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Endotoxin Exposure, Serum Vitamin D, Asthma and Wheeze Outcomes

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Abstract

Background—Endotoxin has been shown to induce neutrophilic asthma and wheeze after binding toll-like receptor 4 to produce pro-inflammatory cytokines. Animal models have demonstrated that vitamin D might inhibit lipopolysaccharide-induced cytokines. However, whether endotoxin exposure and serum vitamin D deficiency interact to affect asthma and wheeze in humans has never been investigated in an epidemiological study.

Methods—Joint associations of house dust endotoxin and vitamin D with asthma and wheeze were examined using logistic regression adjusted for covariates in 5,924 US participants of the National Health and Nutrition Examination Survey (NHANES). Interactions were assessed on the multiplicative as well as additive scale using the relative excess risk, the attributable portion due to additive interaction, and the synergy index.

Results—The median endotoxin concentration was 19.1 EU/mg. Prevalence of vitamin D inadequacy (20–30 ng/ml) and deficiency (<20 ng/ml) were respectively 42.9 and 33.4%. The combination of high endotoxin and low vitamin D was associated with current asthma (OR: 1.56, 1.09, 2.23), wheeze in the past 12 months (OR: 1.72, 95% CI: 1.10, 3.71), recurrent wheeze (OR: 1.97, 95% CI: 1.00, 4.00), asthma diagnosis or recurrent wheeze (OR: 1.88, 95% CI: 1.33, 2.66), and current asthma or recurrent wheeze (OR:1.81, 95% CI: 1.23, 2.68) when compared to low endotoxin and normal vitamin D. Though, the interactions between the exposures were not significant on the multiplicative or additive scale for any of the outcomes.

Conclusions—Combination of high endotoxin exposure and low vitamin D increases the odds of asthma and wheeze, but the exposures do not interact or modify each other's effect in the NHANES cohort.

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Conflict of Interest Statement

The authors have no conflict of interest to disclose.

Keywords

Endotoxin; Vitamin D; Asthma; Wheeze; Interaction; Joint Effect; Multiplicative Effect; Additive Effect

INTRODUCTION

Endotoxin is a lipopolysaccharide constituent of gram-negative bacteria cell walls and is ubiquitous in the environment as part of dust and ambient air (1). Known predictors of higher endotoxin in homes include lower family income, younger age occupants, carpeting, pets, cockroaches or the presence of a smoker in the household (2–4). Endotoxin has been proposed to cause asthma and asthma-like symptoms in both occupational and domestic settings because of its ability to induce T-helper (Th)-1 response with production of interferon (IFN)-γ and neutrophilic inflammation (3, 5). Paradoxically, early life exposure to endotoxin has been suggested to prevent later development of immunoglobulin (Ig) E mediated asthma and allergy by promoting Th-1 type immune development and inducing immune-modulatory effects (6).

Lately, vitamin D has also been of remarkable interest in asthma research because of growing reports of a potential relationship between vitamin D deficiency and asthma symptoms (7). Sources of vitamin D include diet and sun exposure to ultraviolet B (UVB) light which converts 7-dehydrocholestrol in the skin into pre-vitamin D isomerized into vitamin D and later converted into 25-OH Vitamin D (25-OH-D) in the liver (8). This vitamin has been shown to have immunoregulatory properties that could prevent asthma by modifying the effect of Th1, Th2 and regulatory T cells as well as improve asthma control by inhibiting Th17 lymphocytes linked to asthma severity and low steroid responsiveness (9, 10). Hypovitaminosis D is widespread in both developing countries where prevalences range between 30 and 90% and developed nations (11, 12). For example, in Europe, recent data suggest that serum vitamin D was below 20 ng/ml in 28.2 to 44.4% of Austrians, 40% or more of French, 50% or more of Germans and among 67 to 92% of Northern Europeans (Denmark, Finland, Ireland and Poland) (12). In the US, according to 2001–2006 National Health and Nutrition Examination Survey (NHANES) data, 33% of the population have serum vitamin D levels below 20 ng/mL and 77% have levels below 30 ng/mL (13, 14).

Despite the ubiquity of endotoxin in our environment and the high prevalence of low serum vitamin D, no epidemiological study has examined whether these two exposures interact to affect asthma and wheeze, though each of them has been found independently associated with at least one of these conditions. In a previous report using the NHANES, we found that endotoxin was independently associated with wheeze and that the relationship between endotoxin and asthma and wheeze was influenced by allergen-specific sensitization status as well as some environmental exposures (4). Using the same study population, Keet et al observed that lower serum vitamin D levels were positively associated with wheeze, asthma as well as IgE and noted that the relationship between vitamin D and wheeze was modified by age and atopic status (15). The interaction between vitamin D and endotoxin with regard to asthma and wheeze has only been studied in animal models, suggesting a potentially

synergistic effect in inducing these outcomes. It is established that extracellular monomeric endotoxin lymphocyte antigen 96 (LY96) complexes bind to Toll-like receptor (TLR)-4 triggering intracellular signal transduction cascades resulting in production of the proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6, causing airway neutrophilic inflammation (16, 17). Both endotoxin induced cytokines have been found to be inhibited by serum vitamin D levels at or above 30 ng/ml exerting an anti-inflammatory action (18). Moreover, it was demonstrated that vitamin D could upregulate mitogen-activated protein (MAP) kinase phosphatase-1 (MPK-1) expression by monocytes/macrophages and thus impede lipopolysaccharide induced phosphorylation of p38, a critical regulator of pro-inflammatory cytokines (19). Therefore, in the present study, we aimed to test the hypothesis of a potential interactive effect of house dust endotoxin exposure and serum vitamin D on asthma and wheeze outcomes in a large epidemiological study representative of the US population.

METHODS

Data source and study design

We used data from the NHANES conducted from 2005 to 2006 by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) and augmented by the National Institute for Environmental Health Sciences (NIEHS) to include endotoxin exposure assessment. The NHANES is an ongoing cross-sectional survey of the US non-institutionalized civilian population selected using a complex multistage sampling design to derive a representative sample of the US population. A total of 6,185 NHANES participants had data on house dust endotoxin and serum vitamin D. After exclusion of 261 participants with missing poverty income ratio data, the final sample included 5,924 subjects. NHANES protocols were approved by the institutional review boards of the NCHS and CDC and informed consent was obtained from all participants. Details on study design and procedures can be found in the NHANES website (http://www.cdc.gov/nchs/nhanes.htm).

Endotoxin measurement

Combined bed and bedroom floor dust samples were collected at each participant's home using a Sanitaire™ Model 3683 vacuum cleaner and a Mitest™ Dust Collector (Indoor Biotechnologies, Inc., Charlottesville, VA). One-square yard surfaces on beds and the adjacent floors were each vacuumed for two minutes. These composite dust samples were analyzed for endotoxin at our University of Iowa laboratory using a kinetic chromogenic *Limulus* amebocyte lysate assay with expansive quality assurance measures as previously described (4). Sieved dust was extracted with sterile pyrogen-free water plus 0.05% Tween-20™. Control standard endotoxin (*E. coli* 055:B5) was used to develop 12-point standard curves and samples were assayed at four dilutions increasing four-fold from 1:400 to 1:25,600. Endotoxin concentrations were reported in endotoxin units per sieved dust weight (EU/mg of dust). The lower limit of detection was 0.000488 EU/mg.

Serum vitamin D

25-hydroxy vitamin D (25-OH-D) was measured using the DiaSorin assay, a two-step procedure. It consisted of extracting 25-OH-D and other hydroxylated metabolites from serum with acetonitrile and assaying the treated sample with an equilibrium RIA procedure based on an antibody specific to 25-OH-D. The sample, antibody, and tracer were incubated for 90 min at 20–25 °C. Separation was accomplished after a 20-mi nute incubation at 20–25 °C with another antibody-precipitating complex. A NSB buffer was subsequently added after and before centrifugation to help decrease non-specific binding. More detailed information about this procedure is available at http://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/vid_d.pdf.

The Institute of Medicine defines vitamin D deficiency as serum 25-OH-D levels < 20 ng/ml (20). In addition, the Endocrine Society Practice Guideline defines vitamin D as inadequate at serum 25-OH-D levels between 20 and 30 ng/ml (8). Consequently, we categorized serum vitamin D as normal (≥ 30 ng/ml), inadequate (20–30 ng/ml), or deficient (< 20 ng/ml) and defined low serum vitamin D as inadequate or deficient (< 30 ng/ml).

Asthma and wheeze

The prevalence of asthma and wheeze were assessed by responses to the following questions: (1) “Has a doctor or other health professional ever told you that you/Sample Person [SP] had/have/has asthma?” (Asthma diagnosis), (2) “Do you/Does SP still have asthma?” (Current asthma), (3) “In the past 12 months, have you/has SP had any wheezing or whistling in chest?” (Wheeze in past 12 months), (4) “In the past 12 months, how many attacks of wheezing or whistling have you/has SP had?” Past asthma was defined as the presence of asthma diagnosis and the absence of current asthma.

Covariates and sensitization

Data on age, gender, race/ethnicity, family income, presence of a smoker in the household, number of people in the household, number of years lived in the house, pet avoidance because of allergies, as well as presence of mildew, cockroaches, and pets in the homes were collected using questionnaires. Poverty income ratio (PIR) was estimated using guidelines and adjustment for family size, year and state. Physical activity was evaluated by questions related to daily activities, leisure time activities, and sedentary activities (and metabolic-equivalent task (MET) scores were calculated. Participants’ weight and height were measured in a mobile examination center using standardized techniques and equipment. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Sensitization status was defined as specific IgE against any of 15 aeroallergens ≥ 0.35 kU/L (*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, or rye grass). Total and specific IgE levels were measured using the Pharmacia Diagnostics ImmunoCAP 1000 System (Kalamazoo, Michigan).

Statistical analysis

P-values for differences in proportions or means by exposure status were calculated using chi-square test for categorical variables and using Student *t*-test for continuous variables. House dust endotoxin was dichotomized into being exposed to levels below or above the median, while serum vitamin D was categorized into levels defined earlier (normal, low [inadequate or deficient]). Multivariate logistic regression modeling was used to assess the association of exposure to dust endotoxin and serum vitamin D combinations with asthma and wheeze outcomes. Models were adjusted for age, PIR, MET (used as continuous variables), gender, race/ethnicity, number of people in the household, presence of a smoker in the household, BMI, number of years lived in the house, presence of mildew, cockroaches, and pets in the home, pets avoidance because of allergies, as well as sensitization to aeroallergens (used as categorical variables).

Multiplicative interactions were tested by including a product term of endotoxin and vitamin D deficiency in the different models. Additive interaction was assessed by calculating the relative excess risk due to additive interaction (RERI), the attributable portion (AP) due to interaction, and the synergy index (SI) using the methods described by Andersson et al (21). Statistical significance was assessed using the 95% confidence intervals (CI), with the AP and RERI significantly different from 0 and the SI significantly different from 1 indicating the presence of an additive interaction. Age, gender, race/ethnicity, sensitization to allergens, and BMI were tested for effect modification by including a product term in the logistic regression models. All analyses were performed in STATA (Version 13, STATA Corporation, College Station, TX, USA). NHANES sampling weights and STATA survey commands, taking into account the multistage and complex survey design, were used in all statistical procedures so that estimates were nationally representative. Because of sampling weights, traditional procedures for goodness of fit could not be performed; instead STATA, syntax *svylogitgof*, an F-adjusted mean residual test, was used (22). P-values < 0.05 were considered statistically significant.

RESULTS

In our study, the median concentration of endotoxin was 19.1 EU/mg of house dust. Only 23.7% of participants had a normal serum vitamin D, 42.9% had inadequate serum vitamin D level, and 33.4% had serum vitamin D deficiency. Our sample consisted of 5,924 people with a median age of 37 years (IQR: 20–53, range: 1–85) among whom 51.1% were females and 69.3% were non-Hispanic Whites (Table 1). More than half of the participants (54.9%) were overweight or obese, 20.2% lived with a smoker in their household, and 41.4% were sensitized to allergens. The prevalence of asthma outcomes was 14.7% for asthma diagnosis, 5.7% for past asthma and 8.8% for current asthma. The prevalence of wheeze outcomes was 16.5% for any wheeze in the past 12 months and 11.8% for ≥2 wheeze episodes. The prevalence of combined outcomes was 20.9% for asthma diagnosis and recurrent wheeze (recurrent wheeze being defined as ≥2 wheeze episodes) and 16.0% for current asthma and recurrent wheeze.

Participants exposed to lower dust endotoxin levels and with normal serum vitamin D represented 13.0% of the study population. They had a median age of 36 years (IQR: 22–

54); they were more likely to be non-Hispanic White, to have a normal BMI, and to have a higher PIR than other participants. Participants with lower exposure to dust endotoxin and low serum vitamin D accounted for 43.0% of the study population. They were older (median age of 41 years [IQR: 25–54], more likely to be non-Hispanic Blacks, overweight or obese, and to be sensitized to aeroallergens compared to other participants. Higher endotoxin exposure and normal serum vitamin D was prevalent in only 10.7% of participants. These subjects were younger (median age of 24 years [IQR: 8–46]), and more likely to have pets in their homes than other participants. Those with higher endotoxin exposure and low serum vitamin D were 33.7% and had a median age of 35 years (IQR: 16–52). They were more likely to be Hispanic, to have a lower PIR, to have cockroaches in their homes, and to have asthma and/or wheeze than the other participants (Table 1).

In reference to low endotoxin exposure and normal serum vitamin D, the combination of high endotoxin exposure and low serum vitamin D was associated with nearly all asthma and wheeze outcomes: asthma diagnosis (OR: 1.53, 95% CI: 1.20, 1.94), past asthma (OR: 1.47, 95% CI: 1.00, 2.17), current asthma (OR: 1.56, 1.09, 2.23), any wheeze in the past 12 months (OR: 1.72, 95% CI: 1.10, 3.71), recurrent wheeze (OR: 1.97, 95% CI: 1.00, 4.00), asthma diagnosis or recurrent wheeze (OR: 1.88, 95% CI: 1.33, 2.66), and current asthma or recurrent wheeze (OR:1.81, 95% CI: 1.23, 2.68). However, neither the multiplicative nor the additive interactions between high endotoxin exposure and low vitamin D were significant for any of the asthma or wheeze outcomes (Table 2).

DISCUSSION

The present study used a large sample representative of the US population to examine the interaction between house dust endotoxin and serum vitamin D and its relation with asthma and wheeze. Our results suggest that the combination of high endotoxin exposure and low serum vitamin D is associated with higher odds of asthma and wheeze outcomes. Participants with endotoxin exposure above the median and inadequate or deficient serum vitamin D were nearly twice as likely to experience two or more wheezing episodes as those with low endotoxin and normal vitamin D. However, there was no statistically significant interaction between the exposures with regard to their associations with the outcomes

To the best of our knowledge, this is the first report to examine the association of the interaction between endotoxin exposure and vitamin D with asthma and wheeze. Endotoxin has long been recognized to be an independent risk factor for occupational asthma (23). In the first study of national scope conducted in the US (NSLAH), house endotoxin was also found to be associated with asthma diagnosis and symptoms as well as asthma medication use, mainly in adults (3). Using the NHANES, we performed the largest study ever conducted on endotoxin and found bed and bedroom floor endotoxin associated with asthma, wheeze, exercise-induced wheeze, and prescription medication for wheezing (4). However, there is mounting evidence that endotoxin and other microbial exposures could protect against allergic asthma when exposure occurs very early in life (6, 24). Still, the association of endotoxin with wheeze appears more consistent in the literature (25).

Reports on the effect of serum vitamin D on the risk of asthma for their part, have been inconsistent. Several cross-sectional as well as prospective and population studies have concluded that low vitamin D serum levels increased the risk of asthma and wheeze, consistent with our findings of an independent relationship (15, 26–30). Similar associations were found for low maternal serum or cord blood vitamin D with later development of asthma and wheeze in the offspring (31–34). Still, a number of reports have also failed to report an association between vitamin D deficiency and asthma (35–38). A recent meta-analysis comprising 23 studies suggested that pre- or postnatally measured serum vitamin D only plays a role in asthma exacerbation, not asthma development (39). Likewise, clinical trials evaluating the efficacy of vitamin D supplementation on asthma and allergy have produced conflicting outcomes. In the VIDA (Vitamin D Add-on Therapy Enhances Corticosteroid Responsiveness in Asthma) trial, oral vitamin D3 administered 100 000 IU once, then 4000 IU per day for 28 weeks did not reduce first treatment failure rates or asthma exacerbation in adults with persistent asthma and vitamin D insufficiency in comparison to placebo (40). Litonjua et al evaluated the efficacy of prenatal administration of vitamin D in preventing asthma and wheeze and also failed to report significant results. The study compared 48 women randomized to receive 4400 IU of vitamin D daily with 436 women who received a placebo whose offspring were followed up to age 3 years (41). In another placebo controlled trial including both children and adults, vitamin D supplementation (100,000-IU bolus intramuscularly plus 50,000 IU orally weekly for 24 weeks) significantly improved lung function (forced expiratory volume in 1 sec) in mild to moderate persistent asthma (42).

Our analysis failed to find a statistically significant interaction between endotoxin exposure and vitamin D deficiency as we had hypothesized based on previous studies, although the estimated directionality is not entirely inconsistent with those studies. Studying the effect of vitamin D in severe asthma exacerbation and in endotoxin-stimulated cells, Luo et al found that vitamin D deficiency enhanced lipopolysaccharide-induced oxidative stress. In their investigation, administration of vitamin D3 suppressed lipopolysaccharide-induced reactive oxidative species as well as DNA damage and caused a decline in TNF-α and nuclear factor (NF)-κB in epithelial cells (43). Similarly, another study noted that normal vitamin D levels (30–50 ng/ml) are sufficient to inhibit lipopolysaccharide-induced p38 activation and the production of cytokines in monocytes/macrophages. The study additionally identified the upregulation of MKP-1 as a new mechanism this p38 activation and cytokine production (18). Prior to that, other authors found vitamin D supplementation reduced TLR expression and decreased the activation by lipopolysaccharide of MAP kinases. These MAP kinases initiate intracellular signal transduction cascades which are critical regulators of pro-inflammatory cytokine production such as TNF-α and IL-6 (44).

Our study has limitations. Due to the cross-sectional design of the study, temporality between endotoxin exposure, low serum vitamin D, and the outcomes cannot be evaluated and causality cannot be established. House dust was only sampled once, but dust endotoxin and allergens from mattress and bedroom floor have been found to be representative of long time exposures (45). Asthma and wheeze outcomes were self-reported and could not be verified. There may have been other potential confounding variables, such as familial history of atopy and non-residential exposures that were not assessed in the study. Nonetheless,

major strengths of our study were the large sample representative of the US population, precise measurement of endotoxin, and the analysis adjusted for several exposures, some of which could be potential confounders for the associations between endotoxin and asthma or wheeze, and socioeconomic factors (such as PIR) to minimize residual confounding.

In conclusion, individuals with low vitamin D exposed to high dust endotoxin exposure are at higher odds of asthma and wheeze outcomes than those with normal vitamin D and low endotoxin exposure. However, low vitamin D and endotoxin were not significantly interactive on the multiplicative or additive scale in their relation with asthma and wheeze.

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Highlights

- Endotoxin exposure and vitamin D deficiency are independently associated with asthma and wheeze.
- The combination of high endotoxin and low vitamin D is associated with asthma and wheeze.
- Our data do not support an interactive effect of endotoxin and vitamin D on asthma and wheeze.

Table 1

Characteristics of study participants by dust endotoxin and serum vitamin D levels, NHANES 2005 – 2006 (N = 5,924)

Dust Endotoxin / Serum Vitamin D	Low / Normal	Low / Inadequate	High / Normal	High / Inadequate	P	All participants
Prevalence, %	13.0	43.0	10.7	33.7		100
Age, median (IQR), years	36 (22–54)	41 (25–54)	24 (8–46)	35 (16–52)		37 (20–53)
Gender, %						
Men	45.9	49.8	51.7	48.1	.22	48.9
Women	54.1	50.2	48.3	51.9		51.1
Race/ethnicity, %					< .001	
Non-Hispanic Whites	89.5	64.4	84.7	62.9		69.3
Non-Hispanic Blacks	1.7	15.6	1.8	14.4		11.9
Hispanics	5.0	12.4	8.2	16.7		11.9
Other	3.8	7.6	5.3	6.0		6.3
BMI, %					< .001	
Underweight	10.6	7.3	27.7	13.3		11.9
Normal	43.8	28.8	33.3	28.4		31.1
Overweight or obese	43.4	62.5	34.9	55.9		54.9
Missing	2.3	1.4	4.1	2.4		2.1
PIR, mean (SE)	3.33 (0.13)	3.01 (0.09)	2.83 (0.13)	2.51 (0.09)	< .001	2.86 (0.09)
Smoker in household, %	18.9	17.8	23.0	23.0	.06	20.2
MET, mean (SE)	17.43 (0.70)	15.17 (0.26)	25.60 (1.26)	17.95 (0.61)	< .001	17.48 (0.29)
Years lived in house						
<1	15.4	19.4	12.8	17.6	.18	17.6
1 – 2	22.5	18.1	19.9	19.7		19.4
3 – 5	17.7	18.4	20.1	19.9		19.0
6 – 10	15.9	16.4	13.2	16.6		16.1
> 10	28.5	27.6	33.9	26.2		27.9
Mildew or musty smell in home, %	17.5	15.8	16.8	17.3	.86	16.6
Cockroaches in home, %	8.8	12.7	10.5	20.6	< .001	14.6
Pets in home, %	58.4	44.5	64.9	55.3	< .001	52.1
Sensitization to aeroallergens, %	39.0	44.5	33.8	40.8	.001	41.4

Dust Endotoxin / Serum Vitamin D	Low / Normal	Low / Inadequate	High / Normal	High / Inadequate	P	All participants
Asthma outcomes, %						
Asthma diagnosis	12.2	14.0	13.2	17.1	.07	14.7
Past asthma	4.7	5.5	4.9	6.5	.25	5.7
Current asthma	7.3	8.2	7.9	10.5	.03	8.8
Wheeze outcomes, %						
Any wheeze	13.0	15.9	14.4	19.4	.002	16.5
≥ 2 wheezing episodes	8.5	10.9	10.6	14.5	< .001	11.8
Combined wheeze and asthma, %						
Recurrent wheeze & asthma diagnosis	15.9	19.9	19.2	24.7	.02	20.9
Recurrent wheeze & current asthma	12.4	15.0	14.4	19.1	.02	16.0

Abbreviations: NHANES, National Health and Nutrition Examination Survey; IQR, Interquartile Range; SE, Standard Error; BMI, Body Mass Index; PIR, Poverty Income ratio; MET, Metabolic Equivalent Task. P-values indicate statistical significance of characteristics by exposure status. Low endotoxin is <19.1 EU/mg dust; normal serum vitamin D is ≥ 30 ng/ml.

Table 2

Odds ratio for the association of house dust endotoxin – serum vitamin D interaction with asthma and wheeze outcomes

Endotoxin / Vitamin D	Association of endotoxin with asthma and wheeze outcomes				Interactions			
	Low / Normal	Low / Low	High / Normal	High / Low	Multiplicative	RERI (95% CI)	AP (95% CI)	S (95% CI)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)				
Asthma Outcomes								
Asthma Diagnosis	1 (reference)	1.21 (0.89, 1.64)	1.07 (0.74, 1.55)	**1.53 (1.20, 1.94)****	1.19 (0.79, 1.79)	0.25 (−0.22, 0.71)	0.16 (−0.14, 0.46)	1.86 (0.38, 9.04)
Past Asthma	1 (reference)	1.16 (0.70, 1.90)	1.03 (0.64, 1.65)	**1.47 (1.00, 2.17)***	1.23 (0.80, 1.90)	0.29 (−0.16, 0.74)	0.20 (−0.09, 0.48)	2.59 (0.24, 28.05)
Current Asthma	1 (reference)	1.23 (0.75, 2.01)	1.06 (0.60, 1.88)	**1.56 (1.09, 2.23)***	1.23 (0.60, 2.51)	0.28 (−0.48, 1.00)	0.18 (−0.31, 0.66)	1.97 (0.11, 36.49)
Wheeze Outcomes								
Any Wheeze	1 (reference)	1.41 (0.90, 2.20)	1.10 (0.62, 1.94)	**1.72 (1.10, 3.71)***	1.12 (0.66, 1.92)	0.22 (−0.26, 0.69)	0.13 (−0.18, 0.43)	1.42 (0.42, 4.82)
≥ 2 wheezing episodes	1 (reference)	1.48 (0.72, 3.04)	1.19 (0.49, 2.88)	**1.97 (1.00, 4.00)***	1.12 (0.47, 2.67)	0.30 (−0.70, 1.46)	0.15 (−0.36, 1.35)	1.45 (0.14, 96.98)
Combined outcomes								
Asthma diagnosis or recurrent wheeze	1 (reference)	1.44 (0.92, 2.24)	1.30 (0.94, 1.79)	**1.88 (1.33, 2.66)****	1.01 (0.66, 1.53)	0.14 (−0.26, 0.54)	0.08 (−0.14, 0.28)	1.20 (0.62, 6.43)
Current asthma Or recurrent wheeze	1 (reference)	1.39 (0.83, 2.32)	1.22 (0.73, 2.03)	**1.81 (1.23, 2.68)****	1.07 (0.61, 1.86)	0.20 (−0.40, 0.88)	0.11 (−0.21, 0.49)	1.33 (0.35, 15.96)

AP, attributable proportion due to interaction; BMI, body mass index; RERI, relative excess risk due to interaction; S, synergy index;

Models adjusted for age, PIR, MET (used as continuous variables), gender, race/ethnicity, number of people in the household, presence of a smoker in the household, BMI, number of years lived in the house, presence of mildew, cockroaches, and pets in the home, pets avoidance because of allergies, as well as sensitization to aeroallergens (used as categorical variables). RERI and AP are significant when the 95% CI do not include 0.0. SI is significant when the 95% CI do not include 1.0.