomes.

Objective: To examine whether proximity to a major roadway (a traffic-related air pollution proxy) modifies the estimated effects of indoor endotoxin on asthma outcomes in children.

Methods: Cross-sectional study of 200 children with asthma (ages, 6–14 years) living in Puerto Rico. Residential distance to a major roadway was calculated as the distance from the participant's residential US census block centroid to the nearest major road. The outcomes of interest were severe asthma exacerbations, missed school days for asthma, atopy, lung function, and bronchodilator response (BDR). Logistic, linear, or negative binomial regression was used for the multivariable analysis.

Results: In the multivariable analysis, there was an interaction between indoor endotoxin and residential distance to a roadway on severe asthma exacerbations ($P = .02$) and BDR ($P = .07$). In an analysis stratified by distance to a roadway, each $\log_{10}$ -unit increase in endotoxin was associated with 4.21 times increased odds of severe asthma exacerbations among children living within 499 m (the lower 3 quartiles of residential distance) to a road (95% confidence interval, 1.5–12.0). Among subjects living further than 499 m away from a roadway, each $\log_{10}$ -unit increase in endotoxin was associated with reduced odds of severe asthma exacerbations (odds ratio, 0.03; 95% confidence interval, 0.001–0.67). Similar but less substantial findings were observed for BDR.

Conclusion: Our findings suggest that residential proximity to a major road modifies the estimated effect of endotoxin on severe asthma exacerbations in children.

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Introduction

Asthma is the most common chronic respiratory disease of childhood, affecting more than 6 million children in the United

States.¹ In this country, asthma disproportionately burdens minority and economically disadvantaged children.²

Severe asthma exacerbations are a major cause of school absences, caregiver missed work days, and health care costs for asthma.³ Children with persistent asthma and at least 1 severe exacerbation in the previous year have a 2-fold increased risk of subsequent severe exacerbations despite use of controller medications,⁴ a finding that suggests individual susceptibility owing to poorly understood risk factors, including environmental exposures.

Proximity to a major roadway (a proxy for traffic-related air pollution)^{5–8} and household endotoxin (a cell-wall component of gram negative bacteria)^{9,10–13} are both common in urban environments and independently associated with worsening of asthma—including emergency department (ED) visits—in children.

Endotoxin is present not only in household dust but also in particulate matter.¹⁴ Although animal and in vitro studies suggest that

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Disclosures: Dr Celedón has received research materials from GlaxoSmithKline and Merck & Co, Inc (inhaled steroids) and Pharmavite (vitamin D and placebo capsules) to provide medications free of cost to the participants in the National Institutes of Health-funded studies, unrelated to this work. The remaining authors have no conflicts of interest to report.

Funding: This study was supported by grants HL079966, HL117191, and MD011764 from the US National Institutes of Health (NIH). Dr Rosser's contribution was supported by grant KL2 TR001856 from the US NIH. Dr Forno's contribution was supported by grant HL149693 from the US NIH.

<https://doi.org/10.1016/j.anai.2020.09.001>

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co-exposure to air pollution and endotoxin¹⁴ increases airway inflammation, few human studies have examined the relation between concurrent exposure to air pollution and endotoxin and childhood asthma.^{15–17}

A recent study of children and adults living in the contiguous United States revealed synergistic effects between 2 outdoor air pollutants (nitrogen dioxide [NO₂] and particulate matter of <2.5 μm [PM_{2.5}]) and household dust endotoxin on ED visits for asthma in children.¹⁶ Proximity to a major roadway, which was not assessed in that study, differs from the US Environmental Protection Agency assessment of criteria pollutants in several ways. First, proximity to a major roadway encompasses pollutants, such as diesel-exhaust particles and ultrafine particulate matter,^{18,19} which may not be captured by central monitoring stations owing to decremental decay from roadways. Ultrafine particulate matter is small (<0.1 μm), is more likely to reach the pulmonary alveoli than larger PM,²⁰ and has been associated with asthma exacerbations.^{21,22} Second, particulate matter collected near roadways has been found to cause worse inflammation than background (ambient) particle pollution.¹⁴ Third, residential proximity to a major roadway can be easily calculated and thus may have clinical value.

In the United States, Puerto Ricans have a greater burden of asthma than White non-Hispanics or members of other Hispanic subgroups.²³ The reasons for this are insufficiently understood but likely include exposure to environmental risk factors, such as pollutants,²³ which could affect expression of asthma-susceptibility genes through gene-by-environment interactions or epigenetic mechanisms.^{24,25} Given the ubiquity of household endotoxin and recent findings,¹⁶ we hypothesized that concurrent exposure to household endotoxin and living near a major roadway would have joint detrimental effects on asthma among Puerto Rican children living in the island of Puerto Rico. We examined this hypothesis in a cross-sectional study of children with asthma in Puerto Rico.

Methods

Study Population

Subject recruitment and study procedures have been previously described in detail.⁶ In brief, from March 2009 to June 2010, a total of 351 children with asthma (defined as physician-diagnosed asthma and wheezing in the previous year) were recruited from randomly selected households in the metropolitan area of San Juan (Puerto Rico), using multistage probability sampling. All study participants were aged 6 to 14 years, had 4 Puerto Rican grandparents, and had lived in the same household for at least 1 year. Of the 351 study participants, 200 had endotoxin levels measured in house dust and were, thus, included in this analysis.

Study Procedures

At the same study visit, the participants completed a protocol, including questionnaires, spirometry, and collection of blood and house dust samples. The child's parents (usually [93%] the mother) completed a questionnaire slightly modified from one used in the Collaborative Study on the Genetics of Asthma²⁶ and a separate questionnaire on household characteristics. These questionnaires were used to obtain information about the child's general and respiratory health, sociodemographic characteristics, family history, exposure to secondhand smoke (SHS), pet ownership, and respiratory health.

Spirometry was conducted with the EasyOne spirometer (NDD Medical Technologies, Andover, Massachusetts). The participants were free of respiratory infections for at least 4 weeks before testing and were instructed (when possible) to avoid use of inhaled short-acting and long-acting bronchodilators for 4 and 12 or more hours before testing, respectively. Expiratory maneuvers were

judged acceptable if they met or exceeded the American Thoracic Society criteria for children.²⁷ The best forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁) values were selected for analysis. Percentage predicted values were then calculated utilizing Global Lung Initiative (GLI2012) prediction equations.²⁸ After completing spirometry, the subjects were given 200 mg (2 puffs) of an albuterol metered-dose inhaler using a spacer, and spirometry was repeated after 15 minutes.

Dust samples were collected from the following 3 different locations in a participant's household: the one in which the child usually slept, the living or television room, and the kitchen. The dust samples were combined and then sifted through a 50-mesh metal sieve. The fine dust was reweighed, extracted, and aliquoted for analysis of allergens using monoclonal antibody Multiplex array assays that used the same reagents as in the established enzyme-linked immunosorbent assay (dust mite [Der p 1], cockroach [Bla g 1], and mouse urinary protein [Mus m 1]).

After the household dust was used for analysis of allergens, the remaining dust samples were weighed and aliquoted in a gravimetrics laboratory with a filtered air supply and precise temperature and humidity control. The samples were weighed to the nearest microgram using a dual chamber microbalance (Model XP26; Mettler, Columbus, Ohio). Endotoxin was measured by kinetic chromogenic Limulus amoebocyte lysate assay, using multiple dilutions and a 12-point standard curve based on control standard endotoxin from *Escherichia coli* ranging from 0.0244 to 50.0 endotoxin unit (EU)/mL.²⁹ Nondetectable levels were assigned a constant value (half the lowest detectable level). Afterward, endotoxin levels were converted to the log₁₀ scale for data analysis. Given that dust endotoxin levels can vary by season,³⁰ we also recorded the month and season of collection (dry: December to April and wet: May to November) of dust collection in Puerto Rico.

Serum immunoglobulin (Ig)E to each of 5 common allergens in Puerto Rico (dust mite [Der p 1], cockroach [Bla g 2], cat dander [Fel d 1], dog dander [Can f 1], and mouse urinary protein [Mus m 1]) were determined by using the UniCAP 100 system (Pharmacia & Upjohn, Kalamazoo, Michigan). For each allergen, an IgE level of 0.35 IU/mL or greater was considered positive.

Proximity to a major roadway was determined for each participant as previously described.⁶ In brief, home residential addresses were geocoded to US census block (smallest census unit) at the University of Puerto Rico and Euclidean distance in meters from block centroid to nearest major roadway was obtained in ArcMap (ArcGIS 10.1; Esri, Redlands California). A major roadway was defined by Esri 2012 Data and Maps major road layer as any interstate, intermetropolitan area and intrastate highways, and other major roads.

Written parental consent was obtained from the participating children, from whom written assent was also obtained. The study was approved by the institutional review boards of the University of Puerto Rico (San Juan, Puerto Rico), Brigham and Women's Hospital (Boston, Massachusetts), and the University of Pittsburgh (Pittsburgh, Pennsylvania).

Statistical Analysis

Our outcomes of interest were at least 1 severe asthma exacerbation (defined as a hospitalization or visit to the ED or urgent care requiring treatment with systemic corticosteroids or having received a course of systemic steroids for asthma³¹) in the previous year, average number of missed school days per month owing to asthma in the previous year, atopy (defined as at least 1 positive IgE to the allergens tested), FEV₁ and FEV₁/FVC (percentage predicted or <−1.64 z-score based on the GLI2012 prediction equations²⁸), and bronchodilator response (BDR) (defined as an increment in FEV₁ equal or greater than 12% after administration of inhaled albuterol).

Bivariate analyses were conducted using Wilcoxon ranked sum test and χ^2 tests for categorical variables, and the Spearman's correlation test for continuous variables. Logistic (eg, for at least 1 severe asthma exacerbation and atopy), linear (eg, for FEV₁ and FEV₁/FVC), and negative binomial (for average missed school days per month) regression were used for the multivariable analyses of the outcomes of interest. All models included residential distance to a roadway (in meters), endotoxin levels, and an interaction term (residential distance to a roadway $\times$ endotoxin). We decided a priori to adjust for household income (<\$15,000 vs $\geq$ \$15,000 per year, the median annual household income in Puerto Rico in 2008–2009),³² current exposure to SHS (with the exception of models for percentage predicted FEV₁ and FEV₁/FVC), age, and sex. In separate models, we further adjusted for covariates if they were differentially associated with either exposure at $P < .05$. Interaction terms were considered statistically significant at $P < .10$.¹⁵ In the case of a significant interaction term, the analysis was stratified by highest quartile of residential distance to a road vs others, as previously described.¹⁶ All analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina).

Results

eTable 1 indicates a comparison of the main characteristics of children with asthma who were ($n = 200$) and were not ($n = 151$) included in this analysis. Compared with children excluded from this analysis owing to a lack of available endotoxin measurements, those included were less likely to be exposed to SHS but more likely to have an annual household income $\geq$ \$15,000 per year and to have higher levels of dust mite and cockroach allergens in house dust.

Table 1 summarizes the main characteristics of the 200 study participants, whose households had a geometric mean dust endotoxin level of 36.9 EU/mg. Approximately half of all participants reported SHS exposure and having had at least 1 severe asthma exacerbation in the previous year, but only 1% had carpets or rugs in their bedroom or living room. Nearly 80% of the participants had electric stoves and 65% were atopic, but relatively few had a reduced FEV₁ (21%) or FEV₁/FVC (32%). Whereas nearly two-thirds of the participants owned a dog, less than a fifth owned a cat.

Table 2 illustrates a comparison of mean endotoxin or mean residential distance to a major road by selected variables. Endotoxin was significantly higher in homes reporting current cat ownership than in those reporting no cat ownership (57.7 EU/mg vs 34.2 EU/mg, $P < .005$).

An annual household income of $\geq$ \$15,000 per year, no exposure to SHS, having an electric stove, and having had the dust sample collected during the wet season were each significantly associated with greater residential distance to a major roadway ($P < .05$ in all instances).

Children who did not report a severe asthma exacerbation in the previous year or who had fewer reported missed school days lived further away from a major roadway, but these associations were not statistically significant ($P = .08$ –.11). Endotoxin was weakly and positively correlated with mouse urinary protein allergen ($P = .04$) and weakly and negatively correlated with dust mite allergen (eTable 2). Residential distance to a major roadway was weakly and negatively correlated with German cockroach allergen ($P < .01$).

We found an interaction (at $P < .10$) between residential distance to a major roadway and endotoxin level on severe asthma exacerbations ($P = .02$) and BDR ($P = .07$). No such interactions were detected for any of the other outcomes of interest (eTable 3). Figure 1 reveals the results of the analysis of endotoxin level and at least 1 severe asthma exacerbation, after stratification by residential distance to a major roadway (as upper quartile or >499 m vs the others, for ease of exposition). Among children living closer to (or

Table 1

Main Characteristics of Study Participants ($n = 200$)

Variable	Median (IQR) or n (%) ^a
Age, y	10 (8–12)
Female sex, n (%)	82 (41)
Annual household income $\geq$ \$15,000 per year, n (%)	46 (23)
Endotoxin (EU/mg)	
Geometric mean (SE)	36.9 (2.6)
Median (IQR)	33.4 (20.9–58.8)
5th–99th percentile	8.9–189.1
Residential distance to a major roadway, m	
Geometric mean (SE)	243 (2.5)
Median (IQR)	210 (172–499)
5th–99th percentile	50–1007
Secondhand tobacco smoke exposure, current, n (%)	105 (53)
Rugs/carpet in child's bedroom or living room, n (%)	2 (1)
Current dog ownership, ^b n (%)	126 (63)
Current cat ownership, ^b n (%)	28 (14)
Log ₁₀ mouse urinary protein (Mus m 1, ng/g) in house dust ^c	0.78 (0.30–1.62)
Log ₁₀ dust mite allergen (Der p 1, μ g/g) in house dust ^c	0.72 (0.43–1.03)
Log ₁₀ German cockroach allergen (Bla g 2, U/g) in house dust ^c	0.18 (–0.10 to 1.10)
Gas stove (vs electric), n (%)	43 (22)
Wet season of dust collection, ^d n (%)	119 (60)
At least 1 severe asthma exacerbation in previous year, n (%)	102 (51)
BDR, $\geq$ 12%, n (%)	30 (19)
Atopy, ^e n (%)	125 (65)
FEV ₁ % predicted	92.5 (82.2–102.5)
Reduced (z-score $\leq$ –1.64), n (%)	36 (21)
FEV ₁ /FVC ratio % predicted (GLI2012)	92.8 (85.9–97.3)
Reduced (z-score $\leq$ –1.64), n (%)	55 (32)
Average school days missed per month owing to asthma	2 (0–5)

Abbreviations: BDR, bronchodilator response; Dec, December; EU, endotoxin unit; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GLI2012, Global Lung Initiative 2012; IQR, interquartile range; Nov, November.

^aValues displayed as median (IQR) or n (%) unless otherwise specified.

^bCurrent ownership represents current or within the previous 6 months reported ownership.

^cHousehold dust was collected from bedroom, living/television room, kitchen, and combined.

^dDry season is from Dec to April; wet season is from May to Nov.

^eAtopy defined as at least 1 positive specific immunoglobulin E to 1 of 5 aeroallergens (cat, dog, dust mite, cockroach, mouse).

within 499 m of) a major roadway, each log-unit increment in endotoxin level was associated with 2.33 increased odds of at least 1 severe asthma exacerbation in the previous year, after adjustment for age, sex, annual household income, and current exposure to SHS (model 1). After additional adjustment for cat ownership, indoor allergen levels, type of gas stove in the home, and season of dust collection, each log-unit increment in endotoxin level was associated with 4.21 increased odds of at least 1 severe asthma exacerbation in the previous year (model 2). Among children living >499 m away from a major roadway, endotoxin level was associated with 0.03 to 0.077 times decreased odds of at least 1 severe asthma exacerbation, after adjustment for relevant covariates (models 1 and 2). Similar but less substantial associations between endotoxin level and BDR were observed in the analysis stratified by residential distance to a major roadway.

Table 3 lists the predicted probabilities of having had at least 1 severe asthma exacerbation in the previous year, by residential distance to a roadway and endotoxin level. High endotoxin levels had differential effects on predicted probability of severe asthma exacerbations, depending on proximity to a major roadway (close vs further away). No such effect of endotoxin on probabilities for BDR was found in an analysis stratified by residential distance to a roadway.

Figure 2 reveals the predicted probability plots for the analysis using models with continuous household endotoxin $\times$

Table 2

Geometric Mean (95% CI) Endotoxin Level and Proximity to a Major Roadway, by Characteristics of Study Participants (n = 200)

Characteristic	Endotoxin (EU/mg)		Proximity to a major roadway (m)	
	GM (95% CI)	P	GM (95% CI)	P
Age, y		.13		.46
6–10	33.9 (29.0–39.6)		237 (200–280)	
11–15	41.8 (33.1–52.8)		252 (204–312)	
Sex		.52		.80
Female	38.5 (30.8–48.1)		238 (191–296)	
Male	35.9 (30.4–42.3)		247 (210–291)	
Annual household income $\geq$ \$15,000		.61		.04 ^a
Yes	40.2 (30.4–53.1)		299 (240–373)	
No	36.2 (31.0–42.3)		233 (199–272)	
Secondhand tobacco smoke exposure, current		.69		.01 ^a
Yes	38.1 (32.2–45.0)		217 (181–260)	
No	35.8 (28.9–44.4)		274 (227–332)	
Current dog ownership		.83		.17
Yes (currently or in previous 6 mo)	36.5 (30.8–43.3)		261 (223–306)	
No (never or not in previous 6 mo)	37.6 (30.3–46.7)		215 (170–271)	
Current cat ownership		.005		.09
Yes (currently or in previous 6 mo)	57.7 (41.2–80.9)		199 (137–290)	
No (never or not in previous 6 mo)	34.2 (29.7–39.5)		253 (220–291)	
Stove type		.33		.04 ^a
Gas	44.6 (32.8–60.7)		302 (233–393)	
Electric	35.1 (30.3–40.6)		229 (197–266)	
Season of dust collection		.83		<.001 ^a
Dry	35.9 (29.4–43.8)		188 (156–227)	
Wet	37.7 (31.5–45.0)		289 (243–344)	
Severe asthma exacerbation in previous year		.55		.11
Yes	37.8 (31.1–46.0)		213 (177–256)	
No	36.0 (30.1–43.1)		279 (232–335)	
BDR, $\geq$ 12%		.94		.55
Yes	42.6 (29.8–60.8)		218 (156–303)	
No	41.2 (34.8–48.8)		236 (200–278)	
Atopy		.41		.14
Yes	38.7 (32.3–46.4)		255 (216–301)	
No	34.9 (28.5–42.9)		213 (171–265)	
FEV ₁ z-score		.16		.68
$\leq$ -1.64 (reduced)	34.0 (25.9–44.7)		232 (173–310)	
$>$ -1.64 (normal)	42.2 (36.0–49.5)		239 (204–280)	
FEV ₁ /FVC z-score		.93		.48
$\leq$ -1.64 (reduced)	39.1 (30.9–49.3)		259 (203–330)	
$>$ -1.64 (normal)	41.0 (34.5–48.8)		228 (192–270)	
Average asthma school days missed per month		.11		.08
0–3	34.0 (28.8–40.3)		265 (224–314)	
$\geq$ 4	41.8 (33.5–52.1)		202 (163–250)	

Abbreviations: BDR, bronchodilator response; CI, confidence interval; EU, endotoxin unit; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GM, geometric mean.

NOTE: P values obtained from Wilcoxon ranked sum test.

^aP value of <.05.

proximity to a roadway for at least 1 severe asthma exacerbation in the previous year and BDR. For children living closer to a major roadway, increasing endotoxin concentrations were

associated with higher predicted probability of a severe asthma exacerbation and positive BDR. However, when living further away from a major roadway, increasing endotoxin levels are

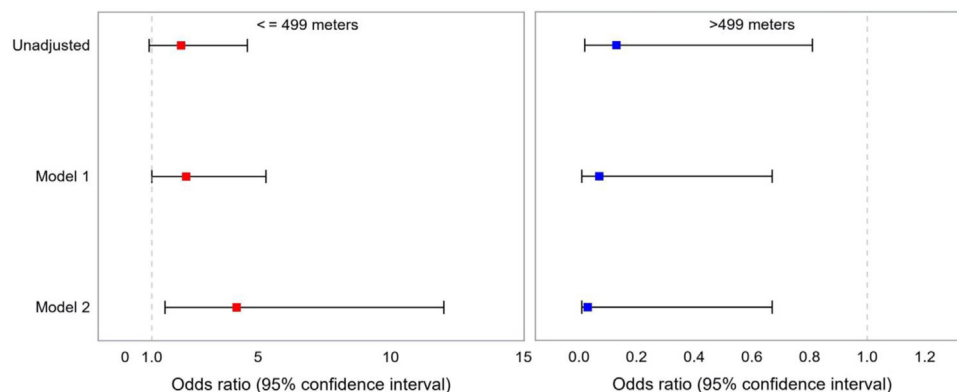


Figure 1. Results of the multivariable analysis of the relation between house dust endotoxin and severe asthma exacerbations, stratified by residential distance to a major road. Model 1 was adjusted for age, sex, annual household income, and current exposure to SHS. Model 2 was additionally adjusted for cat ownership, indoor allergen levels, type of gas stove in the home, and season of dust collection. SHS, secondhand smoke.

Table 3

Predicted Probabilities by Differing Exposures to Endotoxin and Proximity to a Major Roadway

Endotoxin level/ residential distance to a roadway	N	Predicted probability (%)	95% CI
At least 1 severe asthma exacerbation in the previous year			
Low/close	112	51	(35–67)
Low/far	32	50	(29–71)
High/close	33	70	(47–86)
High/far	17	18	(5–46)
BDR, $\geq 12\%$			
Low/close	112	18	(8–36)
Low/far	32	23	(7–54)
High/close	33	25	(9–52)
High/far	17	14	(3–45)

Abbreviations: BDR, bronchodilator response; CI, confidence interval; EU, endotoxin unit; SHS, second hand tobacco smoke.

NOTE: For endotoxin, low is ≤ 58.8 EU/mg and high is >58.8 EU/mg (75th percentile); for proximity to a major roadway, close is ≤ 499 m and far is >499 m (75th percentile). Predicted probabilities obtained from models adjusting for age, sex, current SHS exposure, and annual household income.

associated with decreased predicted probability of an asthma exacerbation or BDR.

Discussion

We reveal that living closer to a major roadway (<499 m) modifies the estimated effect of indoor endotoxin on severe asthma exacerbations in the previous year. Among children living closer to a major roadway, increasing endotoxin levels were associated with increased odds of severe asthma exacerbations. In contrast, increasing endotoxin levels were associated with reduced odds of severe asthma exacerbations among children living further away (>499 m) from a major roadway. Similar but less substantial results were observed for BDR. Our findings add to those of a few studies reporting an interaction between air pollutants and endotoxin on asthma outcomes in children.^{15,16}

The average indoor house dust endotoxin level in our study, conducted in the San Juan metropolitan area of Puerto Rico, was nearly twice as high as that previously reported in studies conducted in the United States and Washington, DC (36.9 EU/mg vs

15.3 EU/mg¹⁶ and 16.2 EU/mg, respectively³³), lower than those reported for homes of children with asthma in Saskatchewan, Canada (mild asthma = 58.7 EU/mg and moderate/severe asthma = 49.2 EU/mg),³⁴ and similar to those reported for the homes of farmers and their spouses living in Iowa and North Carolina (38.4 EU/mg).³⁵ Several factors can affect house dust endotoxin levels, such as home age, presence of children, farm or rural living, flooring materials (the presence of carpet), presence of pets, poverty, geography, and relative humidity.^{33,36,37} Our study is limited to homes with children, which have been found to have increased house endotoxin levels. The reasons for the negative correlation between dust mite and endotoxin in this study are unclear. Dust mite and endotoxin levels have been weakly correlated in some but not all studies.³⁷

Previous studies have reported positive associations between endotoxin and pet ownership or mouse urinary protein.³⁸ Consistent with those findings, current cat ownership and mouse urinary protein were positively correlated with household endotoxin in this study. In contrast to a US study,³⁸ endotoxin levels in our study were not different between those in homes who did or did not have dogs. Notably, a previous study reported that the association between household endotoxin levels and dog ownership varied across US climate regions, perhaps owing to variability in the time that dogs spend outdoors.³³ We are unable to determine if the dog(s) was/were indoor, outdoor, or both in this study.

Our findings are in partial agreement with those of a recent US study which reported that higher levels of outdoor air pollutants (PM_{2.5} and NO₂) and endotoxin synergistically increase odds of ED visits for children with asthma (odds ratio for PM_{2.5}, 3.75; 95% confidence interval, 1.27–11.07 and odds ratio for NO₂, 3.45; 95% confidence interval, 1.65–7.18).¹⁶ Our study expands on those results, because we reveal modification of the estimated effect of endotoxin on severe asthma exacerbations by residential distance to a major roadway.

Endotoxin has previously been reported to interact with air pollutant co-exposures in both in vitro³⁹ and clinical studies.^{15,16,40} A study of urban children with asthma found that increasing household airborne endotoxin was significantly associated with 0.38 times reduced odds of acute visits for asthma in children exposed to no or lower airborne nicotine, but with 1.15 times

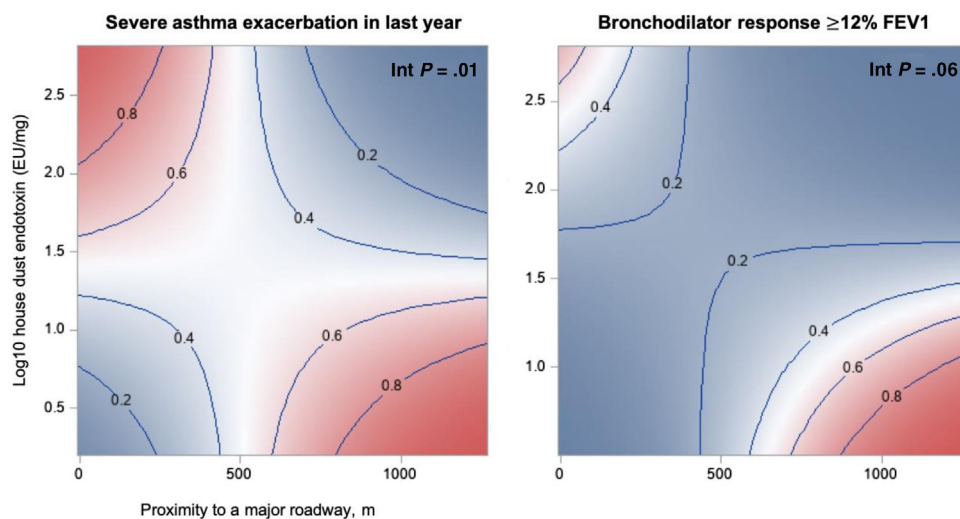


Figure 2. Contour plot of more than or equal to 1 severe asthma exacerbation and BDR, according to the interaction between house dust endotoxin and proximity to a major road. All models were adjusted for age, sex, current exposure to SHS, annual household income, cat ownership, levels of indoor (dust mite, cockroach, and mouse urinary protein) allergens, type of stove, and season of dust collection. Predicted probabilities all fit at median or largest proportion for cohort (age of 10 years, male sex, no exposure to SHS, annual household income $<\$15,000$ per year, no cat ownership, median log₁₀ level for each allergen, electric stove, and wet season for dust collection). BDR, bronchodilator response; EU, endotoxin unit; int P, P for interaction; SHS, secondhand smoke.

significantly increased odds of acute visits for asthma in children exposed to higher nicotine levels.¹⁵ As such, those results suggest that tobacco smoke exposure potentiates the effect of endotoxin on Toll-like receptor 4.¹⁵ This same study did not find an association with airborne PM and endotoxin on asthma outcomes. Nonhuman studies have found synergism between diesel exhaust particles and endotoxin on proinflammatory cytokines, yet they have also revealed reduced levels of the proinflammatory cytokine interleukin (IL)-13 with co-exposure (lipopolysaccharide plus diesel exhaust particles).³⁹

Our finding of a negative association between endotoxin and severe asthma exacerbations among children living further away from a major roadway may be because of endotoxin compositional differences or unmeasured correlated factors. Puerto Rico experiences annual African dust events (ADEs) characterized by the transatlantic Saharan dust deposition. A study in Puerto Rico found higher levels of endotoxin in PM from ADE compared with non-ADE PM; in vitro ADE-PM exposed cells had higher proinflammatory chemokines IL-6 and IL-8 by Toll-like receptors to a greater degree than non-ADE PM; IL-6 and IL-8 decreased with addition of an endotoxin inhibitor.¹⁷ Furthermore, the authors reported that endotoxin extracts from both ADE and non-ADE PM caused cellular cytotoxicity, thus concluding that quantity is likely less important than type of endotoxin (eg, *Pseudomonas aeruginosa* vs *E coli*). A study of Amish vs Hutterite homes supports compositional importance, temporality of exposure, or, both given almost 7-fold higher levels of endotoxin in Amish homes, a population with low asthma and atopic prevalence, than in Hutterites homes, a population with higher asthma and atopy prevalence.⁴¹ A greater abundance of Proteobacteria was found in Amish samples compared with increased abundance of Firmicutes and Bacteroidetes in Hutterite samples.⁴¹ A study of farmers and spouses from 2 US states revealed bacterial microbiome differences by state, farming type, home condition, and season, thus supporting potential differences in endotoxin composition.³⁵ In further support of geographic and compositional differences, a study in the US mainland reported that effects of home endotoxin on respiratory health varied by climate, perhaps owing to the differences in the number of acyl chains attached to lipid A (because penta-acylated and hexa-acylated forms may have different effects on inflammation).³³

We cannot assess temporal relationships in this cross-sectional study. Although adjustment of our analysis for season of dust collection or other covariates did not alter our results, we lack endotoxin speciation/type. Moreover, proximity to a major roadway may correlate with unmeasured factors, such as polyaromatic hydrocarbons or NO₂. Indoor NO₂ has previously been reported to interact with airborne endotoxin on asthma outcomes (increased acute asthma visits with low NO₂, protective with high NO₂).¹⁵ Although we do not have data regarding indoor air pollutants, adjustment for gas stove, a primary source of indoor NO₂,¹¹ did not alter our results. We lack information on additional sources of exposure, such as school proximity to a major roadway, yet findings from a recent study with data on both school and residential distance to a major road suggest that childhood asthma symptoms are predominately driven by residential proximity to a major roadway.⁵ Notably, greater than or equal to 62% of children who participated in that study were likely to attend schools or to live in homes located within 100 m of a major roadway. Thus, in future studies of children living in close proximity to a major roadway, dust endotoxin compositional analysis in the homes may be more important than that in the schools.

We did not find any interaction with household endotoxin and proximity to a major roadway on outcomes other than severe asthma exacerbations, consistent with previous findings of no association between outdoor air pollution and endotoxin on inhalant

sensitization,¹⁶ FEV₁,⁴² or FEV₁/FVC.¹⁵ However, we had limited statistical power owing to relatively small sample size, and thus, our results for BDR may have achieved statistical significance in a larger cohort. For example, a previous study of 146 children and adolescents revealed suggestive but nonstatistically significant evidence that increased airborne endotoxin exposure was associated with increased BDR in homes with high air nicotine but not in those with low air nicotine (*P* for interaction = .16).¹⁵

In conclusion, proximity to a major roadway modifies the estimated effect of indoor endotoxin on severe asthma exacerbations among Puerto Rican children with asthma in Puerto Rico. Large longitudinal studies assessing the composition of dust endotoxin in homes near and farther away from major roadways are needed to confirm and expand our findings, yet this should not preclude continued efforts to implement health policies that ensure clean air for all.

Supplementary Data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.anai.2020.09.001>.

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eTable 1

Comparison of the Main Characteristics of Study Participants Who Were (n = 200) and Were Not (n = 151) Included in the Current Analysis

Characteristic	Endotoxin measured ^a	Endotoxin not measured ^a	P value
Age, y	10 (8–12)	10 (7–12)	.60
Sex, female, n (%)	82 (41)	68 (45)	.42
Annual household income $\geq$ \$15,000, yes, n (%)	46 (23.5)	73 (50)	<.001
Residential distance to a major roadway, m	210 (172–499)	210 (172–378)	.58
Secondhand tobacco smoke exposure, current, n (%)	105 (53)	50 (33)	.001
Current dog ownership, ^b yes, n (%)	126 (63)	96 (64)	.85
Current cat ownership, ^b yes, n (%)	28 (14)	23 (15)	.74
Log ₁₀ mouse urinary protein (Mus m 1, ng/g) in house dust ^b	0.78 (0.30–1.62)	0.85 (0.30–1.38)	.62
Log ₁₀ dust mite allergen (Der p 1, μ g/g) in house dust ^c	0.72 (0.43–1.03)	0.53 (0.31–0.78)	.004
Log ₁₀ German cockroach allergen (Bla g 2, U/g) in house dust ^c	0.18 (–0.10 to 1.10)	0.07 (–0.28 to 0.32)	.01
Stove type, gas (vs electric), n (%)	43 (22)	43 (29)	.11
Wet season of dust collection, ^d n (%)	119 (60)	82 (55)	.37
At least 1 severe asthma exacerbation in past year, n (%)	102 (51)	81 (54)	.58
BDR, $\geq$ 12%, n (%)	30 (19)	23 (23)	.43
Atopy, ^e yes, n (%)	125 (65)	85 (76)	.05
FEV ₁ % predicted (GLI2012)	92 (82–102)	93 (86–104)	.57
Reduced ($\leq$ –1.64 z-score), n (%)	36 (21)	16 (15)	.19
FEV ₁ /FVC ratio % predicted (GLI2012)	93 (86–97)	92 (85–98)	.78
Reduced ($\leq$ –1.64 z-score), n (%)	55 (32)	41 (37)	.33
Average d/mo missed school because of asthma	2 (0–5)	2 (0–5)	.98

Abbreviations: BDR, bronchodilator response; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GLI2012, Global Lung Initiative 2012; IQR, interquartile range.^aValues displayed as median (IQR) or n (%) unless otherwise specified. P value obtained from Wilcoxon ranked sum test and χ^2 test.^bCurrent ownership represents current or within the previous 6 months reported ownership.^cHousehold dust was collected from bedroom, living/television room, kitchen, and combined.^dDry season is from December to April; wet season is from May to November.^eAtopy defined as at least 1 positive specific immunoglobulin E to 1 of 5 aeroallergens (cat, dog, dust mite, cockroach, mouse).**eTable 2**

Correlation Among Endotoxin, Proximity to a Major Roadway, and Home Dust Antigens in Children With Asthma Living in Metropolitan San Juan Area in Puerto Rico

	Endotoxin (EU/mg)	Distance to a major roadway, m	Mouse (Mus m 1) urine protein antigen (ng/g) in house dust	Dust mite (Der p 1) protein antigen (μ g/g) in house dust	Cockroach (Bla g 2) protein antigen (U/g) in house dust
Endotoxin (EU/mg)	1				
	—				
	200				
Proximity to a major roadway, m	0.01	1			
	0.89	—			
	200	200			
Mouse (Mus m 1) urine protein antigen (ng/g) in house dust	0.15	0.12	1		
	0.04	0.09	—		
	189	189	189		
Dust mite (Der p 1) protein antigen (μ g/g) in house dust	–0.15	0.06	–0.16	1	
	0.03	0.37	0.03	—	
	189	189	189	189	
Cockroach (Bla g 2) protein antigen (U/g) in house dust	0.10	–0.23	–0.04	0.003	1
	0.18	0.002	0.58	0.96	—
	189	189	189	189	189

Abbreviation: EU, endotoxin unit.

NOTE: Results presented as Spearman correlation coefficients, P value, and n.

eTable 3

Multivariable Analysis of Endotoxin Level and Asthma Outcomes, Stratified by Upper Quartile (>499 m) of Residential Distance to a Major Roadway

Outcomes	Closer distance to a major roadway (≤ 499 m)			Further distance to a major roadway (>499 m)			Int <i>P</i> value
	Unadjusted	Model 1	Model 2	Unadjusted	Model 1	Model 2	
Severe asthma exacerbation, ≥ 1	2.07 (0.92-4.64)	2.33 (1.01-5.34) ^a	4.21 (1.48-12.0) ^b	0.13 (0.02-0.81) ^a	0.07 (0.01-0.74) ^a	0.03 (0.001-0.67) ^a	.02
BDR, $\geq 12\%$	1.46 (0.51-4.16)	2.00 (0.63-6.37)	2.61 (0.76-8.88)	0.24 (0.02-3.27)	0.21 (0.01-5.14)	0.17 (0.003-10.69)	.07
Atopy	1.16 (0.52-2.59)	1.01 (0.44-2.32)	1.42 (0.55-3.67)	1.75 (0.32-9.64)	2.08 (0.34-12.6)	62.0 (1.24->999)	.82
FEV ₁ % predicted ^c	2.97 (−3.37 to 9.31)	2.80 (−3.59 to 9.20)	1.71 (−5.03 to 8.45)	0.28 (−14.82 to 15.38)	0.61 (−13.35 to 14.57)	−5.40 (−23.48 to 12.68)	.74
Reduced FEV ₁ (≤ -1.64)	0.56 (0.19-1.66)	0.60 (0.19-1.83)	0.68 (0.19-2.36)	0.47 (0.05-4.32)	0.45 (0.04-5.20)	0.66 (0.02-20.0)	.63
FEV ₁ /FVC % predicted ^c	4.31 (−0.02 to 8.64)	4.62 (0.25-8.99)	4.63 (0.15-9.10)	−2.33 (−11.66 to 7.00)	−2.35 (−11.86 to 7.15)	−1.96 (−13.44 to 9.51)	.38
Reduced FEV ₁ /FVC (≤ -1.64)	0.65 (0.26-1.68)	0.69 (0.26-1.84)	0.36 (0.11-1.24)	1.91 (0.32-11.45)	2.10 (0.33-13.46)	1.34 (0.10-17.82)	.42
Missed school days	−0.13 (−0.59 to 0.32)	−0.16 (−0.61 to 0.29)	−0.18 (−0.65 to 0.28)	0.16 (−1.06 to 1.38)	0.14 (−1.02 to 1.30)	0.01 (−1.19 to 1.21)	.76

Abbreviations: BDR, bronchodilator response; CI, confidence interval; EU, endotoxin unit; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; Int, interaction.NOTE: Int *P* value, obtained from models containing roadway and endotoxin (both continuous) plus interaction term in models adjusted for model 1 covariates. Results displayed as odds ratio or Beta (95% CI). Model 1: adjusted for age (years), sex, current reported second hand tobacco smoke exposure, and annual household income $\geq$ \$15,000. Model 2: adjusted for variables in model 1 plus current cat ownership, measured household allergen levels (dust mite, cockroach, mouse), gas stove (vs electric), and season of collection (wet vs dry).^aInteraction *P* value of <.05.^bInteraction *P* value of <.01.^cFor FEV₁ and FEV₁/FVC, models did not include age and sex; percent predicted based on Global Lung Initiative 2012.