



The association between endotoxin in house dust with atopy and exercise-induced bronchospasm in children with asthma

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ABSTRACT

Background: Studies have reported protective and adverse associations between microbial exposure and childhood asthma. However, among children with asthma the relationships between endotoxin and exercise-induced bronchospasm (EIB) is less clear.

Objective: We investigated the association between exposure to endotoxin in house dust with atopy and EIB in children with asthma.

Methods: A cross-sectional survey was conducted among schoolchildren (aged 7–17 years) in the province of Saskatchewan, Canada. A subpopulation with asthma ($n = 116$) were identified from 335 participants using a validated asthma algorithm. We determined atopy among the asthma subpopulation by skin prick testing (SPT) while EIB was evaluated using exercise challenge testing (ECT). Dust samples were collected from mattress and play area floors, and endotoxin was measured in dust extracts. Logistic regression analyses were used to explore associations between endotoxin with atopy and EIB.

Results: Among the 116 children with asthma, 99 completed SPT and all had completed ECT. Of these, 71/99 (71.7%) were atopic and 26/116 (22.4%) had EIB. Exposure to high play area endotoxin concentration [adjusted odds ratio (aOR) = 0.15, 95% CI: 0.03–0.85] and load (aOR = 0.11, 95% CI: 0.02–0.73) were negatively associated with atopy. In contrast, EIB was positively associated with high mattress endotoxin concentration (aOR = 6.01, 95% CI: 1.20–30.13).

Conclusion: Indoor microbial endotoxin exposure has varied associations with atopy and exercise-induced bronchospasm among children with asthma.

1. Introduction

Indoor microbial exposure has been suggested to influence the presence of respiratory disorders, including childhood asthma (Kanchongkittiphon et al., 2015) but the associations are conflicting. Bacterial endotoxin has been reported to have protective (Tischer et al., 2011; Lawson et al., 2012), adverse (Tavernier et al., 2005; Thorne et al., 2005; Chinn and Williams, 2007) as well as no association (Perzanowski et al., 2006; Gehring et al., 2008) for childhood asthma. Reasons for the paradoxical effects are unclear but could be linked to different presentations of the disease in children with asthma now presenting in allergic and non-allergic forms.

Previous studies of endotoxin have shown more consistent associations with allergic sensitization (Gehring et al., 2002, 2007; Tischer et al., 2011). Studies have also shown that exposure to endotoxin is inversely associated with atopic asthma (Braun-Fahrlander et al., 2002; Schram-Bijkerk et al., 2005a) and atopic wheeze (Braun-Fahrlander et al., 2002; Schram-Bijkerk et al., 2005a) among schoolchildren in the general population. However, it is less clear if this relationship also persists among children with asthma since only half of asthma cases in the general population can be attributed to allergic sensitization (Douwes et al., 2002).

Furthermore, children with allergic or non-allergic asthma may also demonstrate bronchial hyperresponsiveness (BHR) in response to

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environmental stimuli such as exercise (Novak and Bieber, 2003; Weiler et al., 2016) or have varied degree of symptom severity associated with microbial exposures (Dannemiller et al., 2016). Exercise-induced bronchospasm (EIB) is one method of assessing BHR, but currently there are limited studies investigating the effects of house dust endotoxin exposures on EIB among children with asthma.

In the present study, we investigated the association between house dust endotoxin exposure with atopic sensitization or EIB among children with asthma. We hypothesized that exposure to high levels of house dust endotoxin will be inversely related to atopy but positively related to EIB. This may provide some insight into the relevant effects of indoor endotoxin exposures and associated response among children with asthma for better childhood asthma management.

2. Methods

2.1. Study population, selection, and recruitment

We conducted a cross-sectional study among schoolchildren with asthma (aged 7–17 years) in the province of Saskatchewan, Canada from 2015 to 2016. Participants in this study were recruited from a 2013 cross-sectional survey ($n = 3509$) as previously described (Lawson et al., 2017). Those who consented to participate in further testing based on a question from the 2013 survey ($n = 1348$) were reapproached in 2015. At this time, we repeated the survey and completed clinical testing (spirometry, exercise challenge testing, and skin prick test) as well as home dust sample collection.

The study was approved by the University of Saskatchewan Biomedical Research Ethics Board (Bio #: 14–162). Completion and return of the survey implied voluntary consent for the questionnaire portion. Children and parents provided written assent and consent, respectively, prior to clinical testing and home dust collection. Furthermore, all school divisions involved approved the study.

2.2. Survey questionnaire

We used standardized and validated questions from the International Study of Asthma and Allergy in Childhood (ISAAC) (Asher et al., 1995, 1998), the American Thoracic Society Children's Respiratory Disease (Ferris, 1978), and questionnaires used previously in the Saskatchewan Lung Health studies (Rennie et al., 2008; Lawson et al., 2012) to obtain information on respiratory health (including physician-diagnosed asthma), general health, parental health history, environmental exposure, sociodemographic factors as well as housing characteristics. A total of 335 schoolchildren completed and returned the 2015 survey questionnaire.

2.3. Spirometry and exercise challenge testing (ECT)

Of the 335 subjects with survey responses, 288 (86%) performed spirometry and ECT. During home or school visits, trained field technicians performed spirometry assessments according to recommended standards for children (Pellegrino et al., 2005; Coates et al., 2013). Measurements of forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, and forced expiratory flow ($FEF_{25\%-75\%}$) were done using the Easy-on PC spirometer (nidd Medical Technologies, Zurich, Switzerland). Some subjects were excluded from testing because they were unable to perform the test due to existing medical conditions ($n = 3$).

ECT was also completed based on recommended protocols (Gerald et al., 2004). Briefly, children stepped up and down on a 6 in. step at a heart rate between 150 and 200 beats per minute for 5 min. Heart rates were monitored throughout the exercise with a Polar heart rate monitor (Polar Electro, Woodbury, NY). Spirometry was repeated 3 and 10 min after cessation of exercise.

2.4. Skin prick test (SPT)

All SPTs were completed at the school or during home visits. Tests were completed on the forearm using a panel of six common and standardized allergen extracts according to recommended protocol (Joint Task Force on Practice Parameters, 2007): cat, local grass (meadow, timothy weed, rye, Kentucky blue, orchard, and red top), *Aspergillus*, *Alternaria*, *Cladosporium*, and house dust mite (Omega Laboratory, Montreal QC, Canada). Two controls: a histamine positive control and a saline negative control were used to reduce false positives and false negatives. The wheal size diameter was measured after 15 min. Subjects was considered positive for atopy if a positive reaction to at least one of the applied allergens is raised ≥ 3 mm compared to the saline control (Joint Task Force on Practice Parameters, 2007). All SPTs in the study were performed by trained technicians who were blinded to the asthma status of each child.

2.5. Identification of subpopulation of children with asthma among survey participants

The asthma classification criteria were based on a validated asthma case detection algorithm (Gerald et al., 2004). Based on the parental response to the questionnaire, children were classified as “diagnosed asthma” if they had positive responses to the questions: “Has this child ever been diagnosed as having asthma by a doctor?” and/or “Has this child taken prescribed asthma medication in the past 12 months?” Children who were otherwise classified as “no asthma” based on survey responses but who had FEV_1/FVC ratio $< 80\%$ upon spirometry testing; or demonstrated a $> 15\%$ decrease in FEV_1 or a $\geq 25\%$ decrease in $FEF_{25\%-75\%}$ from baseline after cessation of exercise were considered to be positive for asthma (Lougheed et al., 2012; Parsons et al., 2013). Overall, a total of 116 children were identified to have asthma.

2.6. Classification of atopy and exercise-induced bronchospasm among children with asthma

Within the subpopulation of children with asthma ($n = 116$), we identified two outcomes. This included: 1) atopy outcome based on atopic vs. non-atopic status, and 2) EIB status using the ECT results (EIB vs. no EIB). Atopy was defined as sensitization (≥ 3 mm in wheal diameter compared to saline control after 15 min) to one or more allergens from SPT in the presence of diagnosed asthma. EIB was defined as $> 15\%$ fall in FEV_1 from baseline after cessation of exercise.

2.7. Collection and analysis of dust samples to quantify endotoxin exposure

Dust samples were obtained from the floor of the play area and from the mattress by trained personnel using a Solaris Turbo Plus vacuum cleaner (Model: Miele S514, Germany), set at 950 W according to the ISSAC protocol (Weiland et al., 2004). Samples were collected into X-Cell-100 dust collection filter socks with pore size between 4.0 and 12.3 μm (Midwest Filtration LLC, OH, USA). Vacuum cleaners were calibrated for flow rate and static pressure pre and post data collection ($R^2 > 0.99$). A separate, pre-weighed and labelled filter sock was used for each of the play area and mattress sample collections. The filter sock was placed into the distal end of the extension tube of vacuum cleaner and sealed with a clean crevice collecting tool placed over the distal end of the extension tube. For each sample collected, a cleaned crevice tool was used. The crevice device was also wiped clean with alcohol swab prior to any dust collection procedure to prevent cross contamination between locations and homes. Vacuum cleaners are further cleaned after each day sample collection procedures. All crevice devices were also cleaned with soapy warm water and treated with alcohol solution in the laboratory at the end of each day sample collection task.

Sampling area and time for dust collection followed the

standardized ISAAC protocol (Weiland et al., 2004). Carpet floors had 2 m² vacuumed for 4 min while smooth floors had 4 m² vacuumed for 2 min. Dust collection from the mattress surfaces was completed after all duvets, blankets and sheets that the child slept under were removed. The entire surface area of the mattress was vacuumed for 2 min. Post sample collection, filter socks containing dust samples were placed in de-identified labelled bags and refrigerated at 4 °C until analysis. Dust samples were post-weighed and then sieved through a 300 µm mesh sieve for extraction and endotoxin analysis.

Prior to analysis, samples were brought to room temperature and 10 mg of sieved dust was weighed out and extracted with 20 mL 0.05% Tween 20 (Fisher Scientific, Mississauga, ON, Canada) in pyrogen-free water (GE Healthcare Bio-Science, Mississauga, ON, Canada) (Schram-Bijkerk et al., 2005b) and shaken at 325 revolutions per minute (RPM) for 2 h. The extracted solution was then centrifuged (Sorvall ST 16 R, Thermo Fisher Scientific, Mississauga, ON, Canada) at 1000 × g for 15 min to obtain supernatant and 1 mL aliquots were stored at −80 °C until analysis. An aliquot of the supernatant was diluted 1 in 10 and used to measure endotoxin in duplicate by the chromogenic *Limulus* Amoebocyte Lysate (LAL) Kinetic QCL assay according to manufacturer's specifications (Lonza, Walkersville, MD, USA). To ensure quality, the assays were conducted using reagents from a single lot.

The absorbance of endotoxin was monitored at 405 nm for 2 h using the Biotek ELx808 plate reader and Gen5 v 2.06 software (BioTek, Winooski, VT, USA). Values were compared to standard curves prepared for endotoxin (0.005 EU/mL–50 EU/mL). Endotoxin concentration in dust samples was expressed as endotoxin units (EU) per mg of sieved dust while endotoxin load was expressed as EU per m² of the area vacuumed. Both endotoxin concentration and endotoxin load were reported in this study given that concentration and load represent different aspects of indoor microbial exposure (dose and burden, respectively) (Committee on Damp Indoor Spaces and Health, 2004). Laboratory technicians were blinded to disease status of each child and to the indoor location where each dust was sampled (i.e. whether sample was obtained from child's play area or mattress surface).

2.8. Statistical analysis

Two outcomes were assessed, separately, in this study among children with asthma: atopy and EIB. Among the 116 children with asthma, 88 (75.9%) had complete dust samples and SPT results and formed the study population for examining the relationships between endotoxin and atopy. Similarly, 102 (87.9%) had complete dust samples and ECT results and formed the study population for examining the associations between endotoxin and EIB. Categorical variables were compared, separately for atopic vs. non-atopic children with asthma and for EIB vs. no EIB children with asthma, using the independent samples chi-square (χ^2) and Fisher's exact tests as appropriate while continuous variables were compared using the independent sample Student *t*-test. Correlation between play area and mattress endotoxin was also assessed using Pearson's correlation coefficient (*r*).

Multiple logistic regression models were then fitted to test the association between endotoxin exposure with atopy expressed as either atopic vs. non-atopic (non-atopic as reference) or EIB vs. no EIB (no EIB as reference). Separate independent models were fitted for each of play area and mattress dust including: i) a univariate model for each endotoxin (concentration and load); and ii) main effects model for endotoxin which included a variable for endotoxin as a predictor variable as well as potential confounders. Variables were included in the models based on statistical significance identified in the univariate analyses, clinical importance, and the effect the removal of a variable had on the beta coefficient of other variables in the model (Hosmer et al., 2013). The additional variables included in the model were sex, age, parental education ($\leq$ high school vs. $>$ high school), parental smoke (if either father or mother smoke: yes vs. no), parental history of asthma and allergy (either father or mother reported history of asthma and/or

allergy: yes vs. no), pet ownership (presence of cat and/or dog in the home in the past 12 months: yes vs. no), home dampness (water or dampness in the home in the past 12 months: yes vs. no), and location of residence (urban vs. rural). Endotoxin remained, *a priori*, in all models. Levels of endotoxin were modelled as categorical variables in tertiles to define high (3rd tertile), medium (2nd tertile), and low (1st tertile) levels because the relationships with atopy as well as EIB did not meet the linear assumptions. Low (1st tertile) was used as reference level. In addition, by modelling endotoxin as categorical variables (in tertiles), the effects of magnitude due to some outliers—if modelled as continuous variables—were eliminated. Throughout the analyses, generalized estimating equations (GEE) were used to account for clustering within families. The Hosmer-Lemeshow test was used to assess model Goodness-of-Fit.

The strength of the associations was assessed using odds ratios (ORs) and their 95% confidence interval (CI). Statistical significance was defined by an alpha level of $p \leq 0.05$. All analyses were completed with the Statistical Package for the Social Science (SPSS) Version 24 (SPSS Inc. Armonk, NY: IBM Corp.) and the Statistical Analysis System (SAS) Version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Subject characteristics, respiratory symptoms and exposure characteristics

Of the 116 children identified with asthma, 99 (85.3%) completed SPT while all completed ECT; thus 99 (85.3%) of the children had complete SPT and ECT results. Of the 99 children that completed SPT, 71/99 (71.7%) were atopic and of the 116 children with ECT results, 26/116 (22.4%) had EIB. When we considered overlap in atopy and EIB in the 99 children with both SPT and ECT results, 17 (17.2%) with EIB were also atopic while 54 (54.5%) had atopic sensitization alone, 6 (6.1%) had EIB alone, and 22 (22.2%) were non-atopic and no EIB.

Table 1 presents an overview of the distribution of socio-demographic, home characteristics, and early life characteristics by atopic (atopic vs. no atopic) as well as by EIB (EIB vs. no EIB) status. The distribution of sex and age were similar between atopic and non-atopic groups. However, compared to children with atopic sensitization, children in the non-atopic group were more likely to live in modern homes (homes built between 1980–present; $p = 0.03$). Children with no EIB, on average, were older with a higher BMI and were more likely to have parents with history of allergic disease compared to children with EIB ($p < 0.05$). The distribution of all other characteristics was similar and not statistically significant between groups whether expressed as atopic vs. non-atopic or as EIB vs. no EIB.

Respiratory symptoms were also similar between the atopic vs. non-atopic or EIB vs. no EIB groups except for ever wheeze which was significantly higher in no EIB compared to EIB group (Table 2).

Endotoxin were at detectable levels in all house dust samples. Overall, endotoxin ranges were: play area (2.20 EU/mg–6.55 EU/mg and 8.19 EU/m²–12.76 EU/m²) mattress (0.69 EU/mg–6.21 EU/mg and 6.02 EU/m²–12.18 EU/m²). Endotoxin levels in play areas and mattresses did not differ between atopic and non-atopic group or between EIB and no EIB group (Fig. 1). Correlation between play area and mattress endotoxin concentration was weak and non-significant ($r = 0.10$, $p = 0.32$). Similarly, play area and mattress endotoxin load were weakly correlated and not significant ($r = 0.11$, $p = 0.29$).

3.2. Associations between house dust endotoxin exposure levels and atopic sensitization and exercise-induced bronchospasm among children with asthma

Among the 116 children with asthma, 88 (75.9%) had complete dust samples and SPT results (atopic: $n = 67$; nonatopic: $n = 21$) and 102 (87.9%) had complete dust samples and ECT results (EIB: $n = 20$;

Table 1Characteristics of the study population ($n = 116$ children with asthma) by atopic and exercise-induced bronchospasm (EIB) status.

	Atopic ($n = 71$) [†]	Non-atopic ($n = 28$) [†]	<i>p</i> -value	EIB ($n = 26$)	Absence of EIB ($n = 90$)	<i>p</i> -value
Mean age ($\pm$ SD), years	10.9 (2.7)	11.0 (2.5)	0.86	10.0 (2.8)	11.3 (2.6)	0.04
Body mass index ($\pm$ SD), kg/m ²	20.3 (5.0)	21.0 (4.8)	0.55	18.3 (2.6)	21.2 (5.3)	< 0.001
% Female	32.4	32.1	0.98	26.9	37.8	0.31
Ethnic background						
% Caucasian	81.0	75.0	0.52	79.2	82.1	0.77 [†]
% Other ethnic background	19.0	25		20.8	17.9	
Physical activity						
% Low	1.4	7.1	0.31	0.0	4.4	0.55
% Moderate	31.0	32.1		34.6	34.4	
% High	67.6	60.7		65.4	61.1	
Parental education level						
% > high school (maternal)	80.0	88.9	0.38	84.0	83.1	1.00 [†]
% > high school (paternal)	76.1	84.6	0.37	91.7	75.6	0.09
Tobacco smoke exposure						
% Parental smoking	13.2	14.3	1.00 [†]	15.4	11.5	0.73 [†]
% Environmental tobacco smoke (ETS)	7.0	10.7	0.68 [†]	0.0	11.2	0.11
Home characteristics						
% Modern home (1980–Present)	36.9	61.5	0.03	36.0	47.6	0.31
% Gas heating	97.1	92.6	0.61 [†]	96.2	94.2	0.36 [†]
% Home air filter	47.9	50.0	0.85	57.7	48.9	0.43
% Home humidifier	28.2	28.6	0.97	30.8	28.9	0.85
% Pet ownership	57.7	57.1	0.96	57.7	58.9	0.91
% Carpet flooring	58.2	71.4	0.28	70.0	54.9	0.22
% Home dampness	25.7	22.2	0.72	15.4	27.3	0.22
% Visible mold	10.4	7.7	1.00 [†]	12.5	11.6	1.00 [†]
Family history						
% Parental history of asthma	36.6	39.3	0.81	26.9	36.7	0.36
% Parental history of allergic disease	60.6	57.1	0.76	42.3	65.6	0.03
Early life characteristics						
% Breastfed	81.5	96.4	0.10	84.0	88.2	0.73
% Attended daycare	70.8	69.2	0.89	72.0	66.3	0.59
% Consumed raw farm milk	5.6	0	0.5 [†]	3.8	5.6	1.00 [†]
% Contact with farm buildings	16.4	11.1	0.75 [†]	15.4	18.1	1.00 [†]
% Contact with farm animals	15.9	14.8	1.00 [†]	11.5	18.6	0.56 [†]
Intrauterine exposure characteristics						
% Mother consumed farm milk	0	3.6	0.28 [†]	0.0	1.1	1.00 [†]
% Mother contact with farm animals	12.7	14.3	1.00 [†]	3.8	14.4	0.19 [†]
Location of residence						
% Urban	91.5	85.7	0.46 [†]	96.2	84.4	0.19 [†]
% Rural	8.5	14.3		3.8	15.6	

* 99/116 of the children identified to have asthma completed SPT.

[†] Statistical difference assessed by the Fisher's exact test due to small cell sizes (expected values < 5).no EIB: $n = 82$).

Univariate logistic regression analyses showed that high play area endotoxin concentration and load were inversely associated with atopy (OR = 0.25, 95% CI: 0.07–0.91) (Table 3, Model I). Similarly, multiple logistic regression analysis (Model II) showed statistically significant inverse associations with atopy in the high endotoxin group [endotoxin concentration: adjusted odds ratio (aOR) = 0.15, 95% CI: 0.03–0.85; endotoxin load: aOR = 0.11, 95% CI: 0.02–0.73].

Table 4 presents the associations between endotoxin with EIB, based on ECT results, among children with asthma irrespective of atopic status. Exposure to high level of mattress endotoxin concentration was positively associated with EIB both at the univariate level (OR = 4.64,

95% CI: 1.15–18.75; Model I) and after adjusting for potential covariates (aOR = 6.01, 95% CI: 1.20–30.13; Model II).

4. Discussion

In the current study, high endotoxin levels in play area dust were inversely associated with atopy while high mattress endotoxin concentration was positively associated with EIB among children with asthma. The results suggest that endotoxin exposure in the indoor environment shows opposite effects on atopic sensitization and bronchial hyperresponsiveness among children with asthma.

In the Allergy and Endotoxin Study (ALEX) conducted among

Table 2

Respiratory symptoms among children with asthma by atopic and exercise-induced bronchospasm (EIB) status.

	Atopic ($n = 71$)	Non-atopic ($n = 28$)	<i>p</i> -value	EIB ($n = 26$)	Absence of EIB ($n = 90$)	<i>p</i> -value
Respiratory symptoms						
% Ever wheeze	75.4	80.0	0.64	50	84.7	< 0.001
% Wheeze past 12 month	51.4	48.0	0.77	52.9	40.0	0.26
% Cough past 12 months	47.9	42.9	0.65	30.8	47.8	0.12
% Chest congestion	13.8	22.2	0.36 [†]	19.2	15.9	0.69
% Chronic bronchitis	1.4	3.6	0.49 [†]	3.8	4.4	1.00 [†]
% Nasal congestion	43.7	33.3	0.35	46.2	40.4	0.60
% Hay fever	9.9	7.1	1.00 [†]	3.8	10.0	0.45 [†]

[†] Statistical difference assessed by the Fisher's exact test due to small cell sizes (expected values < 5).

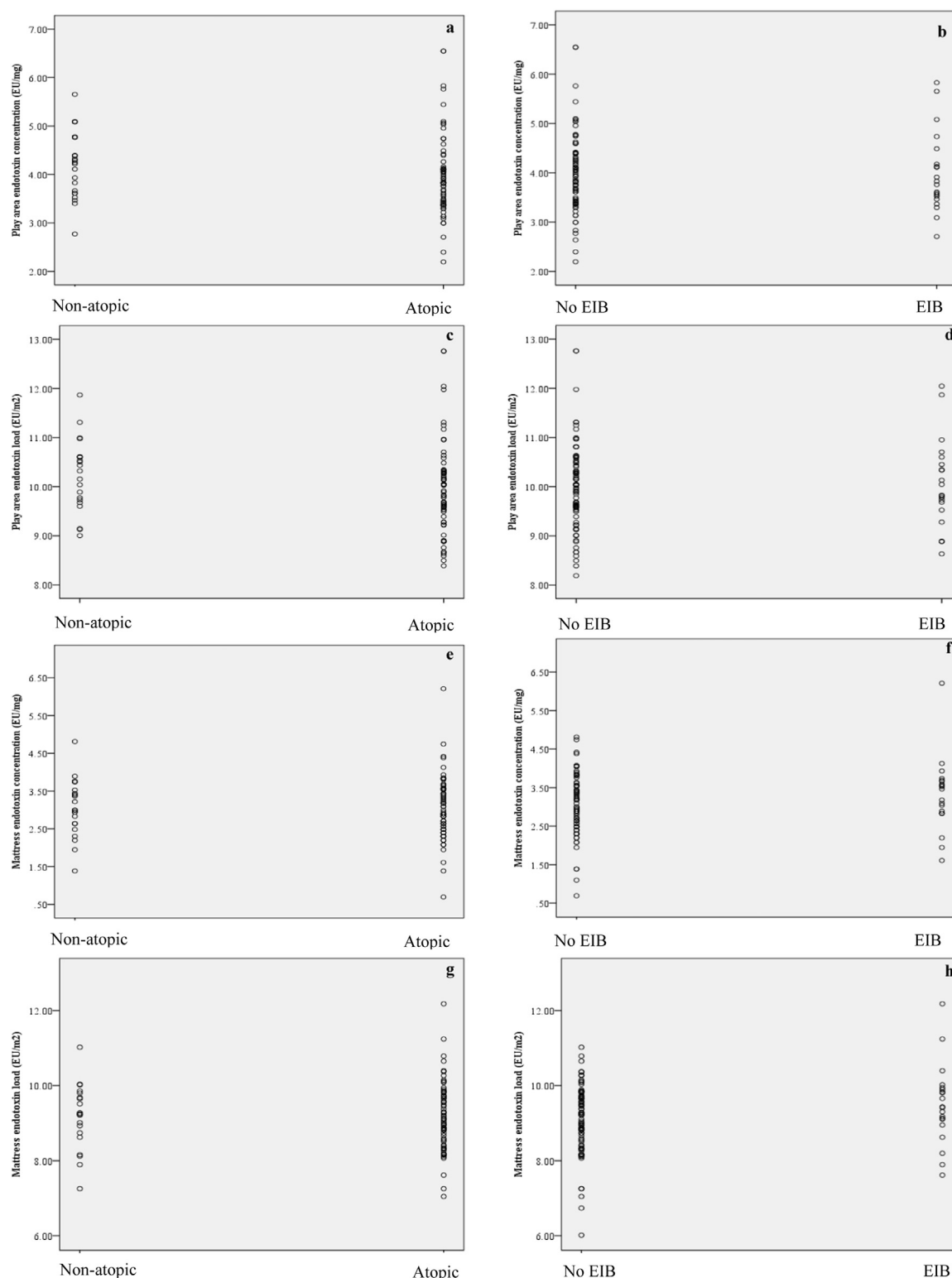


Fig. 1. Distribution of log transformed play area endotoxin concentration (a, b) and load (c, d) levels and mattress endotoxin concentration (e, f) and load (g, h) levels in homes of children with asthma, stratified by atopy and EIB status.

children (aged 6–13 years) in Austria, Germany, and Switzerland, Braun-Fahrlander et al. demonstrated that endotoxin load in samples of dust derived from children's mattresses were inversely associated with atopic sensitization in the general population of children with and without asthma (Braun-Fahrlander et al., 2002). While it may be difficult to directly compare our results with the ALEX study because that study included a control group (children without asthma), our results

complement the ALEX results as we showed that among children with established asthma, a similar inverse association between endotoxin and atopy was observed, but the association may be limited to play area. Reasons for the differences in association between play area and mattress dust is unclear in this study but may be related to higher levels of endotoxin found in the play area compared to the mattress, differences in the determinants of endotoxin in different locations in the

Table 3

Multiple logistic regression analyses describing the associations between endotoxin and atopy among children with asthma^a.

	Model I OR (95% CI)	Model II ^b aOR (95% CI) ^c
Play area		
Endotoxin concentration (EU/mg)		
Low (< 35.33)	1.00	1.00
Medium (35.33–63.33)	0.67 (0.17–2.65)	0.51 (0.10–2.55)
High (> 63.33)	0.25 (0.07–0.91)[‡]	0.15 (0.03–0.85)[‡]
Endotoxin Load (EU/m ²)		
Low (< 15274.17)	1.00	1.00
Medium (15274.17–29603.37)	0.67 (0.17–2.65)	0.46 (0.07–2.90)
High (> 29603.37)	0.25 (0.07–0.91)[‡]	0.11 (0.02–0.73)[‡]
Mattress		
Endotoxin concentration (EU/mg)		
Low (< 15)	1.00	1.00
Medium (15–30)	0.71 (0.22–2.27)	0.52 (0.13–2.13)
High (> 30)	0.90 (0.26–3.09)	0.72 (0.14–3.69)
Endotoxin Load (EU/m ²)		
Low (< 6939.32)	1.00	1.00
Medium (6939.32–15204.89)	0.69 (0.21–2.30)	0.52 (0.11–2.49)
High (> 15204.89)	0.75 (0.22–2.59)	0.47 (0.09–2.42)

EU: Endotoxin units.

Model I: Model with no adjustments for atopic sensitization; Models II: Adjusted model for atopic sensitization with endotoxin as an independent variable.

aOR: Adjusted odds ratio.

^a 88 children had complete data for SPT and dust samples (atopic = 67; nonatopic = 21).

^b Statistical comparisons between atopic and non-atopic groups were completed using logistic regression with GEE to account for clustering within families.

^c Model adjusted for sex, age, parental education, parental smoke, parental history of asthma and allergy, pet ownership, home dampness, and location of residence.

[‡] $p < 0.05$.

Table 4

Multiple logistic regression analyses describing the associations between endotoxin and exercise-induced bronchospasm (EIB) among children with asthma^a.

	Model I OR (95% CI)	Model II ^b aOR (95% CI) ^c
Play area		
Endotoxin concentration (EU/mg)		
Low (< 35.33)	1.00	1.00
Medium (35.33–63.33)	1.00 (0.31–3.24)	0.60 (0.13–2.82)
High (> 63.33)	0.83 (0.25–2.78)	0.60 (0.12–3.05)
Endotoxin Load (EU/m ²)		
Low (< 15274.17)	1.00	1.00
Medium (15274.17–29603.37)	1.50 (0.43–5.31)	1.05 (0.20–5.35)
High (> 29603.37)	1.79 (0.52–6.15)	1.60 (0.29–8.75)
Mattress		
Endotoxin concentration (EU/mg)		
Low (< 15)	1.00	1.00
Medium (15–30)	2.77 (0.65–11.75)	2.18 (0.41–11.72)
High (> 30)	4.64 (1.15–18.75)[‡]	6.01 (1.20–30.13)[‡]
Endotoxin Load (EU/m ²)		
Low (< 6939.32)	1.00	1.00
Medium (6939.32–15204.89)	1.94 (0.51–7.38)	1.09 (0.23–5.21)
High (> 15204.89)	2.70 (0.74–9.83)	2.82 (0.63–12.70)

EU: Endotoxin units.

Model I: Model with no adjustments for EIB; Models II: Adjusted model for EIB with endotoxin as an independent variable.

aOR: Adjusted odds ratio.

^a 102 children had complete data for ECT and dust samples (EIB = 20; no EIB = 82).

^b Statistical comparisons between EIB and no EIB groups were completed using logistic regression with GEE to account for clustering within families.

^c Model adjusted for sex, age, parental education, parental smoke, parental history of asthma and allergy, pet ownership, atopic sensitization, home dampness, and location of residence.

[‡] $p < 0.05$.

homes (Rennie et al., 2008; Lawson et al., 2012) or differences in endotoxin structures within locations in the indoor environment (Park et al., 2004).

There is evidence that inhaled endotoxin exposure can induce BHR. However, these effects have only been found in adults (Michel et al., 1989; Kitz et al., 2006) and an animal study (Tulic et al., 2000). In a clinical bronchial challenge test among adults with asthma in Belgium, Michel et al. found a significant reduction in FEV₁ (≥ 10% decrease) which lasted between 15 and 45 min following inhalation of 20 µg of endotoxin extract (Michel et al., 1989). We found similar trends of associations in the current study, albeit with a different bronchial challenge test (exercise challenge test) and source of endotoxin exposure (settled house dust). Furthermore, our result suggests that, among children with asthma, EIB response to endotoxin may be dose related with a statistically significant and stronger association found for high endotoxin exposure (aOR = 6.01, 95%CI: 1.20–30.13) than for the association at medium endotoxin exposure (aOR = 2.18, 95%CI: 0.41–11.72). This is consistent with dose-response relationship results observed in an endotoxin exposure challenge test study among 77 adults which demonstrated significant bronchoconstriction (decreased in FEV₁) following inhalation of 200 µg of endotoxin extract compared to inhalation of 30 µg (Rylander et al., 1989).

Associations between play area endotoxin levels and EIB were not found in the present study. Possible explanations for the presence of EIB and mattress endotoxin concentration and the lack of it for play area endotoxin levels may be that children come into closer contact with microbial agents while sleeping and or differences in play and mattress areas endotoxin's structure (Park et al., 2004). Studies have shown mattress dust to be the most reproducible source of house dust exposure (Park et al., 2000) with nonsignificant variation over a six-month period (Gereda et al., 2000) compared to play areas which may be regularly vacuumed.

We acknowledge that EIB as a form of BHR as used in this study may also serve as a marker of asthma severity (Perin et al., 1994). However, this only occurs in situations where there is persistence of bronchial hyperreactivity despite consistent and intensive therapy with inhaled corticosteroid over time (Perin et al., 1994). Despite this, as seen in our results, the prevalence of the report of symptoms and other markers of asthma severity (frequent wheeze) were higher among those with EIB.

Limitations of our study should be considered. The participation rate for this study was low. It is important to note that participants in this study were recruited from urban and rural locations and were frequently hard to reach, especially the rural locations. As our results indicated statistical significance for endotoxin, we believe that the power of the study was sufficient. However, the results of the study should be interpreted with caution as conclusions from the study were based on a smaller number of subjects identified as non-atopic ($n = 21$) and EIB ($n = 20$). Classifications by atopic sensitization and EIB response among children with asthma were not entirely distinct in this study as there was some overlap. For example, 17/116 (17.2%) children with asthma in this study were both atopic and positive for EIB. We used objective measures to minimize misclassification of atopy and EIB in this study. We completed analyses for atopic sensitization and EIB response, separately, recognizing that these outcomes do overlap. As such the associations reported in the current study were based on classification atopy or EIB among children with asthma in relation to endotoxin exposure, irrespective of the overlap between the two groups.

The high proportion of children with positive indication of asthma in this study (34.6%; 116/335) may indicate the possibility of response bias and a tendency towards a population with a higher prevalence of asthma for this study. This may also explain the reason for the high frequency of atopic children (71.7%) in this population of children with asthma where more children with atopic sensitization were also more likely to respond to the survey. However, since only children identified as positive for asthma were considered in this study, the associations

reported will remain valid within this subpopulation given the well-defined asthma definition and objective measures used in selecting these participants.

Endotoxin in settled dust may only represent part of the indoor total microbial exposure which also includes other agents such as allergens, muramic acid, ergosterol, and peptidoglycan that may exert independent effects on asthma and asthma morbidity (Karvonen et al., 2014). Bronchial challenge studies in adults demonstrated that atopy modifies the response to endotoxin exposure with decrease lung function (FEV₁) observed in atopic compared with nonatopic subjects (Michel et al., 2001). Similarly, in a study in the US assessing asthma severity among children with asthma microbial exposure was associated with asthma severity but the observed response differed in atopic compared with nonatopic children (Dannemiller et al., 2016). Due to the small sample size, we were unable to assess modification effects of atopy on EIB in relation to endotoxin exposure in this study.

Finally, dust sample collection for this study was limited to children with asthma. The lack of a control group (children without asthma) in this study limited our assessment of the associations to the presence of atopy and EIB among children with asthma as opposed to an assessment of the association with atopic asthma and EIB asthma, which would require a control group without asthma. However, our results showed a similar pattern of an inverse association between endotoxin exposure and atopy and BHR (measured as response to methacholine challenge test) found in a study in the Netherlands where high occupational endotoxin exposure was inversely associated with atopy and positively associated with BHR in adult population with asthma (Smit et al., 2010).

The strengths of our study included the use of objective measures for exposure and outcome assessments thus limiting the possibility of recall bias for the associations reported herein. We also used an established algorithm of case finding to minimize misclassification of asthma status (Gerald et al., 2004). Clinical data and dust samples were collected by trained technicians using standardized protocols (Weiland et al., 2004; Pellegrino et al., 2005; Coates et al., 2013). Laboratory personnel were blinded to asthma status of each child. Furthermore, home dust collection was conducted concurrently with clinical data. We also adjusted for potential confounders known to be associated with asthma, atopic sensitization and BHR, thus strengthening the validity of our findings.

In summary, we found paradoxical effects of indoor endotoxin exposure on atopy and EIB in children with preexisting asthma conditions. Exposure to house dust endotoxin levels was inversely associated with atopy but positively associated with EIB among children with asthma.

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Ethical approval

The study was approved by the University of Saskatchewan Biomedical Research Ethics Board (Bio #: 14–162).

Author contributions

OO conceptualized and designed the study, conducted data collection and management, interpreted the data, and drafted the initial manuscript; JAL contributed to the study concept, design, data management and interpretation, reviewed and revised the manuscript; DCR contributed to the study concept, data collection, results interpretation, reviewed and revised the manuscript; AS contributed to the study design, data analysis methods, results interpretation, reviewed and revised the manuscript; RD contributed to study design, reviewed and revised the manuscript; AA contributed to data collection, results interpretation, and reviewed the manuscript. SK and GK contributed to samples preparation, laboratory analysis of dust samples, result interpretation, reviewed and revised the manuscript. Both OO and JAL had full access to the data and take responsibility for the integrity of the data and for the accuracy of the data analysis. All authors have approved the final manuscript as submitted and take responsibility for its content.

Conflict of interest

The authors have no conflict of interest related to this study to disclose.

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