Endotoxin Exposure: Predictors and Prevalence of Associated Asthma Outcomes in the United States

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Abstract

Rationale: Inhaled endotoxin induces airway inflammation and is an established risk factor for asthma. The 2005–2006 National Health and Nutrition Examination Survey included measures of endotoxin and allergens in homes as well as specific IgE to inhalant allergens.

Objectives: To understand the relationships between endotoxin exposure, asthma outcomes, and sensitization status for 15 aeroallergens in a nationally representative sample.

Methods: Participants were administered questionnaires in their homes. Reservoir dust was vacuum sampled to generate composite bedding and bedroom floor samples. We analyzed 7,450 National Health and Nutrition Examination Survey dust and quality assurance samples for their endotoxin content using extreme quality assurance measures. Data for 6,963 subjects were available, making this the largest study of endotoxin exposure to date. Log-transformed endotoxin concentrations were analyzed using logistic models and

forward stepwise linear regression. Analyses were weighted to provide national prevalence estimates and unbiased variances.

Measurements and Main Results: Endotoxin exposure was significantly associated with wheeze in the past 12 months, wheeze during exercise, doctor and/or emergency room visits for wheeze, and use of prescription medications for wheeze. Models adjusted for age, sex, race and/or ethnicity, and poverty-to-income ratio and stratified by allergy status showed that these relationships were not dependent upon sensitization status but were worsened among those living in poverty. Significant predictors of higher endotoxin exposures were lower family income; Hispanic ethnicity; participant age; dog(s), cat(s), cockroaches, and/or smoker(s) in the home; and carpeted floors.

Conclusions: In this U.S. nationwide representative sample, higher endotoxin exposure was significantly associated with measures of wheeze, with no observed protective effect regardless of sensitization status.

Keywords: allergy; asthma; house dust; indoor air; wheeze

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At a Glance Commentary

Scientific Knowledge on the

Subject: Endotoxin exposure is a risk factor for wheeze outcomes among both sensitized and nonsensitized individuals. Levels of specific IgE antibodies to dog, mouse, and rat significantly modify the relationship between endotoxin exposure and current asthma and wheeze. Endotoxin exposures are higher for those who live in poverty and those who have children, pets, cockroaches, carpeted floors, or a smoker in the home.

What This Study Adds to the

Field: In the National Health and Nutrition Examination Survey 2005–2006, endotoxin exposure was positively associated with wheeze, independent of sensitization status but with increased susceptibility among those living in poverty. No threshold was observed in the relationship between endotoxin exposure and asthma outcomes. Predictors of high household endotoxin levels are poverty, participant age, pet keeping, cockroach problems, indoor smoking, carpeting, and age of the home.

Inhaled endotoxin induces airway inflammation via Toll-like receptor (TLR) 4 and is an established risk factor for asthma (1). However, most studies on domestic endotoxin exposure have been limited to children and have demonstrated a potent immunomodulating effect in reducing the likelihood of developing asthma and allergies (1, 2). Very few studies have been conducted in adults, and only one, the National Survey of Lead and Allergens in Housing (NSLAH), has been done on a national scale (3, 4). The NSLAH study researchers analyzed 2,456 residents of 831 homes across the United States and were the first to find household endotoxin to be associated with doctor-diagnosed asthma, wheeze, and taking medications for wheeze (3). In the National Health and Nutrition Examination Survey (NHANES), we aimed to investigate these relationships in a much larger sample representative of the United States, considering a wider variety of asthma and wheeze outcomes than were studied in the NSLAH study

and with specific IgE and home allergen data. We also hypothesized that the relationship between house dust endotoxin and prevalence of asthma and wheeze could differ with environmental exposures and sensitization to specific allergens.

Researchers in our laboratory analyzed 7,450 NHANES samples for content of endotoxin, along with extensive quality assurance (QA) samples supporting those analyses. The NHANES is an ongoing series of national cross-sectional studies performed to examine the health and nutritional status of adults and children in the United States (5). Together with the National Institute of Allergy and Infectious Diseases and the National Center for Health Statistics, the National Institute of Environmental Health Sciences expanded the scope of the 2005–2006 NHANES with a component that evaluated exposures to allergens and endotoxins (5). This component was developed to enhance understanding of the relationships between allergen and endotoxin exposures and allergic sensitization and of the modification of these relationships by demographic factors. Some of the results of these studies were previously reported in the form of abstracts (6–8).

Methods

Study Framework

We used data from the NHANES, which is a continuous, cross-sectional survey of the U.S. noninstitutionalized civilian population. The 2005–2006 NHANES researchers used a complex, multistage design to derive a representative sample and oversampled people below the poverty level, aged 12–19 years, aged 60 years or older, pregnant women, African Americans, and Mexican Americans to ensure adequate subgroup analyses. The study design and protocol were approved by the National Center for Health Statistics Ethics Review Board. Written informed consent was obtained from all participants who were enrolled. Details are available at http://www.cdc.gov/nchs/nhanes/search/nhanes05_06.aspx.

Endotoxin Analysis

Combined bed and bedroom floor dust samples were collected at each participant's home. Sieved, frozen house dust samples were shipped to our laboratory for

extraction and analysis of endotoxin at four dilutions as previously described (9) (*see online supplement*). A number of QA measures were adopted. These included rigorous chain of custody verification, internal and external audits, bar coding of samples, use of a single lot of assay reagents, blind repeats of NHANES dust samples ($n = 665$), use of a single microplate reader, and application of Westgard rules to accept or reject a run. The lower limit of detection was 0.000488 endotoxin units (EU) per milligram of house dust.

Assessment of Respiratory and Allergic Diseases

Asthma- and allergy-related outcomes were assessed using validated survey instruments administered via an interviewing system. Wheeze-related outcomes in the past 12 months included any wheeze, sleep disturbance because of wheezing, exercise-induced wheezing, doctor and/or emergency room (ER) visits for wheezing attacks, prescriptions for wheezing, limitations of usual activities because of wheezing, and work or school missed because of wheezing (ages 6–69 yr). Asthma-related outcomes were defined by ever having had a diagnosis of asthma, current asthma, asthma attack in the past 12 months, and use of asthma medication in the past 30 days. Allergy-related outcomes and symptoms included current hay fever, diagnosed allergies, allergy symptoms, sneezing or runny or blocked nose, allergy medication in the past 30 days, itchy rash for at least 6 months, itchy rash in the past 12 months, diagnosed eczema, any eczema medications in the past 30 days, and diagnosed sinus infection.

Dust Allergens, Allergic Sensitization, and Sociodemographic Data

Dust sample extracts from our laboratory were analyzed for a panel of allergens (cockroach, Bla g 2; dog, Can f 1; cat, Fel d 1; dust mites, Der p 1 and Der f 1; mouse, Mus m 1; and rat, Rat n 1) using a Multiplex Array for Indoor Allergens assay (MARIA; Indoor Biotechnologies, Charlottesville, VA). Bla g 1 and *Aspergillus fumigatus* were assayed by enzyme immunoassay. Serum IgE specific to 15 aeroallergens (*Alternaria alternata*, *Aspergillus fumigatus*, Bermuda grass, birch, cat dander, cockroach, dog dander, dust mites [Der p 1 and Der f 1], mouse urine proteins, oak, ragweed, rat urine proteins, Russian thistle, and rye

Table 1. Respiratory and Allergy-related Outcomes in Relation to Endotoxin Concentration

Disease Outcomes	Subjects (n)	GM (GSE) (EU/mg)	P Value
Overall	6,963	15.49 (0.50)	
Wheeze-related outcomes in past 12 mo			
Any wheeze			
Yes	974	17.82 (1.27)	0.040
No	5,986	15.07 (0.52)	
Sleep disturbance because of wheeze			
Yes	515	17.64 (1.97)	0.227
No	6,445	15.31 (0.51)	
Exercise-induced wheeze			
Yes	490	18.47 (1.62)	0.039
No	6,464	15.23 (0.50)	
Prescription medication for wheeze			
Yes	607	17.84 (1.24)	0.025
No	6,353	15.25 (0.50)	
Doctor or ER visit for wheezing attack			
Yes	474	19.32 (2.28)	0.056
No	6,486	15.22 (0.49)	
Limitation in usual activities because of wheezing			
Yes	425	18.01 (2.00)	0.139
No	6,535	15.31 (0.48)	
Missed work or school because of wheezing (ages 6–69)			
Yes	193	17.54 (2.30)	0.162
No	5,247	14.54 (0.47)	
Asthma-related outcomes			
Diagnosed asthma			
Yes	1005	17.02 (1.12)	0.135
No	5,949	15.24 (0.54)	
Current asthma			
Yes	639	17.44 (1.48)	0.112
No	6,297	15.33 (0.49)	
Asthma attack in past 12 mo			
Yes	340	17.47 (1.55)	0.115
No	6,596	15.40 (0.49)	
Any asthma medication in past 30 d			
Yes	526	16.57 (1.49)	0.332
No	6,437	15.38 (0.45)	
Combined respiratory outcomes			
Current asthma and any wheeze			
Yes	438	17.59 (1.51)	0.103
No	6,495	15.36 (0.50)	
Current asthma and exercise-induced wheeze			
Yes	284	16.83 (1.86)	0.458
No	6,643	15.42 (0.53)	
Allergy-related outcomes			
Diagnosed hay fever			
Yes	449	15.66 (0.94)	0.829
No	6,499	15.47 (0.51)	
Episode of hay fever in past 12 mo			
Yes	268	17.44 (1.41)	0.116
No	6,679	15.37 (0.50)	
Diagnosed allergies			
Yes	1,802	14.79 (0.96)	0.354
No	5,143	15.83 (0.55)	
Allergy symptoms in past 12 mo			
Yes	1,191	14.65 (1.13)	0.379
No	5,749	15.74 (0.52)	
Sneezing/runny or blocked nose in past 12 mo			
Yes	1,875	15.47 (0.82)	0.944
No	5,085	15.53 (0.52)	
Any allergy medication in past 30 d			
Yes	262	15.99 (1.97)	0.765
No	6,701	15.46 (0.47)	

(Continued)

Table 1. (Continued)

Disease Outcomes	Subjects (n)	GM (GSE) (EU/mg)	P Value
Itchy rash coming and going ≥ 6 mo			
Yes	660	15.48 (1.29)	0.970
No	6,298	15.53 (0.53)	
Itchy rash at any time in past 12 mo			
Yes	537	16.71 (1.35)	0.325
No	6,421	15.43 (0.51)	
Diagnosed eczema			
Yes	631	14.57 (1.30)	0.441
No	6,322	15.59 (0.50)	
Eczema medications in past 30 d			
Yes	40	13.61 (3.99)	0.664
No	6,923	15.50 (0.51)	
Diagnosed sinus infection in past 12 mo			
Yes	780	13.77 (0.87)	0.045
No	6,167	15.82 (0.54)	

Definition of abbreviations: ER = emergency room; EU/mg = endotoxin units per milligram of dust; GM = geometric mean; GSE = geometric standard error of the mean.

Boldface type indicates statistically significant difference.

grass) were measured (*see online supplement*). For children ages 1–5 years, nine specific IgE antigens were tested (10). Sensitization status was defined as specific IgE antigen against any measured aeroallergen of at least 0.35 kU/L. Data on sociodemographics, family income, household size, home characteristics, environmental tobacco smoke, and the presence of pets or cockroaches in homes were collected using questionnaires.

Statistical Analysis

Endotoxin concentrations were log transformed to establish normality. Geometric means (GMs) were calculated for each respiratory and allergy outcome, and *P* values for differences in means between their absence and presence were estimated using Student's *t* test. Univariate and multivariate (adjusted for age, sex, race and/or ethnicity, and poverty-to-income ratio [PIR]) logistic regression analyses between log-transformed endotoxin concentrations or dichotomized endotoxin levels (below vs. above median) and health outcomes were performed. Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) for log₁₀ endotoxin were reported for a 10-fold increase in exposure. Age, allergic sensitization, and home environmental exposures were tested for effect modification by including a product term in the model, and, when a significant effect modification was found, we reported the changes in odds of current asthma and wheeze associated with a 10-fold increase in endotoxin for each value of the moderator kept constant (11, 12).

To explore predictors of endotoxin levels in homes, a forward stepwise linear regression with log₁₀ endotoxin as the outcome was constructed with a *P* value for entry less than 0.10 and a *P* value for removal greater than 0.20. Candidate variables for this analysis are listed in Table E1 in the online supplement. Descriptive analyses, predictive modeling, and logistic modeling were performed with SAS software (version 9.3; SAS Institute, Cary, NC). To account for the complex survey design, survey nonresponse, and post-stratification, sampling weights and design variables were applied to these analyses. *P* values less than 0.05 were considered statistically significant.

The relationship between endotoxin and respiratory outcomes was illustrated graphically to exhibit trends in outcome prevalence across a range of concentrations. These smooth plots were generated using R statistical software, and modeling was done using the “gam” package in R.

Results

Endotoxin assay quality control data demonstrated a high degree of reliability from assay to assay, with low control samples having a GM of 16.16 EU/mg (95% CI, 15.73–16.59; *n* = 361) and high control samples having a GM of 82.17 (95% CI, 80.18–84.21; *n* = 361) (Table E2). A log-log plot of the blind endotoxin repeat samples returned an *r*² value of 0.929 (*n* = 665). As shown in Table 1, the overall GM for endotoxin concentration in sieved dust from bed and bedroom floor was 15.49 EU/mg of dust (adjusted for sample design

and post-stratification). Although elevated endotoxin levels were associated with the presence of all wheeze outcomes and asthma-related outcomes, only wheezing in the past 12 months (*P* = 0.040), exercise-induced wheeze (*P* = 0.039), and use of prescription medication for wheezing (*P* = 0.025) were significantly associated with elevated endotoxin concentrations. No significant difference in endotoxin concentration was observed in house dust for participants with asthma- or allergy-related outcomes, whereas a lower concentration was noted in participants with sinus infections than in those without sinus infections (*P* = 0.045) (Table 1).

In logistic regression analysis, a 10-fold increase in endotoxin concentration was associated with all four wheeze-related outcomes, including any wheeze (OR, 1.25; 95% CI, 1.03–1.51), exercise-induced wheeze (OR, 1.29; 95% CI 1.03–1.62), use of prescription medication for wheezing (OR, 1.23, 95% CI, 1.05–1.44), and visit to a doctor or ER for wheeze (OR, 1.38; 95% CI, 1.01–1.88), all in the past 12 months (overall data are provided in Table 2, columns 2–4 and 9–11, and Figure E1). The results remained similar after adjusting for age, sex, race and/or ethnicity, and PIR. Endotoxin associations with asthma outcomes were modest, and CIs included 1.0. When exposure–outcome associations were stratified by sensitization status, exercise-induced wheeze in the past 12 months was associated primarily with participants who were not sensitized (OR, 1.46; *P* = 0.011), whereas wheeze and use of prescription medications for wheeze in the

Table 2. Odds Ratios and 95% Confidence Intervals Associated with a 10-fold Increase in Endotoxin in Logistic Regression Models for the Presence of Wheeze and Asthma-related Outcomes, Overall and by Sensitization Status, Using Log Endotoxin Exposure Levels

Health Outcome	Unadjusted Model						Adjusted Model*					
	Overall			Sensitized			Not Sensitized			Overall		
	n	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	n	OR	95% CI
Wheeze-related outcomes in the past 12 mo												
Any wheeze	6,960	1.25	1.03–1.51	1.28	1.00–1.65	1.22	0.95–1.56	1.22	0.95–1.56	6,651	1.26	1.02–1.56
Exercise-induced wheeze	6,954	1.29	1.03–1.62	1.23	0.88–1.72	1.46	1.09–1.95	1.21	0.84–1.74	6,645	1.30	1.01–1.68
Prescription medication for wheeze	6,960	1.23	1.05–1.44	1.24	1.02–1.51	1.17	0.85–1.60	1.21	1.01–1.44	6,651	1.21	1.01–1.44
Doctor/ER visit for wheeze	6,960	1.38	1.01–1.88	1.27	0.90–1.80	1.41	0.86–2.31	1.35	0.97–1.87	6,651	1.24	0.86–1.79
Asthma-related outcomes												
Diagnosed asthma	6,954	1.15	0.97–1.38	1.17	0.92–1.50	1.21	0.91–1.61	1.10	0.93–1.30	6,644	1.16	0.90–1.48
Current asthma	6,936	1.18	0.98–1.44	1.26	0.97–1.64	1.17	0.79–1.74	1.13	0.92–1.38	6,626	1.21	0.90–1.61
Asthma attack in past 12 mo	6,936	1.18	0.97–1.42	1.17	0.87–1.56	1.23	0.78–1.95	1.11	0.90–1.36	6,626	1.13	0.81–1.57
Any asthma medication in past 30 d	6,963	1.10	0.91–1.33	1.02	0.74–1.39	1.15	0.94–1.41	1.17	0.94–1.45	6,653	1.17	0.94–1.47
Combined outcomes in past 12 mo												
Current asthma and any wheeze	6,933	1.19	0.98–1.46	1.14	0.89–1.47	1.37	0.89–2.11	1.12	0.91–1.39	6,624	1.09	0.83–1.43
Current asthma and exercise-induced wheeze	6,927	1.12	0.84–1.50	1.21	0.78–1.86	1.12	0.73–1.70	1.05	0.78–1.41	6,618	1.16	0.75–1.78

Definition of abbreviations: CI = confidence interval; ER = emergency room; OR = odds ratio.
Overall data include participants with missing sensitization status. Boldface type indicates statistically significant difference.
*Adjusted for age, sex, race and/or ethnicity, and poverty-to-income ratio.

past 12 months were more pronounced in sensitized participants (ORs, 1.28 and 1.24, respectively). After stratification and adjusting for age, sex, race and/or ethnicity, and PIR, fewer outcomes were significantly related to endotoxin exposure; however, exercise-induced wheeze remained significantly elevated (adjusted OR, 1.52) (Table 2).

To assess variation in the endotoxin–respiratory outcome associations by sensitization status, we did additional modeling, stratifying endotoxin exposure above versus below the median value of 16.2 EU/mg (Table E3). Consistent with the analysis of endotoxin as a continuous variable, shown in Table 2, higher endotoxin in house dust was associated with exercise-induced wheezing in the past 12 months, use of prescription medications for wheeze, and doctor and/or ER visits for wheeze. As before, the magnitude of the association of endotoxin and exercise-induced wheeze was stronger among nonsensitized participants than in sensitized subjects. Adjusting for covariates did not substantially impact the magnitude of the associations, and none of the interactions by sensitization status were significant in the unadjusted or adjusted models. Other wheeze outcomes yielded similar ORs between sensitized and nonsensitized participants. Additional analyses with stratification by age group did not yield significant differences in the relationship between endotoxin exposure and respiratory health outcomes.

We also investigated the role of poverty in the association of endotoxin and asthma outcomes (Table 3). The stratified analysis showed an increased risk of wheezing, taking medication for wheezing, and doctor and/or ER visits for wheeze, with higher endotoxin exposure that was more pronounced observed in those living in poverty as defined by a PIR below the U.S. federal food assistance cutoff of 1.85. The effect of poverty was even more pronounced when we evaluated endotoxin exposure above versus below the median (Table E4).

In effect modification testing, age, presence of mildew or musty smell, cockroaches, or pets, as well as sensitization status, were not found to be significant. Specific IgE levels to dog allergen significantly modified the relationship between endotoxin and wheeze ($P = 0.02$); specific IgE to mouse significantly modified the relationship between endotoxin and current asthma ($P = 0.026$); and specific IgE to rat significantly modified the relationship between endotoxin and current asthma ($P = 0.024$), wheeze ($P = 0.027$), or the combined outcome of current asthma and wheeze ($P = 0.04$) (Table E5). At higher levels of specific IgE, endotoxin was associated with decreased odds of current asthma and wheeze in each case (Table E6).

We next characterized the associations using generalized additive modeling. As illustrated in Figure 1, locally weighted scatterplot smoothing demonstrated a positive

exposure–response relationship between log-transformed endotoxin concentration and asthma diagnosis, current asthma, wheeze in the past 12 months, and current asthma and wheeze in the past 12 months.

In forward stepwise linear regression, variables initially entered in the model that were significant predictors of higher endotoxin concentration in house dust were income less than \$20,000 ($P < 0.001$); Mexican American compared with non-Hispanic white race and/or ethnicity ($P < 0.001$); living in a house built before 1978 ($P = 0.012$); age younger than 18 years ($P < 0.001$); presence of a dog ($P < 0.001$), a cat ($P < 0.001$), cockroaches ($P = 0.003$), and/or a smoker living in the home ($P = 0.016$); or carpeted floors ($P < 0.001$) in the home (Table 4). Younger age (< 6 yr and 6–17 yr vs. ≥ 18 yr) was a significant predictor of higher endotoxin levels (2.15-fold higher for homes with young children). Homes with smooth floors had significantly lower endotoxin concentrations in combined bedroom bed and floor dust ($P < 0.001$) than homes with carpeting.

Discussion

NHANES 2005–2006 provides the largest and most detailed study to date of endotoxin exposure and associated respiratory health outcomes and predictors of exposure. With extensive QA for the endotoxin analyses and nearly complete data for over 6,935 participants, we are able to relate endotoxin exposures to outcomes while accounting for sensitization status for 15 aeroallergens. The results suggest that house dust endotoxin from bed and bedroom floor is associated with a higher prevalence of wheeze, exercise-induced wheeze, use of prescription medication for wheezing, and doctor and/or ER visits for wheeze. Further, this effect is strongly associated with living in poverty. The association of endotoxin with asthma and wheeze was modified by specific IgE levels against dog, mouse, and rat, with significantly higher odds of the conditions observed at low levels and significantly lower odds at high concentrations against mouse and rat. The relationships of endotoxin with wheeze did not exhibit a threshold effect but did show a positive dose–response association over the range of values. Predictors of higher concentrations of endotoxin were poverty; living in an

Table 3. Adjusted Odds Ratios Showing the Effect of a 10-fold Increase in Endotoxin Exposure on Asthma Outcomes, Stratified by Poverty-to-Income Ratio

Health Outcome	PIR <1.85 [OR* (95% CI)]	PIR $\geq$ 1.85 [OR* (95% CI)]
Any wheeze	1.30 (1.02–1.67)	1.19 (0.88–1.62)
Exercise-induced wheeze	1.12 (0.88–1.43)	1.33 (0.89–2.01)
Prescription medication for wheeze	1.29 (1.05–1.58)	1.13 (0.90–1.41)
Doctor/ER visit for wheeze	1.49 (1.02–2.19)	1.19 (0.81–1.77)
Diagnosed asthma	1.03 (0.82–1.29)	1.12 (0.87–1.43)
Current asthma	1.11 (0.87–1.41)	1.10 (0.82–1.47)
Asthma attack in past 12 mo	1.09 (0.77–1.55)	1.06 (0.79–1.44)
Any asthma medication in past 30 d	0.99 (0.73–1.35)	1.25 (0.96–1.64)
Current asthma and any wheeze	1.09 (0.83–1.42)	1.12 (0.84–1.49)
Current asthma and exercise-induced wheeze	0.90 (0.70–1.15)	1.13 (0.75–1.70)

Definition of abbreviations: CI = confidence interval; ER = emergency room; OR = odds ratio; PIR = poverty-to-income ratio.

Boldface type indicates statistically significant difference.

*Adjusted for age, sex, and race/ethnicity.

Figure 1. Smoothed plots of prevalence of disease outcomes versus the unweighted, combined bed and bedroom floor dust endotoxin concentration (expressed as endotoxin units per milligram of dust [EU/mg]). (A) The prevalence of wheeze in the past 12 months. (B) The prevalence of doctor-diagnosed asthma. (C) The prevalence of current asthma. (D) The prevalence of the combined outcomes of current asthma and wheeze in the past 12 months. All four plots show a nearly linear increase of the respiratory health outcomes with the endotoxin concentration log values. *Solid lines* show the smoothed mean prevalence, and *dashed lines* indicate upper and lower 95% confidence intervals.

older home (built before 1978); younger age; presence of dogs, cats, cockroaches, or carpeted floor in the home; and having a smoker in the household.

In a previous national study on endotoxin conducted from 1998 to 1999, we assayed 2,456 samples from 831 U.S. homes from the National Survey of Endotoxin in United States Housing (a component of the NSLAH) to measure both endotoxin concentration and endotoxin load in reservoir dust from bedroom bed and floor, family room and kitchen floors, and sofa upholstery (4). Table 5 compares unweighted endotoxin data for the

NHANES and the NSLAH studies, both of which were analyzed in our laboratory using similar methods. The GM for the NHANES composite bedroom floor and bedding sample was very close to that for the NSLAH bedding sample, but it was considerably lower than that for the NSLAH bedroom floor sample. Although it is possible that endotoxin levels in homes decreased over the 8 years separating these two studies, a more likely explanation is that the much larger NHANES sample was not well represented by the smaller NSLAH study. Bedroom floor dust samples, also analyzed for endotoxin in our laboratory,

from New York City households in East and West Harlem, the South Bronx, and Washington Heights had a high GM of 75.9 EU/mg, likely reflecting the influence of poverty and substandard housing on endotoxin exposure (13).

Measuring endotoxin at just two dilutions in mattress dust collected in 22 study centers across 10 European countries, the European Community Respiratory Health Survey II researchers observed much lower levels, with a GM of 2.45 EU/mg (range, 0.098–402.7 EU/mg). These investigators cautioned that multiple shipping and long-term storage of the dust

Table 4. Major Predictors of Endotoxin Concentration Derived from Forward Stepwise Regression

Main Effect	Subjects (n)	β	SE	10 ^B	P Value
Intercept	6,611	0.62	0.07	4.12	<0.001
Family income					
<\$20,000	1,821	0.16	0.03	1.44	<0.001
≥\$20,000	4,790	0.00	0.00	Ref.	
Race/ethnicity					
Other	540	0.00	0.03	1.01	0.915
Mexican American	1,774	0.13	0.02	1.34	<0.001
Non-Hispanic black	1,903	−0.01	0.03	0.97	0.688
Non-Hispanic white	2,394	0.00	0.00	Ref.	
When was home built?					
Missing data	1,800	0.11	0.05	1.28	0.063
Before 1978	2,438	0.10	0.03	1.25	0.012
1978–present	2,373	0.00	0.00	Ref.	
People in household (n)					
1	487	−0.00	0.04	1.00	0.954
2	1,180	0.00	0.00	Ref.	
3	1,241	0.05	0.04	1.12	0.263
4	1,354	−0.04	0.05	0.91	0.413
>4	2,349	0.09	0.05	1.24	0.092
Age (categorical)					
1–5 yr	906	0.33	0.02	2.15	<0.001
6–17 yr	2,057	0.20	0.03	1.57	<0.001
≥18 yr	3,648	0.00	0.00	Ref.	
Years family has lived in home					
1 yr	1,363	0.03	0.04	1.08	0.454
1–2 yr	1,413	0.06	0.04	1.15	0.154
3–5 yr	1,319	0.00	0.00	Ref.	
6–10 yr	1,029	0.03	0.02	1.07	0.252
≥11 yr	1,487	0.06	0.03	1.14	0.057
Dog in house now					
Yes	1,907	0.15	0.02	1.41	<0.001
No	4,704	0.00	0.00	Ref.	
Cat in house now					
Yes	1,131	0.07	0.01	1.18	<0.001
No	5,480	0.00	0.00	Ref.	
Have you seen cockroaches in your home?					
Yes	1,496	0.11	0.03	1.29	0.003
No	5,115	0.00	0.00	Ref.	
Does anyone smoke in home?					
Yes	1,255	0.08	0.03	1.20	0.016
No	5,356	0.00	0.00	Ref.	
Floor surface carpeted					
Yes	5,820	0.23	0.03	1.70	<0.001
No	791	0.00	0.00	Ref.	
Type of home					
Detached housing	4,595	0.05	0.03	1.12	0.079
Attached housing	2,016	0.00	0.00	Ref.	

Definition of abbreviation: Ref. = reference.

Coefficient estimates for factors in the model represent the estimated additional effect above the intercept value associated with the indicated level of each factor.

before endotoxin analysis may have diminished the integrity of the samples (14). In other studies performed in large U.S. inner cities (15, 16) and in a European consortium study (2), investigators reported higher endotoxin levels in bedroom floor and/or bedding (Table 5). Although endotoxin measurements in several national and regional studies were done by our laboratory, caution must be

exercised when comparing endotoxin data across laboratories, owing to differences in sample collection, storage, extraction, and assay techniques (17–20).

The ability of endotoxin to induce asthma and asthma-like symptoms was first recognized in occupational settings, especially in the cotton, grain, and livestock industries (21). Subsequent investigators concluded that domestic endotoxin

exposure was also associated with asthma severity measured by asthma symptoms and medication use (22, 23). Our findings of a higher prevalence of wheeze (Table 2) and asthma (Table E3) outcomes, as well as wheezing severity or medication use, associated with elevated dust endotoxin concentration, is consistent with findings of the first U.S. nationwide study (NSLAH) that endotoxin levels were associated with asthma diagnosis, asthma symptoms in the past 12 months, and use of asthma medication (4). However, stronger associations with wheeze-related outcomes were found in NHANES versus NSLAH. In a case-control investigation, Tavernier and colleagues observed higher levels of endotoxin in living room carpet among children with asthma compared than in age- and sex-matched children without asthma (24). Timing and duration of exposure have been conjectured to influence the effect of endotoxin on the development of asthma. Though endotoxin may be associated with increased risk of asthma in adulthood, it has been reported to be protective against the disease if exposure occurs very early in life (25). The mechanisms underlying immunomodulation by endotoxin are not entirely clear, but they are thought to include regulatory T lymphocytes and both proinflammatory and antiinflammatory cytokines and their upstream and downstream signaling pathways (26). The binding of LPS-binding protein to CD14 associated with lymphocyte antigen 96 (also referred to as LY96 or MD2) and TLR4 triggers a cascade that includes nuclear factor- κ B and activation of type 1 T helper cell and regulatory T lymphocyte pathways (27, 28). Exposures lead to chronic, low-level activation of innate immunity, which regulates immune responses away from type 2 inflammation pathways (29). Endotoxin may act, in part, as a proxy for immunomodulating microorganisms colonizing the gut or the lung.

A varying effect of endotoxin on asthma has been postulated by Matsui and colleagues, who hypothesized that endotoxin might be a risk factor for wheeze in certain environments (e.g., nonfarming settings, including the inner city) but could protect against wheeze when exposure occurs early in life in other surroundings (e.g., farming settings) (30). We found that endotoxin was strongly positively

Table 5. Comparison of Reservoir Endotoxin House Dust Values with Those in Other Studies

Study and Sample Type	Subjects (n)	GM or Median	5th–95th Percentile	Range
Studies done at our laboratory using the same methods				
NHANES (unweighted data)				
Bedroom floor, bedding	6,963	19.0	2.1–149	0.024–454,500
NSLAH (unweighted data) (1, 2)				
Bedroom floor	588	37.7	5.0–264	0.114–10,120
Bedding	470	20.6	2.0 – 149	0.024–1,147
New York City (4)				
Bedroom floor	301	75.9	12.5–501	1.2–3,388
Studies done at other laboratories				
Boston (6)				
Bedroom floor	323	63		2–761
East Baltimore (7)				
Bedroom floor	85	31.7		4.8–643.5
Bavarian, Austrian, Swiss homes (8)				
Farm bedding	319	37.8	14.4–88.9	
Nonfarm bedding	493	22.8	8.2–62.9	
European homes (5)				
Bedding	996	2.45	0.35–17.70	0.098–402.7

Definition of abbreviations: GM = geometric mean; NHANES = National Health and Nutrition Examination Survey; NSLAH = National Survey of Lead and Allergens in Housing.

All values are in endotoxin units per milligram of dust.

associated with asthma at low levels of specific IgE against dog, mouse, and rat but negatively associated with asthma at high levels of IgE against mouse and rat. Likewise, in a sample of laboratory scientists and technicians, Pacheco and colleagues looked at the association of aerosolized endotoxin with symptoms related to mouse by sensitization status and concluded that endotoxin predicts respiratory symptoms related to mice in non-mouse-sensitized participants (31). Researchers have suggested that allergen-specific IgE could be a surrogate measure of both exposure to a given allergen and sensitization (32). In a birth cohort assessment of the relationship of allergens and bacteria in dust with the development of recurrent wheeze or atopy, Lynch and colleagues found exposure to cockroach, mouse, and, to a lesser extent, cat allergens was protective against recurrent wheeze (30). They further observed that both cockroach and mouse allergens positively correlated with beneficial dust bacterial taxa of Bacteroidetes phylum, such as *Prevotellaceae*, known to contain a pentaacylated type of LPS (33, 34). Brix and colleagues suggested that distinct forms of endotoxin may trigger TLR4 differently, with the result that the pentaacylated endotoxin is protective against asthma and allergic sensitization and the more common hexaacylated variety poses

increased risk (35). Likewise, Hađina and colleagues noted lower inflammation and differing cytokine profiles in mice exposed to pentaacylated versus hexaacylated forms of endotoxin (36).

Predictors of endotoxin in house dust have been examined in several previous studies. We formerly reported in the NSLAH study that census region, race and/or ethnicity, poverty, education level, and presence of an electric heating source predicted higher endotoxin concentration in bedroom floor, whereas census region, metropolitan status, and poverty predicted endotoxin concentration on bedding (3). Bischof and colleagues measured endotoxin concentrations in settled house dust from the living room floors of 405 randomly selected homes in two German cities (Erfurt and Hamburg) and found higher endotoxin levels predicted by older buildings; lower-story residence; longer residence; irregular vacuum cleaning; and presence of a dog, cat, or mouse in the home (37). In two other German cities (Munich and Leipzig), Gehring and colleagues described endotoxin levels in dust samples from 2,157 infants' and 2,108 mothers' mattresses and found the presence of dog and a high number of occupants in the home to be predictors of high endotoxin concentrations (38). In the Allergy and Endotoxin Study, endotoxin was measured

in dust from the living room floors and children's mattresses of 319 farm families and 493 nonfarm families. Pet ownership and regularity of floor cleaning were associated with high endotoxin levels in nonfarm environments, whereas farm activities, study area, time since last cleaning, mattress type, and presence of young children predicted high endotoxin in farm settings (39). Gram-negative bacteria can be transported by pets and humans in the gut and on the skin, which may explain the presence of children, dogs, or cats as potential contributors to increased endotoxin levels in house dust. Cockroach carcasses contain endotoxin. Poverty, presence of cockroaches, and cigarette smoking may be indicators of poor general home hygiene and housing quality and thus auspicious of high endotoxin (3). We found that homes with smooth floors had significantly lower endotoxin concentrations in combined bedroom bed and floor dust, suggesting that, in addition to holding more dust, carpet dust contains more endotoxin per unit of mass than dust recovered from smooth floors. We observed 35% higher endotoxin in homes of Mexican Americans than homes of people of other races and ethnicities, apart from the effects of family size, housing type, or income. This finding may reflect that Hispanics are more likely than non-Hispanic whites to

live disproportionately in the southern United States in geographical locations more favorable to microbial growth. Analyses that include participant-specific geographic and climate variables are needed to test this hypothesis.

Our study has some limitations. Because NHANES is a cross-sectional study, we cannot infer that endotoxin exposures were causally associated with respiratory outcomes. Asthma and wheeze were self-reported and not verified. Recurrent wheeze appeared to be a more sensitive outcome measure than asthma. We suggest that participants reactive to endotoxin exposures in the absence of an allergy phenotype may not carry a diagnosis of asthma. Further, some patients resist the diagnosis of asthma, fearing loss of medical insurance coverage or restriction from participation in sports. Endotoxin was measured only once. Researchers in one study reported that a single measurement may not provide a precise estimate of dust endotoxin in homes over a short period of time (1–6 mo), although their measurements were based on children's and mothers' bedroom floors (40). Researchers in other studies observed no change in

endotoxin concentrations in beds and/or bedroom floors for up to a 1-year period (16, 41). Furthermore, the concentration of endotoxin in reservoir dust may poorly correlate with the amount of airborne endotoxin available to be inhaled (37). However, Rabinovitch and colleagues measured personal endotoxin cloud in schoolchildren and found results similar to ours (42). Dust endotoxin could be a proxy for other environmental exposures, explaining to some degree its associations with wheeze, asthma, and other allergic diseases. Certain microorganism-associated molecular patterns may act as ligands, binding to specific TLRs (e.g., lipoteichoic acids on TLR2 and TLR6, flagellin on TLR5, CpG DNA on TLR9) and produce proinflammatory cytokines and/or chemokines (43). However, unlike endotoxin, the potency of these other microbial agents is not established, despite their known ability to induce inflammation.

In conclusion, endotoxin levels in house dust from bed and bedroom floor in NHANES are lower than those reported in a previous study of the U.S. population (NSLAH) and those conducted in

farmhouses or in inner-city housing. However, even at these low levels, endotoxin exposure is associated with a higher prevalence of current wheeze, exercise-induced wheeze, use of prescription medication for wheezing, and doctor and/or ER visits for wheeze, regardless of sensitization status. Poverty, younger age, carpeting, pets, cockroaches, and/or a smoker in the household predict higher endotoxin levels. We propose that mitigating endotoxin indoors through reducing vermin in housing and mitigating poverty could lower the burden of wheeze outcomes with potential savings in medical expenditures for this condition. ■

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