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Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood

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Abstract

Background—There has been no longitudinal study of the relation between concurrent exposure to dust mite allergen and endotoxin in early life and asthma and atopy at school age.

Objectives—To examine the relation between exposure to dust mite allergen and endotoxin at age 2 to 3 months and asthma, wheeze, and atopy in high-risk children.

Methods—Birth cohort study of 440 children with parental history of atopy in the Boston metropolitan area.

Results—In multivariate analyses, early exposure to high levels of dust mite allergen (≥10 μg/g) was associated with increased risks of asthma at age 7 years (odds ratio [OR], 3.0; 95% CI, 1.1-7.9) and late-onset wheeze (OR, 5.0; 95% CI, 1.5-16.4). Exposure to endotoxin levels above the lowest quartile at age 2 to 3 months was associated with reduced odds of atopy at school age (OR, 0.5; 95% CI, 0.2-0.9). In contrast with its inverse association with atopy, endotoxin exposure in early life was associated with an increased risk of any wheeze between ages 1 and 7 years that did not change significantly with time (hazard ratio for each quartile increment in endotoxin levels, 1.23; 95% CI, 1.07-1.43).

Conclusion—Among children at risk of atopy, early exposure to high levels of dust mite allergen is associated with increased risks of asthma and late-onset wheeze. In these children, endotoxin exposure is associated with a reduced risk of atopy but an increased risk of wheeze.

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Clinical implications—Early endotoxin exposure may be a protective factor against atopy but a risk factor for wheeze in high-risk children.

Keywords

Endotoxin; dust mite; wheeze; atopy; asthma

The relation between exposure to dust mite allergen and/or endotoxin in early life and the development of asthma and atopy is not clear. For example, an association between exposure to dust mite allergen in infancy and asthma in children at risk for atopy[1] was not replicated in subsequent longitudinal studies with variable duration of follow-up.[2-6] Exposure to endotoxin in house dust has been associated with reduced risks of atopy[7] and eczema[8,9] but an increased risk of wheeze in longitudinal studies from birth to infancy.[7,9] There has been no longitudinal analysis of the relation between concurrent exposure to dust mite allergen and endotoxin in early life and asthma and atopy at school age.

We examined the relation between exposure to dust mite allergen and endotoxin at age 2 to 3 months and asthma and atopy at school age in a prospective birth cohort study of children with parental history of atopy (the Epidemiology of Home Allergens and Asthma Study).

METHODS

Study population

Study participants were recruited between September 1994 and August 1996. The screening and recruitment of families have been described in detail elsewhere.[10,11] In brief, eligibility criteria included residence in the Boston metropolitan area; maternal age ≥18 years; and history of hay fever, asthma, or allergies in at least 1 of the child's parents. Families were not screened if the newborn was hospitalized in the intensive care unit, if his/her gestational age was < 36 weeks, or if he/she had a congenital anomaly. After written informed consent was obtained from the child's primary caretaker, a home visit was made when the child was 2 to 3 months of age, and a questionnaire regarding demographics, home characteristics, environmental exposures, tobacco use, and health outcomes was administered by trained research assistants. Every 2 months, beginning when the child was 2 months of age, a telephone questionnaire (modified from the American Thoracic Society-Division of Lung Diseases questionnaire)[12] was administered by trained research assistants to the child's primary caretaker until the child's second birthday. Afterward, interviews were conducted every 6 months. Of the 505 children enrolled in the study, 7 were excluded because they were followed for ≤4 months during their first year of life. The study was approved by the Institutional Review Board of Brigham and Women's Hospital.

Dust extraction and analysis of allergens and endotoxin

The methods used for collection of dust samples at the home visit and for measurement of dust mite allergen and endotoxin have been described previously.[10,11,13,14] In brief, separate dust samples were collected by vacuuming the baby's bed, the baby's bedroom floor, the parent's bed (if the child slept there at least half the time), the living room/family room (heretofore referred to as family room), and the kitchen floor. When limited dust was available from the baby's bed, the baby's bedroom floor, or the family room, Der f 1 (more prevalent than Der p 1 in the Boston area) was analyzed first and then the remaining extract was analyzed for Der p 1. As in previous analyses,[15] dust mite allergen assayed from the baby's bed was used in the current analysis because that sample had the highest concentration and the most complete information for dust mite allergen and was considered a location where children would be highly exposed. When an adequate amount of dust from

the family room was available, an assay for endotoxin was also performed. All allergens were measured by monoclonal antibody ELISA assays,[16] with antibodies and reference allergens obtained from Dr Chapman's laboratory at the University of Virginia (Charlottesville, Va).

Consistent with previous analyses in our cohort, concentrations of dust mite allergen were grouped in categories potentially relevant to sensitization.[8,10,15] For dust mite allergen, we used the highest concentration of Der f 1 or Der p 1 in the baby's bed at the following levels: (1) ≥ 10 μg/g (including concentrations exceeding detectable limits), (2) 2 to < 10 μg/g, (3) 0.05 to < 2 μg/g, and (4) < 0.05 μg/g (including assays below the limit of detection). Samples with no dust in the bed were also assigned to category 4. The correlation between dust mite concentration per unit area and per unit mass in 484 living room samples was 0.98, supporting the assumption that homes with no dust would have levels < 0.05 μg/g.

Endotoxin activity was determined by the kinetic limulus assay with the resistant-parallel-line estimation method.[17,18] Limulus amebocyte lysate was obtained from BioWhittaker (Walkersville, Md), reference standard endotoxin from the US Pharmacopeia, Inc (Rockville, Md), and control standard endotoxin from Associates of Cape Cod (Woods Hole, Mass). Results are reported in endotoxin units adjusted to account for lot-to-lot variation in limulus amebocyte lysate sensitivity to house dust-associated endotoxin[17,18] and referenced to standard endotoxins EC5 and EC6 (1 ng EC5 and EC6 5 10 endotoxin units [EU]).

Definition of other variables

Variables considered for inclusion in the multivariate analyses were the child's ethnicity and annual household income,[19] sex, *in utero* exposure to smoking, birth weight,[20] maternal age at delivery,[20] gestational age,[20] breast-feeding,[21] bottle-feeding in the bed or crib before sleep time,[21] day care attendance in the first year of life,[22] paternal history of asthma, maternal history of asthma, paternal history of hay fever, maternal history of hay fever, number of older siblings, type of home (single family vs multiapartment building), and average number of cigarettes per day smoked by adults in the household.

Assessment of allergen sensitization

At a mean age of 7.4 years (range, 6.5-10.1 years), allergy skin testing was performed in 248 children, and IgEs specific to common allergens were measured in an additional 23 children.

Allergy skin testing was performed on the volar aspect of the lower arms. The allergens tested included common indoor (cat dander, dog dander, cockroach [*Blatella germanica*], dust mite [Dermatophagoides pteronyssinus and Dermatophagoides farinae], and mouse epithelial extract) and outdoor (ragweed, mixed trees, *Aspergillus, Alternaria*, mixed grasses, *Cladosporium*, and *Penicillium*) allergens (Hollister Steir Labs, Spokane, Wash). Glycerinated saline and histamine were used as the negative and positive controls, respectively. Skin tests to specific allergens were considered positive if the mean diameter of the wheal was ≥ 3 mm after subtraction of the control wheal.

Serum from 23 children who declined skin testing was assayed for IgE to the allergens listed by using the UniCAP 250 system (Pharmacia & Upjohn, Kalamazoo, Mich). IgEs to specific allergens were considered positive at a level ≥ 0.35 IU/mL.

Because of low statistical power to test for interaction between atopy (objectively defined by allergy skin testing or measurement of allergen-specific IgEs) and endotoxin on some of the outcomes of interest (eg, repeated measures of wheeze), we used an alternative definition of atopy ("atopic status," broadly defined as objectively defined sensitization to ≥1 allergen

and/or a physician's diagnosis of allergic rhinitis or eczema by age 7 years) solely for this purpose.

Definition of outcomes

We used 2 types of outcomes: endpoints at age 7 years (or school age) and repeated measures of wheeze over the course of follow-up. At age 7 years, asthma was defined as physician-diagnosed asthma (at any time since birth) and ≥ 1 episode of wheezing in the previous year, and allergic rhinitis as physician-diagnosed allergic rhinitis (at any time since birth) and a history of nasal discharge or sneezing apart from colds in the previous year. At age 7 years, transient wheeze was defined as ≥ 1 episode of wheezing before the age of 3 years but not thereafter, persistent wheeze as ≥ 1 episode of wheezing before the age of 3 years and ≥ 1 episode of wheezing between ages 6 and 7 years, and late-onset wheeze as no episodes of wheezing before the age of 3 years and ≥ 1 episode of wheezing between ages 6 and 7 years.[23] Sensitization to ≥ 1 allergen (atopy) was considered present at school age if there was at least 1 positive skin test or specific IgE to the allergens tested. For the longitudinal analysis of repeated measures of wheeze, wheeze was considered present at any time point between 12 and 84 months of age if an affirmative response was given to the question, "Has your child had wheezing or whistling in the chest since we last spoke?"

Statistical analysis

Bivariate analyses were conducted by χ^2 or Fisher exact tests for categorical variables and 2-tailed t tests for a categorical and a continuous variable. Logistic regression was used to study the relation between dust mite allergen or endotoxin exposure at age 2 to 3 months and the endpoints of interest (asthma, wheeze [transient, persistent, and late-onset], allergic rhinitis, and atopy) while adjusting for potential confounders. In the final models, we included those variables that satisfied a change-in-estimate criterion (≥10% in the odds ratio [OR]) or that were significant at $P < .05$. Selected interactions were examined after the final models were chosen.

To assess further the relation between endotoxin exposure and childhood wheeze, we performed a confirmatory longitudinal analysis of the relation between endotoxin exposure at age 2 to 3 months and repeated measures of any wheeze between ages 1 and 7 years. For that analysis, we used proportional hazard models, with repeated events on the same child handled by the method of Andersen and Gill[24] and adjustment for correlations between these repeated events handled by methods described by Therneau and Grambsch.[25] To examine age-dependent associations, we calculated interaction terms between the age of the children at each survey and the variables in the model.

RESULTS

The characteristics of the 498 study subjects have been described in detail elsewhere.[26,27] Of the 498 study participants, 440 (88.4%) were followed to the age of 7 years, and 271 (54.4%) had an assessment of allergic sensitization at school age. There was no statistically significant difference in exposure to endotoxin or dust mite allergen at age 2 to 3 months among children at baseline, children followed up to age 7 years, and children with assessment of allergic sensitization (Table I). Subjects who dropped out of the study before age 7 years were significantly more likely to come from low-income families and to be black than those with complete follow-up. Similar results were obtained for the comparison of children with and without assessment of allergic sensitization (Table I).

Of the 271 children with assessment of allergic sensitization, 149 (55%) were sensitized to ≥ 1 allergen (Table II). Of the 440 children followed up to age 7 years, 404 were included in

the analysis of wheeze categories. Of these 404 children, 157 (38.9%) had transient wheeze, 64 (15.8%) had persistent wheeze, and 32 (7.9%) had late-onset wheeze (Table III).

Exposure to dust mite allergen

Children exposed to high levels (≥10 μg/g) of dust mite allergen in their bed at age 2 to 3 months had a 3-fold increase in the odds of asthma at age 7 years compared with those exposed to low levels (<0.05 μg/g) of dust mite allergen (Table II). In the multivariate analysis (Table II), there was a significant trend for a dose-response relationship between dust mite allergen exposure and asthma (OR for each 1-category increment in dust mite allergen exposure, 1.4; 95% CI, 1.02-1.90; $P = .04$). Exposure to high levels of dust mite allergen at age 2 to 3 months was associated with a 5-fold increase in the odds of late-onset wheeze but was not significantly associated with transient or persistent wheeze (Table III).

Early exposure to ≥ 2 μg/g dust mite allergen was associated with 2-fold increased odds of sensitization to dust mite (OR, 2.1; 95% CI, 1.1-6.3). These results were not appreciably changed after adjustment for sex and household income (OR for exposure to ≥ 2 vs < 2 μg/g, 2.6; 95% CI, 1.5-4.6). Increased levels of dust mite allergen in the child's bed at age 2 to 3 months were also associated with increased odds of allergic rhinitis at age 7 years (Table II), but there was no clear dose-response relationship or threshold effect. Consistent with our findings for dust mite sensitization, however, children exposed to ≥ 2 μg/g dust mite allergen had increased odds of allergic rhinitis (adjusted OR, 2.3; 95% CI, 1.2-4.3).

To examine whether our results were dependent on selection of categories of dust mite allergen exposure, we conducted a confirmatory analysis in which tertiles of levels of dust mite allergen ≥0.05 μg/g were compared with levels < 0.05 μg/g. This analysis yielded similar results (see this article's Tables E1 and E2 in the Online Repository at www.jacionline.org).

Exposure to endotoxin

Endotoxin exposure in the family room at age 2 to 3 months was not significantly associated with asthma at age 7 years (Table II). On the other hand, exposure to the highest quartile of endotoxin at age 2 to 3 months was associated with 3.5-fold increased odds of persistent wheeze (Table III). In addition, exposure to endotoxin was linearly associated with both transient and persistent wheeze (Table III). Each quartile increment in endotoxin exposure in early life was associated with increased odds of transient (OR, 1.3; 95% CI, 1.0-1.6; $P = .049$) and persistent (OR, 1.4; 95% CI, 1.0-1.9; $P = .03$) wheeze. There was no significant association between endotoxin exposure and late-onset wheeze.

The results of the confirmatory longitudinal analysis were similar to those obtained for the logistic regression analysis of transient, persistent, and late-onset wheeze. In bivariate and multivariate longitudinal analyses, each quartile increment in exposure to endotoxin at age 2 to 3 months was significantly associated with an increased risk of any wheeze between ages 1 and 7 years that did not change appreciably over time. Because of lack of variability in the estimates of association between endotoxin exposure and wheeze over time and a nonsignificant interaction between endotoxin exposure and age on wheeze ($P = .92$), we repeated the multivariate analysis without time indicator variables. In this parsimonious model, each quartile increment in early exposure to endotoxin was associated with a 23% excess risk of wheezing between ages 1 and 7 years (Table IV). Compared with children exposed to the lowest quartile of endotoxin levels at age 2 to 3 months, those exposed to the highest quartile had an 88% excess risk of wheezing between ages 1 and 7 years (95% CI for hazard ratio, 1.48-2.39). To examine potential modification of the effect of endotoxin on wheeze by atopy, we tested for interaction between endotoxin exposure and atopic status

(broadly defined as sensitization to ≥ 1 allergen and/or a physician's diagnosis of allergic rhinitis or eczema by age 7 years); this interaction was not statistically significant. Very similar results were obtained for the analyses of children with (n = 203) and without (n = 237) positive atopic status (Table IV).

Exposure to endotoxin levels above the lowest quartile at age 2 to 3 months was associated with reduced odds of atopy at school age (Table II). After adjustment for sex and household income, exposure to endotoxin levels above the lowest quartile was associated with significantly lower odds of atopy at school age (OR, 0.5; 95% CI for OR, 0.2-0.9). After adjustment for dust mite allergen exposure and other covariates, exposure to the highest quartile of endotoxin was associated with a 70% reduction in the odds of allergic rhinitis (Table II).

We found no significant modification of the effect of endotoxin on any of the outcomes of interest by dust mite allergen exposure, and vice versa ($P > .10$ in all cases). However, we had limited statistical power to detect such effect modification.

DISCUSSION

Sporik et al[1] first reported a strong association between exposure to ≥ 10 μg/g house dust mite in infancy and asthma at age 11 years among children with parental history of asthma or hay fever. That study was limited by small sample size (n = 59) and loss of follow-up of study participants. Our finding of a positive association between early dust mite allergen exposure and asthma in a larger cohort of children precisely replicate the results of Sporik et al,[1] and together with those of a randomized clinical trial of avoidance of dust mite allergen on asthma[28] suggest that early exposure to high levels of dust mite allergen increases the risk of asthma in children at risk for atopy.

In contrast with our results, other longitudinal studies and a randomized clinical trial of dust mite allergen avoidance found no significant association between early dust mite allergen exposure and asthma. The discrepant findings of those studies and ours could be explained by differences in duration of follow-up,[4-6,29] level of exposure,[2] retention of study participants,[2,3] selection of participants with regard to parental atopy,[2,3] and nonmeasurement of dust mite allergen in the child's bed (where exposure is likely most intense and prolonged).[2,3] Our finding of an association between dust mite allergen exposure and late-onset—but not transient or persistent—wheeze suggests that any effect of dust mite allergen exposure on asthma in high-risk children may be missed without sufficiently long duration of follow-up. Selection bias is an unlikely explanation for our findings because there was no significant difference in dust mite allergen levels at age 2 to 3 months between children who were and were not followed up to age 7 years.

Among children in our cohort, we previously reported that endotoxin exposure at age 2 to 3 months is associated with a reduced risk of eczema,[8] an increased risk of recurrent wheeze in the first year of life,[14] and reduced allergen-stimulated lymphocyte proliferation and IL-13 production by age 2 to 3 years.[30] Three birth cohort studies of children not selected on the basis of parental history of atopy also found that early exposure to house dust endotoxin is associated with an increased risk of wheeze up to age 2 years.[7,9,31] Results of cross-sectional studies of German schoolchildren[32] and US adults[33] further suggest that indoor endotoxin exposure is associated with reduced risk of atopy but increased risk of nonatopic wheeze. Among 493 European schoolchildren in nonfarming households, endotoxin exposure was associated with reduced risk of hay fever but increased risk of nonatopic wheeze.[34] In a cross-sectional of adults in 831 homes in the United States, endotoxin

exposure was associated with increased risks of asthma, asthma symptoms, and medication use for asthma.[33]

Our findings suggest that early endotoxin exposure is associated with a reduced risk of atopy but an increased risk of wheeze among high-risk children. Consistent with recent findings in adults,[33] we found that the strength of the association between endotoxin and wheeze was very similar in children who did and did not become atopic at age 7 years. Although there was no significant association between early endotoxin exposure and asthma at age 7 years, we had greater statistical power to detect an association of weak to moderate strength and assess modification of the effect of endotoxin exposure by atopy in the analysis of repeated measures of wheeze.

Wheeze can result from bronchoconstriction caused by airway inflammation. In human beings, inhalation of endotoxin and organic dusts containing endotoxin induces airway inflammation and upregulation of proinflammatory cytokines such as TNF-α in subjects with and without asthma.[35] On the other hand, inhalation of endotoxin can have systemic effects on the developing immune system, including increased production of IL-12 by antigen-presenting cells and upregulation of IFN-γ and other cytokines produced by T_H1 cells.[36,37] IFN-γ inhibits the development of T_H2 cells, thus leading to downregulation of T_h2-mediated immune responses characteristic of atopy.[38] We recognize, however, that the observed associations between exposure to endotoxin at age 2 to 3 months and wheeze or atopy may partly reflect persistent exposure to increased levels of endotoxin throughout childhood.

In summary, our results suggest that exposure to high levels of dust mite allergen in early life is associated with an increased risk of asthma at school age among children at risk for atopy. In children with parental history of atopy, early endotoxin exposure was associated with reduced risks of allergic rhinitis and atopy at school age but with increased risk of wheeze from ages 1 to 7 years. Because endotoxin has opposite effects on atopy and wheeze, a change in endotoxin exposure is by itself an unlikely explanation for the high prevalence of asthma in industrialized countries.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

EU	Endotoxin unit
OR	Odds ratio

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TABLE I

Characteristics of participating children

Variable	Number (percent)		Children with assessment of allergen sensitization†
	At baseline†	At 7 years of follow-up†	
Female sex	230 (46.2)	200 (45.5)	116 (42.8)
Household income < $30,000	45 (9.3)	27 (6.3) *	13 (4.9) *
Ethnicity			
White	375 (75.3)	351 (79.8) *	219 (80.8) *
Black	60 (12.1)	36 (8.2) *	23 (8.5) *
Hispanic	28 (5.6)	21 (4.8)	11 (4.1)
Asian and other	35 (7.0)	32 (7.2)	18 (6.6)
Day care attendance in the first year of life	238 (47.8)	208 (47.3)	140 (51.7)
Maternal history of asthma	152 (30.5)	130 (29.6)	76 (28.0)
Paternal history of asthma	116 (23.7)	100 (23.2)	61 (22.8)
Der p or Der f (highest), baby's bed (μg/g)			
<0.05	223 (44.9)	198 (45.1)	123 (45.4)
≥0.05 to <2	159 (32.0)	135 (30.7)	79 (29.2)
≥2 to <10	68 (13.7)	64 (14.6)	43 (15.9)
≥10	47 (9.4)	42 (9.6)	25 (9.2)
Quartiles of endotoxin, family room (EU/mg)			
2.14-52.48	100 (24.9)	83 (23.5)	50 (22.6)
52.49-79.99	100 (24.9)	90 (25.5)	53 (24.0)
80.48-125.59	101 (25.2)	92 (26.1)	60 (27.2)
125.6-713.20	100 (24.9)	88 (24.9)	58 (26.2)

* $P < .05$ for comparison with children at baseline.

† Numbers and percentages reflect missing information on some variables.

TABLE II

Allergen or endotoxin exposure at age 2 to 3 months and asthma, allergic rhinitis, and atopy at school age

Allergen or endotoxin levels	Asthma				Allergic rhinitis				Sensitization to ≥1 allergen (atopy)			
			OR, 95% CI				OR, 95% CI				OR, 95% CI	
	Yes	No	Unadjusted	Adjusted‡	Yes	No	Unadjusted	Adjusted§	Yes	No	Unadjusted	Adjusted‖
Der p 1 or Der f 1, child's bed (μg/g)												
<0.05	14	184	1.0	1.0	24	173	1.0	1.0	67	57	1.0	
≥0.05 to <2	16	119	1.8 (0.8-3.8)	1.3 (0.6-2.9)	18	117	1.1 (0.6-2.1)	1.2 (0.6-2.5)	41	38	0.9 (0.5-1.6)	
≥2 to <10	5	59	1.1 (0.4-3.2)	1.4 (0.5-4.1)	16	48	2.4 (1.2-4.9) *	3.2 (1.5-7.0) **	26	17	1.3 (0.7-2.7)	
≥10	8	34	3.1 (1.2-7.9) *	3.0 (1.1-7.9) *†	6	36	1.2 (0.5-3.2)	1.5 (0.5-4.3)	15	10	1.3 (0.5-3.1)	
Endotoxin quartiles, family room												
First	8	75	1.0		13	70	1.0	1.0	34	16	1.0	1.0
Second	7	83	0.8 (0.3-2.3)		12	78	0.8 (0.4-1.9)	0.8 (0.3-1.9)	26	27	0.5 (0.2-1.0)	0.4 (0.2-0.9) *
Third	11	81	1.3 (0.5-3.3)		17	74	1.2 (0.6-2.7)	1.0 (0.4-2.4)	29	31	0.4 (0.2-1.0) *	0.4 (0.2-0.9) *
Fourth	11	77	1.3 (0.5-3.5)		8	80	0.5 (0.2-1.4)	0.3 (0.1-0.9) *	35	23	0.7 (0.3-1.6)	0.6 (0.3-1.4)

* $P < .05$,

** $P \leq .01$ for category of allergen or endotoxin exposure.

† P for linear trend < .05.

‡ Adjusted for sex, household income, maternal asthma, living in a single-family home, and bottle-feeding before bedtime in the first year of life.

§ Adjusted for maternal hay fever, living in a single-family home, low birth weight, and number of older siblings, in addition to the variables listed in the column (dust mite allergen and endotoxin levels at age 2 to 3 months).

‖ Adjusted for sex and household income.

TABLE III

Allergen or endotoxin exposure at age 2 to 3 months and transient, persistent, and late-onset wheeze

Allergen or endotoxin levels	Wheeze‡									
	Never (reference)	Transient	Unadjusted	Adjusted§	OR, 95% CI			Late-onset	Unadjusted	Adjusted§
					Persistent	Unadjusted	Adjusted§			
Der p 1 or Der f 1, child's bed (μg/g)										
<0.05	76	70	1.0		28	1.0		10	1.0	1.0
≥0.05 to <2	43	54	1.3 (0.8-2.3)		18	1.1 (0.6-2.3)		10	1.7 (0.7-4.5)	1.6 (0.6-4.2)
≥2 to <10	22	22	1.1 (0.5-2.1)		10	1.2 (0.5-2.9)		5	1.7 (0.5-5.5)	2.1 (0.6-7.2)
≥10	10	11	1.2 (0.5-2.9)		8	2.1 (0.8-6.0)		7	5.3 (1.6-16.9) *	5.0 (1.5-16.4) **
Endotoxin quartiles, family room										
First	34	28	1.0	1.0	7	1.0	1.0	6	1.0	
Second	37	24	0.8 (0.4-1.6)	0.7 (0.3-1.5)	16	2.1 (0.8-5.7)	2.1 (0.8-5.9)	4	0.6 (0.2-2.4)	
Third	28	37	1.6 (0.8-3.2)	1.7 (0.8-3.5)	9	1.6 (0.5-4.7)	1.7 (0.5-5.1)	14	2.8 (1.0-8.3)	
Fourth	25	31	1.5 (0.7-3.1)	1.6 (0.8-3.5) ***	18	3.5 (1.3-9.6) *	3.5 (1.3-9.8) ***	6	1.4 (0.4-4.7)	

* $P < .05$,

** $P \leq .01$,

† P for linear trend < .05.

‡ Thirty-six children not included because of missing information (n = 19) or not fitting any wheeze category (n = 17).

§ All models were adjusted for sex and household income, in addition to the variables listed in each column (dust mite allergen or endotoxin levels at age 2-3 months).

TABLE IV

Endotoxin exposure at age 2 to 3 months and wheezing between ages 1 and 7 years

	Wheezing between ages 1 and 7 years Hazard ratio, 95% CI, *P* value		
		Atopy or atopic diseases at age 7 years*	
	All children†‡	Yes†§	No†‖
Each quartile increment in endotoxin levels (EU/mg)	1.23 (1.07-1.43), .004	1.23 (1.02-1.49), .03	1.23 (1.0-1.51), .05

*Sensitization to at least 1 allergen or physician-diagnosed allergic rhinitis or physician-diagnosed eczema by age 7 years.

†All multivariate models were adjusted for sex and household income.

‡Also adjusted for maternal age at delivery, gestational age, and exposure to other children in the first year of life (day care attendance or having 2 older siblings at home).

§Also adjusted for exposure to other children in the first year of life.

‖Also adjusted for maternal age at delivery.