House Dust Endotoxin Levels Are Associated with Adult Asthma in a U.S. Farming Population

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Abstract

Rationale: Endotoxin initiates a proinflammatory response from the innate immune system. Studies in children suggest that endotoxin exposure from house dust may be an important risk factor for asthma, but few studies have been conducted in adult populations.

Objectives: To investigate the association of house dust endotoxin levels with asthma and related phenotypes (wheeze, atopy, and pulmonary function) in a large U.S. farming population.

Methods: Dust was collected from the bedrooms (n = 2,485) of participants enrolled in a case–control study of current asthma (927 cases) nested within the Agricultural Health Study. Dust endotoxin was measured by *Limulus* amebocyte lysate assay. Outcomes were measured by questionnaire, spirometry, and blood draw. We evaluated associations using linear and logistic regression.

Measurements and Main Results: Endotoxin was significantly associated with current asthma (odds ratio [OR], 1.30; 95% confidence interval [CI], 1.14–1.47), and this relationship was modified by early-life farm exposure (born on a farm: OR, 1.18; 95% CI, 1.02–1.37; not born on a farm: OR, 1.67; 95% CI, 1.26–2.20; Interaction $P = 0.05$). Significant positive associations were seen with both atopic and nonatopic asthma. Endotoxin was not related to either atopy or wheeze. Higher endotoxin was related to lower FEV_1/FVC in asthma cases only (Interaction $P = 0.01$). For asthma, there was suggestive evidence of a gene-by-environment interaction for the *CD14* variant rs2569190 (Interaction $P = 0.16$) but not for the *TLR4* variants rs4986790 and rs4986791.

Conclusions: House dust endotoxin was associated with current atopic and nonatopic asthma in a U.S. farming population. The degree of the association with asthma depended on early-life farm exposures. Furthermore, endotoxin was associated with lower pulmonary function in patients with asthma.

Keywords: pulmonary function; atopy; wheeze; CD14; TLR4

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Asthma is a chronic respiratory disease estimated to affect 8.4% of the U.S. population (1). Environmental exposures, including endotoxin, are thought to influence asthma onset and progression (2). Endotoxin is a type of lipopolysaccharide located on the outer cell wall of gram-negative bacteria that, when released during lysis or cell division, initiates an innate immune response via the production of inflammatory cytokines (3, 4). Studies on the association of endotoxin and asthma have focused primarily on children (5). Most studies of endotoxin exposure in adults have been limited to the association of occupational exposure in the textile industry (6), animal feed industry (7), large animal farms (8), and other workplaces (9). A study of farmers in southeastern Norway found airborne endotoxin collected during farming tasks to be protective for atopic asthma but a risk factor for nonatopic asthma (10). These studies suggest that occupational endotoxin exposure may be an important risk factor for asthma. However, few studies have investigated the relationship of house dust endotoxin and adult asthma (11–13). House dust is a common and consistent source of exposure, so understanding its role in adult asthma is important.

The largest studies of house dust endotoxin and adult asthma in the United States were conducted within the National Survey of Lead and Allergens in Housing (NSLAH) and the National Health and Nutrition Examination Survey (NHANES)—both surveys of children and adults designed to be representative of the U.S. population (12, 13). The NSLAH included only questionnaire-based outcomes whereas the NHANES also included atopic sensitization. A study of mattress endotoxin levels nested within the European Community Respiratory Health Survey (ECRHS) additionally included spirometric measures of pulmonary function (11).

In the present study, we aimed to investigate the association of house dust endotoxin with current asthma and the related phenotypes of atopic sensitization and pulmonary function in a case–control study nested within the Agricultural Health Study, a U.S. adult farming cohort (14), where exposures might be higher and more variable than in the general U.S. population (15, 16) and potentially different from European settings where farming practices differ. To date, there has not been a study of house dust endotoxin exposure and adult asthma of this size. We also tested for effect modification by both early-life farming exposure, which has been found to protect against childhood allergic diseases but is less studied in adult populations (17), and by the presence of polymorphisms in two key endotoxin receptor genes (*CD14* [OMIM: 158120] and *TLR4* [OMIM: 603030]) reported to modify the response to endotoxin (18, 19).

By the nature of our study design, we were able to investigate the effect of endotoxin levels on these various outcomes in patients with asthma and those without asthma separately, which may provide some insight into the clinically relevant effects of endotoxin on respiratory health. A preliminary analysis of this study has been previously reported in abstract form (20).

Methods

Full methods are provided in the online supplement.

Study Population and Outcome Assessment

The Agricultural Lung Health Study (ALHS) is a case–control study of current asthma nested within the Agricultural Health Study (AHS) (14), a cohort of predominantly farmers and spouses of farmers. Total enrollment into the ALHS included 3,301 individuals. Participants were classified as "asthma cases" or "noncases" (*see* Figure E1 in the online supplement for more details).

We enrolled three categories of asthma cases: self-reported doctor-diagnosed current asthma (n = 876), potential undiagnosed asthma based on the presence of current asthma symptoms and asthma medication use in nonsmokers (n = 309), and overlapping diagnoses of current asthma and either chronic obstructive pulmonary disease (COPD) or emphysema in nonsmokers (n = 38). Noncases included individuals who did not report current asthma, asthma symptoms in the past 12 months, or current use of asthma medication.

The current study is an analysis of ALHS participants who had a bedroom dust sample collected for endotoxin analysis (2,485 independent households, 207 repeat home visits) (Figure E1). The study was approved by the Institutional Review Board at the National Institute of Environmental Health Sciences.

Participants were classified as having current wheeze if they indicated, via questionnaire, having had wheezing or whistling in their chest at any time or during exertion in the past 12 months. During home visits, trained field staff collected spirometry measures (FEV_1 and FVC). We measured specific immunoglobulin E (IgE) in blood to Bermuda grass, ragweed, Timothy grass, mountain cedar, *Alternaria*, dust mite, cat dander, milk, egg, and wheat. Atopy was defined as passing a threshold of 0.70 IU/ml to one or more antigens (21). Allergic rhinitis symptoms were assessed by questionnaire.

Endotoxin Exposure Assessment

Field technicians collected a vacuum dust sample from bedroom floors and sleeping surfaces. After sieving, samples were frozen (–20°C) and shipped to the University of Iowa for analysis. Endotoxin levels were measured using the *Limulus* amebocyte lysate assay (Lonza Walkersville, Inc., Walkersville, MD), as described (12, 13, 22). To ensure quality, the assays were conducted with reagents from a single lot and included high- and low-quality control samples. Measurements below the limit of detection (LOD) (0.00048 endotoxin units [EU]/mg, n = 15/2,692) were assigned a value equal to the LOD divided by the square root of two.

Gene-by-Environment Interaction Analysis

Three single-nucleotide polymorphisms (SNPs), rs2569190 in *CD14*, and rs4986790 and rs4986791 in *TLR4*, were selected for gene-by-environment analysis and genotyped from DNA extracted from blood or saliva. All SNPs passed standard quality metrics.

Statistical Analyses

We used multivariable linear and logistic regression models to test for the association of endotoxin with each outcome. Endotoxin was analyzed using quartile-defined categories and as a $\log_{10}$-transformed continuous variable. All models were adjusted for age, sex, and state (Iowa vs. North Carolina). Models of atopy, wheeze, and pulmonary function were also adjusted for asthma status. Additional models included smoking (never/past/current), pack-years, race, and season of collection.

Models of pulmonary function were additionally adjusted for age squared, height, height squared, and for FVC and weight. All analyses were conducted with SAS version 9.3 (SAS Institute, Inc., Cary, NC). The analysis used the following releases of Agricultural Health Study data: P3REL201209.00, P1REL201209.00, and AHSREL201304.00.

Results

For the main analyses, we restricted the data set to samples collected from independent households (n = 2,485) (Figure E1). This study population consisted of 927 (37%) asthma cases and 1,558 (63%) noncases. The majority were white (98.3%), never-smokers (64.1%), and from Iowa (68.5%) (Table 1). Farmers were mainly male (96.7%), and spouses of farmers were mainly female (99.7%). The ages at the home visits were similar between asthma cases and noncases, and those with asthma smoked less than those without asthma, on average (Table 1).

The overall geometric mean endotoxin concentration of the bed and bedroom floor house dust was 30.4 EU/mg (median, 44.4 EU/mg; interquartile range [IQR], 20.2–74.7 EU/mg; minimum, 0.0003 EU/mg [adjusted LOD]; maximum, 4,452 EU/mg). The geometric mean was higher in asthma cases (Table 2). In repeat home visits (n = 207), endotoxin levels were correlated (Spearman $\rho = 0.46$; $P < 0.0001$) despite a long lag time between visits (median, 9 mo; SD, 9.8 mo; IQR, 4–16 mo).

Increasing endotoxin was significantly associated with higher odds of asthma whether modeled categorically using quartile cut-points (Table 3) or treated continuously as a $\log_{10}$-transformed variable (OR, 1.30; 95% CI, 1.14–1.47) (Table E1). The analysis using quartile cut-points suggests that this relationship may not be linear; quartile 2 (OR, 1.39; 95% CI, 1.09–1.77), quartile 3 (OR, 1.58; 95% CI, 1.24–2.01), and quartile 4 (OR, 1.44; 95% CI, 1.13–1.84) have similar odds ratios compared with the first quartile (Table 3). To better visualize the relationship between endotoxin levels and asthma odds, we created smooth plots using a spline model, confirming the potential nonlinear relationship identified by the quartile analysis. The smooth plot shows a gradual increase in asthma case proportion across the lower and higher endotoxin concentrations, with a steeper increase around the median (Figure 1).

Table 1. Study population characteristics

Characteristic	Overall (*n = 2,485*) [*n* (%)*/*mean* (*SD*)]	Asthma Cases (*n = 927*) [*n* (%)*/*mean* (*SD*)]	Noncases (*n = 1,558*) [*n* (%)*/*mean* (*SD*)]
Participant type†			
Farmer	1,433 (57.7)	477 (51.5)	956 (61.4)
Spouse	1,052 (42.3)	450 (48.5)	602 (38.6)
Sex			
Male	1,389 (55.9)	451 (48.7)	938 (60.2)
Female	1,096 (44.1)	476 (51.4)	620 (39.8)
State			
Iowa	1,703 (68.5)	648 (69.9)	1,055 (67.7)
North Carolina	782 (31.5)	278 (30.1)	503 (32.3)
Smoking status			
Never	1,569 (64.1)	632 (69.2)	937 (61.0)
Former	762 (31.1)	254 (27.8)	508 (33.1)
Current	118 (4.8)	28 (3.1)	90 (5.86)
Missing	36	13	23
Smoking, pack-years‡	18.5 (22.0)	14.4 (18.6)	20.5 (23.2)
Currently living on farm			
Yes	1,892 (77.3)	699 (76.6)	1,193 (77.7)
No	556 (22.7)	214 (23.4)	342 (22.3)
Missing	37	14	23
Currently farming			
Yes	1,468 (59.9)	511 (55.9)	957 (62.3)
No	982 (40.1)	403 (44.1)	579 (37.3)
Missing	35	13	22
Living on farm at birth			
Yes	1,857 (75.7)	668 (73.2)	1,189 (77.2)
No	596 (24.3)	245 (26.8)	351 (22.8)
Missing	32	14	18
Race			
White	2,418 (98.3)	903 (98.6)	1,515 (98.2)
Black	24 (1.0)	8 (0.8)	16 (1.0)
Other	17 (0.7)	5 (0.6)	12 (0.7)
Missing	26	11	15
Dust collection season			
Winter	591 (23.8)	200 (21.6)	391 (25.1)
Spring	664 (26.7)	217 (23.4)	447 (28.7)
Summer	715 (28.8)	276 (29.8)	439 (28.2)
Fall	515 (20.7)	234 (25.2)	281 (18.0)
Current wheeze			
Yes	840 (34.3)	644 (70.5)	196 (12.8)
No	1,607 (65.7)	269 (29.5)	1,338 (87.2)
Missing	38	14	24
Atopic§			
Yes	454 (18.7)	257 (27.8)	200 (13.2)
No	1,976 (81.3)	659 (72.2)	1,317 (86.8)
Missing	55	14	41
Pulmonary function			
FEV_1, ml	2,600 (837)	2,373 (827)	2,734 (813)
FVC, ml	3,552 (1,027)	3,341 (1,025)	3,677 (1,008)
FEV_1/FVC, %	72.9 (9.3)	70.6 (10.5)	74.2 (8.2)
Age, yr	62.8 (11.2)	62.3 (10.9)	63.2 (11.4)

*Percentages in each category are based on nonmissing values. Some add to more than 100% because of rounding.
†Farmers and spouses do not represent a married pair. Rather, they are farmers or spouses of farmers from independent households.
‡From current and former smokers only.
§Atopy was defined as a specific IgE level of 0.70 IU/ml in response to 1 of 10 common allergens: Bermuda grass, ragweed, Timothy grass, mountain cedar, *Alternaria*, dust mite, cat dander, milk, egg, or wheat.

The majority of participants reported currently living on a farm and currently farming (Table 1). However, inclusion of

Table 2. Distribution of endotoxin overall and by asthma status

Subset	Geometric Mean (*EU/mg*)	Interquartile Range (*EU/mg*)
Overall (n = 2,485)	30.4	20.2–74.7
Asthma cases (n = 927)	36.2	23.1–76.0
Noncases (n = 1,558)	27.5	18.2–73.0

Definition of abbreviation: EU = endotoxin unit.

these variables in the regression model did not alter the association between endotoxin, treated as a continuous variable, and asthma (OR, 1.31; 95% CI, 1.15–1.49). We also assessed additional potential confounders including dogs currently living inside of the home, a known source of endotoxin, ever/never use of pesticides previously associated with asthma (23, 24), and body mass index. Similarly, inclusion of these variables in the models did not alter the results.

Because early-life exposure to high levels of endotoxin, like those often found on farms, has been reported to protect against the development of asthma (17), we examined whether living on a farm at birth modified the association between endotoxin and asthma. Associations between dust endotoxin and asthma were seen in both strata, but were significantly stronger for individuals not born on a farm (OR, 1.67; 95% CI, 1.26–2.20) compared with those who were (OR, 1.18; 95% CI, 1.02–1.37) (Interaction $P = 0.05$) in an analysis of $\log_{10}$ endotoxin treated as a continuous variable. Trend analyses based on a spline model were used to visualize this relationship (Figure E2).

We did not observe a statistically significant association between endotoxin and wheezing in the past 12 months when endotoxin was categorized by quartiles after adjusting for asthma status (Table 3). As with asthma, odds ratios were higher in the top three quartiles compared with the bottom quartile. When treated as a $\log_{10}$-transformed continuous variable, endotoxin showed no appreciable association with wheeze in the overall data set adjusted for asthma status (OR, 1.02; 95% CI, 0.87–1.18) (Table E1) or among asthma cases and noncases considered separately (asthma cases: OR, 1.08; 95% CI, 0.86–1.36; noncases: OR, 0.97; 95% CI, 0.79–1.18; Interaction $P = 0.62$). Because inhaled steroid use in patients with asthma could blunt associations between endotoxin exposure and wheezing, we considered models stratified by or adjusted for this variable, but found no evidence of effect modification or confounding.

Endotoxin was not associated with atopy (Table 3 and Table E1). When atopy and asthma were analyzed together by polytomous regression, endotoxin was significantly related to both atopic asthma (OR, 1.38; 95% CI, 1.09–1.74) and nonatopic asthma (OR, 1.24; 95% CI, 1.07–1.43) relative to no asthma or atopy, supporting the interpretation that endotoxin is related to asthma in both atopic and nonatopic persons (Table 4). Seventy-four percent of atopic participants reported having allergic rhinitis symptoms in the past 12 months; this variable was not associated with endotoxin and did not modify the association of endotoxin and asthma.

When we examined measures of pulmonary function in the overall data set; endotoxin was not appreciably associated with FEV_1, FVC, or their ratio (Table 5). However, when we stratified by asthma status, endotoxin was related to lower FEV_1/FVC (%) in patients with asthma ($\beta = -1.0\%$; SE = 0.5) compared with noncases ($\beta = -0.05\%$; SE = 0.2) (Interaction $P = 0.01$). A similar pattern was observed for FEV_1 (Table 5).

We tested the hypothesis that SNPs in *CD14* and *TLR4* may modify the association between endotoxin and asthma. Using standard additive models, no significant interactions were observed (Interaction $P > 0.33$) (Table E2). We also fit secondary models designed to mirror those used in previous studies where evidence for effect modification by the SNPs was reported (Table E2) (25, 26). For the *TLR4* variants, we combined two

Table 3. Odds ratios and 95% confidence intervals for the association of house dust endotoxin concentrations with asthma, wheeze, and atopy

Outcome and Endotoxin Category (*EU/mg*)	Number of Cases/Noncases*	Base Model† [*OR* (*CI*)]	Full Model‡ [*OR* (*CI*)]
Current asthma (n = 2,485)			
Quartile 1	194/428	Ref.	Ref.
Quartile 2	237/384	1.34 (1.06–1.70)§	1.39 (1.09–1.77)‖
Quartile 3	255/366	1.56 (1.24–1.98)¶	1.58 (1.24–2.01)¶
Quartile 4	241/380	1.45 (1.14–1.84)‖	1.44 (1.13–1.84)‖
Any current wheeze (n = 2,447)			
Quartile 1	180/432	Ref.	Ref.
Quartile 2	216/397	1.14 (0.85–1.54)	1.12 (0.83–1.52)
Quartile 3	230/384	1.18 (0.88–1.59)	1.15 (0.85–1.55)
Quartile 4	214/394	1.11 (0.82–1.50)	1.09 (0.80–1.47)
Atopy (n = 2,430)			
Quartile 1	116/492	Ref.	Ref.
Quartile 2	126/485	1.08 (0.81–1.44)	1.08 (0.80–1.45)
Quartile 3	108/492	0.90 (0.67–1.22)	0.89 (0.66–1.21)
Quartile 4	104/507	0.87 (0.64–1.17)	0.86 (0.63–1.17)

Definition of abbreviations: CI = confidence interval; EU = endotoxin unit; OR = odds ratio.
Endotoxin quartiles are defined by the following cutoff points: quartile 1: less than 20.2 EU/mg; quartile 2: 20.2–44.4 EU/mg; quartile 3: 44.4–74.7 EU/mg; and quartile 4: more than 74.7 EU/mg.
**Number of Cases/Noncases* refers to the number of participants with and without asthma, current, wheeze, or atopy for each given analysis.
†Base model adjusted for age, sex, and state. Wheeze and atopy analyses also adjusted for asthma status.
‡In addition to the covariates included in the base model, the full model is adjusted for smoking (never/past/current), pack-years, race, and season.
§$P < 0.05$.
‖$P < 0.01$.
¶$P < 0.001$.

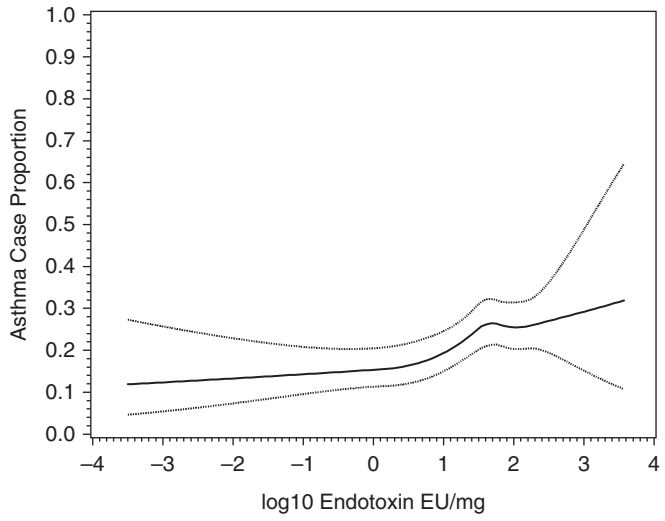

Figure 1. Smooth plot of asthma case proportion (*black line*) and 95% confidence interval (*dashed lines*) by $\log_{10}$ endotoxin units (EU) per milligram from bedroom floor and bedding house dust adjusted for age, sex, and state (20). The *y* axis represents the proportion of asthma cases at the given endotoxin level and cannot be interpreted as a prevalence because of the case–control study design used.

variants (rs4986790 and rs4986791) for a haplotype analysis. Individuals were categorized as noncarriers (89%) if they were homozygous for both major alleles or as a variant carrier (11%) if one of the minor alleles was present, as described (25, 27), resulting in no evidence of a gene-by-environment interaction (Interaction $P = 0.48$; Table E2). The *CD14* SNP (rs2569190) was tested using a recessive genetic model based on the findings by Zambelli-Weiner and colleagues (26). This model was somewhat suggestive that an interaction may exist (Interaction $P = 0.16$; Table E2), and the observed direction was consistent with previously published reports.

Table 4. Polytomous regression analysis of atopic and nonatopic asthma and endotoxin

Asthma Subtype	n	OR (CI)
Noncases	1,306	Ref.
Asthma cases		
Atopy only	172	0.91 (0.75–1.11)
Nonatopic asthma	647	1.24 (1.07–1.44)*
Atopic asthma	241	1.38 (1.09–1.74)*

Definition of abbreviations: CI = confidence interval; OR = odds ratio.
Shown are odds ratios and 95% confidence intervals for the association of $\log_{10}$-transformed house dust endotoxin concentrations (treated as a linear variable) with asthma adjusted for age, sex, state, smoking (never/past/current), pack-years, race, and season.
*$P < 0.01$.

Discussion

We conducted a large study of current asthma (2,485 independent households with 927 current asthma cases) and bedroom dust endotoxin from the homes of farmers and spouses of farmers. We found that higher endotoxin levels were associated with increased odds of asthma, irrespective of atopy assessed by specific IgE measurements, and that the relationship between house dust endotoxin levels and adult asthma was modified by early-life farm exposure. In addition, we observed a decrease in pulmonary function (FEV_1/FVC) associated with increasing endotoxin in patients with asthma.

Our asthma findings are consistent with a smaller study (831 total households with 588 bedroom floor dust data points) of house dust endotoxin conducted within the NSLAH (12). In that study, higher endotoxin concentrations in bedroom floor dust were associated with asthma without effect modification by self-reported allergy status (12). Subset analyses revealed that the association with endotoxin and asthma was restricted to adults. Smooth plots from that study show a general increase in asthma prevalence as endotoxin concentrations increase, with a prominent elevation around the median, which closely resemble the shape of the smooth plot generated with our ALHS data.

In an analysis of the NHANES survey, Thorne and coauthors reported statistically significant associations between bedroom house dust endotoxin levels and wheeze, while the association with current asthma was slightly weaker and not statistically significant (13). It is difficult to directly compare results because adults and children were analyzed together in that study whereas our study is limited to adults. In our study, we found an association with wheeze, but it was not significant after adjusting for asthma status. The NHANES population had fewer patients with asthma and the results were not adjusted for asthma status. The outcome of asthma with any wheeze in NHANES would be more comparable to the outcome in our study, where two-thirds of patients with asthma wheezed in the past year. Of note, when they analyzed current asthma with any wheeze they found a positive association of a similar magnitude as in our study. However, given the smaller number of cases in their study (639, including both children and adults) the association just barely missed statistical significance (OR, 1.19 per $\log_{10}$ EU/mg; 95% CI, 0.98–1.46).

As Thorne and coauthors (13) caution, it can be difficult to compare endotoxin measurements from different studies; however, our dust samples were processed and analyzed using the same techniques and the same laboratory as both the NSLAH and NHANES endotoxin studies, albeit at different points in time (Thorne laboratory, University of Iowa). It is difficult to compare the endotoxin levels in our study with those of the NSLAH study because they measured bedroom floor and bedding dust separately (geometric mean [GM], 37.7 EU/mg from the bedroom floor; GM, 20.6 EU/mg from the bedding). However, the GM for endotoxin in the combined bed and bedroom floor dust sample in our study (30.4 EU/mg) was more than 50% higher than in the NHANES study (GM, 19.0 EU/mg).

A previous study of farmers in southeastern Norway reported that higher

Table 5. Association of dust endotoxin and pulmonary function

Outcome	Combined (*n* = 2,396)		Asthma Cases (*n* = 865)		Noncases (*n* = 1,470)		Interaction *P* Value
	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	
FEV_1, ml	−13.3 (14.3)	0.35	−39.1 (29.4)	0.18	−2.4 (16.0)	0.88	0.16
FVC, ml	0.65 (16.3)	0.97	−13.4 (32.3)	0.68	6.7 (18.7)	0.72	0.47
FEV_1/FVC, %	−0.3 (0.2)	0.15	−1.0 (0.5)	0.06	−0.05 (0.2)	0.85	0.01

β and SE are given for the difference in pulmonary function per unit increase in $\log_{10}$ endotoxin units per milligram. Results shown are from adjusted linear regression models that include age, age squared, sex, state, smoking (never/past/current), pack-years, race, season, height, and height squared. FVC models also include weight. The combined analysis is additionally adjusted for asthma status. Numbers differ from those in Table 1 because of missing covariate information.

airborne endotoxin exposure during farming tasks was protective for atopic asthma (33 cases) but a risk factor for nonatopic asthma (131 cases) (10). In our larger study of house dust endotoxin, we found that higher exposure was significantly, positively related to both atopic asthma (241 cases) and nonatopic asthma (647 cases). Endotoxin was not related to atopy in either study. The study of Norwegian farmers did not examine effect modification by early-life farming exposure.

Because our study included both farmers and farm spouses, many of whom did not grow up on a farm, we were able to examine this issue. We found that being born on a farm modified the association between endotoxin and asthma; increasing endotoxin was associated with asthma in both groups, but this effect was significantly greater in individuals who were not born on a farm. Many studies have shown a general "protective farm effect," although the exact causal mechanism(s) remains unclear (17). These studies have primarily investigated childhood outcomes, and few have been conducted in U.S. populations (28). This putative protective farm effect could be due to early exposure to higher levels of endotoxin (15, 17).

Some studies have looked directly at early endotoxin exposure in childhood outcomes of asthma, atopy, and wheeze (29–31). However, the potential protection of early exposures in adults is less clear; our data suggest that some aspect(s) of farm life reduces the association of household dust endotoxin and asthma persisting into adulthood. Although the exact biological mechanism is not fully understood, it has been hypothesized that these early exposures may help promote a shift to a helper T-cell type 1–mediated immune response, protecting against numerous inflammatory diseases (17).

We evaluated associations with spirometric measures of pulmonary function. Previous studies of endotoxin effects on pulmonary function in adults have focused on exposure from occupational settings. Neither of the two U.S. national surveys (12, 13) investigated the relationship of house dust endotoxin with pulmonary function. Our case–control study design enabled us to examine the association between endotoxin and pulmonary function in patients with asthma and subjects without asthma separately with sufficient power. We found evidence of effect modification by asthma status; FEV_1/FVC was moderately reduced in relation to increasing exposure in patients with asthma only. The study of 972 adults nested within the ECRHS (11) did not find any association between mattress endotoxin levels and pulmonary function overall; results were not presented separately by asthma status. However, Michel and colleagues in two separate studies found a significant reduction in FEV_1/FVC in relation to house dust endotoxin levels in patients with asthma (n = 29 and n = 69) (32, 33). Together, these studies and ours suggest that endotoxin may have a more dramatic effect on pulmonary function in patients with asthma compared with subjects without asthma.

Endotoxin is recognized by the innate immune system through its interaction with key receptor proteins. Endotoxin aggregates are released from gram-negative bacterial cells during lysis or replication; they are then bound to lipopolysaccharide-binding protein and transferred to membrane-bound CD14, and then to a Toll-like receptor 4 (TLR4)–myeloid differentiation factor 2 (MD-2) complex that dimerizes and initiates a proinflammatory cytokine signaling cascade (3, 4, 18). This pathway is not dependent on lipopolysaccharide-binding protein or membrane-bound CD14, as soluble CD14 can act to transfer endotoxin to TLR4 (4). On the basis of these observations, we narrowed our literature search for potential genetic modifiers located in the key receptor proteins (CD14, TLR4, and MD-2) limited to studies specifically of endotoxin and asthma in adults where the author concluded that a gene-by-environment interaction was present.

Using these criteria, three variants in *CD14* and *TLR4* stood out as candidates (25–27). However, we found little evidence of a gene-by-environment interaction, consistent with other studies (18, 34–37). We did observe a marginal interaction by rs2569190 in *CD14*, using a recessive model, and the direction mirrors that reported by Zambelli-Weiner and coauthors, who found that individuals homozygous for the minor allele had reduced asthma risk at lower endotoxin concentrations and increased asthma risk at higher endotoxin concentrations (26). A genotype-dependent effect of endotoxin inhalation may be acute, as several previous studies found *TLR4* genotype–specific hyporesponsiveness (e.g., reduced decline in FEV_1) to direct endotoxin inhalation challenge, driven by the two variants tested in the study (rs4986790 and rs4986791) (27, 38, 39). It is also possible that other variants either within or outside the key endotoxin receptor that were not analyzed here may modify the effects of endotoxin.

Strengths and Limitations

Our study has several strengths. We analyzed a large data set from a farming population, members of which might be expected to have higher household endotoxin exposure levels. In fact, a study by Barnig and colleagues found that house dust endotoxin levels were higher in floor and mattress samples taken from farmhouses than nonfarmhouses and houses in urban settings (15). Von Mutius and coauthors also found that houses of farming families had higher endotoxin levels than those of nonfarming families and that nonfarming families in regular contact with livestock via neighboring farms had higher home endotoxin levels (16), suggesting that the bedroom dust endotoxin levels in our study are likely a surrogate for the occupational and lifestyle exposures of our participants. Because of the proximity of the home to the farming operations that confer higher exposures, participants may track dust, containing endotoxin, into the home on their skin, clothing, and footwear. We focused on current asthma in adults; previous house dust endotoxin studies have been primarily focused on childhood exposures and childhood asthma outcomes or analyzed childhood and adult patients with asthma together. In addition, we were able to investigate several different outcomes beyond asthma including wheeze, atopy, pulmonary function, and potential modifiers that have not been examined in all studies.

Our study has some limitations. All studies of house dust endotoxin assume that the endotoxin concentrations measured are a proxy for inhaled endotoxin levels. However, we measured endotoxin from bedroom floors and bedding, where people spend a large part of their day. In addition, bed dust has been shown to be the most reproducible household sampling location, with greater between-home variation than within-home variation (40). Although we analyzed only a single measurement of endotoxin, which is subject to exposure measurement error if levels change markedly over time, we had repeated measures from 207 houses and found the two measures to be correlated (Spearman $\rho = 0.46$); a similar correlation has been seen in another study (41). Nonetheless, the use of a single dust collection to reflect chronic exposure is likely to have introduced some degree of measurement error that could have attenuated the observed associations. Finally, we are not able to determine the specific bacterial source of the measured endotoxin. It would be of interest to investigate the full microbiota of house dust and how that relates to asthma in adults.

Conclusions

We have conducted the largest adult asthma case–control study of house dust endotoxin levels of this size in a U.S farming population. We have shown that increasing endotoxin levels are positively associated with increased odds of current atopic and nonatopic asthma. The association with asthma was modified by early-childhood farming exposures, and we found suggestive evidence of modification by a polymorphism in *CD14*. In addition, our results suggest that household dust endotoxins are related to lower FEV_1/FVC in adult patients with asthma. ■

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References

1 Walford HH, Doherty TA. Diagnosis and management of eosinophilic asthma: a US perspective. *J Asthma Allergy* 2014;7:53–65.
2 Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? *Lancet* 2015;386:1075–1085.
3 Nijland R, Hofland T, van Strijp JA. Recognition of LPS by TLR4: potential for anti-inflammatory therapies. *Mar Drugs* 2014;12: 4260–4273.
4 Kitchens RL. Role of CD14 in cellular recognition of bacterial lipopolysaccharides. *Chem Immunol* 2000;74:61–82.
5 Rennie DC, Lawson JA, Senthilselvan A, Willson PJ, Dosman JA. Domestic endotoxin exposure and asthma in children: epidemiological studies. *Front Biosci* (*Elite Ed*) 2012;4:56–73.
6 Lai PS, Hang JQ, Valeri L, Zhang FY, Zheng BY, Mehta AJ, Shi J, Su L, Brown D, Eisen EA, *et al*. Endotoxin and gender modify lung function recovery after occupational organic dust exposure: a 30-year study. *Occup Environ Med* 2015;72:546–552.
7 Smid T, Heederik D, Houba R, Quanjer PH. Dust- and endotoxin-related acute lung function changes and work-related symptoms in workers in the animal feed industry. *Am J Ind Med* 1994;25:877–888.
8 May S, Romberger DJ, Poole JA. Respiratory health effects of large animal farming environments. *J Toxicol Environ Health B Crit Rev* 2012;15:524–541.
9 Liebers V, Raulf-Heimsoth M, Brüning T. Health effects due to endotoxin inhalation [review]. *Arch Toxicol* 2008;82:203–210.
10 Eduard W, Douwes J, Omenaas E, Heederik D. Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. *Thorax* 2004;59:381–386.
11 Bakolis I, Doekes G, Heinrich J, Zock JP, Heederik D, Kogevinas M, Guerra S, Norbäck D, Ramasamy A, Nevalainen A, *et al*.; Indoor Group of the ECRHS; Work Package 10 Group of HITEA. Respiratory health and endotoxin: associations and modification by CD14/-260 genotype. *Eur Respir J* 2012;39:573–581.
12 Thorne PS, Kulhánková K, Yin M, Cohn R, Arbes SJ Jr, Zeldin DC. Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. *Am J Respir Crit Care Med* 2005; 172:1371–1377.
13 Thorne PS, Mendy A, Metwali N, Salo P, Co C, Jaramillo R, Rose KM, Zeldin DC. Endotoxin exposure: predictors and prevalence of associated asthma outcomes in the United States. *Am J Respir Crit Care Med* 2015;192:1287–1297.
14 Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, Pennybacker M, Rothman N, Dosemeci M, Bond AE, *et al*. The Agricultural Health Study. *Environ Health Perspect* 1996;104: 362–369.
15 Barnig C, Reboux G, Roussel S, Casset A, Sohy C, Dalphin JC, de Blay F. Indoor dust and air concentrations of endotoxin in urban and rural environments. *Lett Appl Microbiol* 2013;56:161–167.
16 von Mutius E, Braun-Fahrländer C, Schierl R, Riedler J, Ehlermann S, Maisch S, Waser M, Nowak D. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000;30:1230–1234.
17 von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol* 2010;10:861–868.
18 Ferwerda B, McCall MB, Verheijen K, Kullberg BJ, van der Ven AJ, Van der Meer JW, Netea MG. Functional consequences of Toll-like receptor 4 polymorphisms. *Mol Med* 2008;14:346–352.
19 Lau MY, Dharmage SC, Burgess JA, Lowe AJ, Lodge CJ, Campbell B, Matheson MC. CD14 polymorphisms, microbial exposure and

allergic diseases: a systematic review of gene–environment interactions. *Allergy* 2014;69:1440–1453.
20 London SJ. House dust endotoxin levels are associated with adult asthma in the agricultural lung health study [abstract]. *Am J Respir Crit Care Med* 2016;193:A2781.
21 Van Hoeyveld E, Nickmans S, Ceuppens JL, Bossuyt X. Defining thresholds of specific IgE levels to grass pollen and birch pollen allergens improves clinical interpretation. *Clin Chim Acta* 2015;450: 46–50.
22 Vojta PJ, Friedman W, Marker DA, Clickner R, Rogers JW, Viet SM, Muilenberg ML, Thorne PS, Arbes SJ Jr, Zeldin DC. First National Survey of Lead and Allergens in Housing: survey design and methods for the allergen and endotoxin components. *Environ Health Perspect* 2002;110:527–532.
23 Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MC, Sandler DP. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. *Am J Respir Crit Care Med* 2008;177:11–18.
24 Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Coble J, Alavanja MC, Beane Freeman LE, Sandler DP. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur Respir J* 2009;34:1296–1303.
25 Werner M, Topp R, Wimmer K, Richter K, Bischof W, Wjst M, Heinrich J. *TLR4* gene variants modify endotoxin effects on asthma. *J Allergy Clin Immunol* 2003;112:323–330.
26 Zambelli-Weiner A, Ehrlich E, Stockton ML, Grant AV, Zhang S, Levett PN, Beaty TH, Barnes KC. Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol* 2005;115:1203–1209.
27 Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;25:187–191.
28 Genuneit J. Exposure to farming environments in childhood and asthma and wheeze in rural populations: a systematic review with meta-analysis. *Pediatr Allergy Immunol* 2012;23:509–518.
29 Braun-Fahrländer C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, *et al*.; Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;347:869–877.
30 Karvonen AM, Hyvärinen A, Gehring U, Korppi M, Doekes G, Riedler J, Braun-Fahrländer C, Bitter S, Schmid S, Keski-Nisula L, *et al*.; PASTURE Study Group. Exposure to microbial agents in house dust and wheezing, atopic dermatitis and atopic sensitization in early childhood: a birth cohort study in rural areas. *Clin Exp Allergy* 2012; 42:1246–1256.
31 Lawson JA, Dosman JA, Rennie DC, Beach JR, Newman SC, Crowe T, Senthilselvan A. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: a case–control study. *BMC Pulm Med* 2012;12:56.
32 Michel O, Ginanni R, Duchateau J, Vertongen F, Le Bon B, Sergysels R. Domestic endotoxin exposure and clinical severity of asthma. *Clin Exp Allergy* 1991;21:441–448.
33 Michel O, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, Pauwels R, Sergysels R. Severity of asthma is related to endotoxin in house dust. *Am J Respir Crit Care Med* 1996;154:1641–1646.
34 von Aulock S, Schröder NW, Gueinzius K, Traub S, Hoffmann S, Graf K, Dimmeler S, Hartung T, Schumann RR, Hermann C. Heterozygous Toll-like receptor 4 polymorphism does not influence lipopolysaccharide-induced cytokine release in human whole blood. *J Infect Dis* 2003;188:938–943.
35 van der Graaf C, Kullberg BJ, Joosten L, Verver-Jansen T, Jacobs L, Van der Meer JW, Netea MG. Functional consequences of the Asp299Gly Toll-like receptor-4 polymorphism. *Cytokine* 2005;30: 264–268.
36 Douville RN, Lissitsyn Y, Hirschfeld AF, Becker AB, Kozyrskyj AL, Liem J, Bastien N, Li Y, Victor RE, Sekhon M, *et al*. TLR4 Asp299Gly and Thr399Ile polymorphisms: no impact on human immune responsiveness to LPS or respiratory syncytial virus. *PLoS One* 2010;5:e12087.
37 Kljaic-Bukvic B, Blekic M, Aberle N, Curtin JA, Hankinson J, Semic-Jusufagic A, Belgrave D, Simpson A, Custovic A. Genetic variants in endotoxin signalling pathway, domestic endotoxin exposure and asthma exacerbations. *Pediatr Allergy Immunol* 2014;25:552–557.
38 Long H, O'Connor BP, Zemans RL, Zhou X, Yang IV, Schwartz DA. The Toll-like receptor 4 polymorphism Asp299Gly but not Thr399Ile influences TLR4 signaling and function. *PLoS One* 2014;9:e93550.
39 Michel O, LeVan TD, Stern D, Dentener M, Thorn J, Gnat D, Beijer ML, Cochaux P, Holt PG, Martinez FD, *et al*. Systemic responsiveness to lipopolysaccharide and polymorphisms in the Toll-like receptor 4 gene in human beings. *J Allergy Clin Immunol* 2003;112:923–929.
40 Park JH, Spiegelman DL, Burge HA, Gold DR, Chew GL, Milton DK. Longitudinal study of dust and airborne endotoxin in the home. *Environ Health Perspect* 2000;108:1023–1028.
41 Abraham JH, Gold DR, Dockery DW, Ryan L, Park JH, Milton DK. Within-home versus between-home variability of house dust endotoxin in a birth cohort. *Environ Health Perspect* 2005;113: 1516–1521.