



The association between endotoxin and beta-(1 → 3)-D-glucan in house dust with asthma severity among schoolchildren

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ABSTRACT

Background: Asthma severity can be affected by microbial exposures. However, less is known about the specific indoor agents aggravating the disease in children. We examined the associations between indoor endotoxin and beta-(1 → 3)-D-glucan exposures and asthma severity in children with asthma.

Methods: A clinical cross-sectional study of schoolchildren (aged 7–17 years) was conducted in the province of Saskatchewan, Canada. Children with asthma (n = 116) were identified from 335 participants using a combination of survey responses and objective clinical assessments. We then ascertained asthma severity based on recommended guidelines (continuous daytime asthma symptoms, frequent nighttime asthma symptoms, and ≤ 60% predicted FEV₁). Levels of indoor endotoxin and beta-(1 → 3)-D-glucan were measured in dust samples obtained from play area floors and child's mattresses.

Results: The study population of 116 children with asthma was comprised of 75.9% mild asthma and 24.1% moderate/severe asthma. Higher mattress endotoxin concentration was associated with increased odds of moderate/severe asthma [adjusted odds ratio (aOR) = 11.40, 95% confidence interval (CI): 1.45–89.43] while higher beta-(1 → 3)-D-glucan concentration (aOR = 0.16, 95% CI: 0.03–0.89) and load (aOR = 0.10, 95% CI: 0.02–0.72) in play areas were inversely associated with moderate/severe asthma. Furthermore, higher mattress endotoxin concentration was associated with lower FVC (p = 0.01) and FEV₁ (p = 0.03). These associations were not seen for beta-(1 → 3)-D-glucan.

Conclusion: Our results showed differential effects of microbial exposures on childhood asthma severity and further highlight domestic endotoxin exposure effects on respiratory health outcomes in children with asthma.

1. Introduction

Asthma is the most common chronic disease [1] and a leading cause of morbidity among children in Canada, accounting for a great deal of economic burden per year [2]. Examination of risk factors for asthma severity could identify exposures that aggravate the disease among children and aid attempts to reduce morbidity and subsequent health-care utilization and costs. The National Asthma Education and Prevention Program (NAEPP) Expert Panel Report 2 guidelines [3] recommend that asthma severity be assessed using a combination of frequency of clinical respiratory symptoms (day- and night-time symptoms) and objective lung function criteria (determined with forced expiratory volume in one second [FEV₁]).

Exposures to dust mite [4,5], furred pets [6,7], and tobacco smoke [8,9] have been shown to be associated with asthma severity. While exposure to indoor mold or dampness have also been reported to worsen asthma symptoms [10,11], to date, the impact of many indoor microbial components in the exacerbation of asthma remains poorly assessed.

Evidence of associations between endotoxin, a component of gram-negative bacteria [12], and childhood asthma is controversial with some studies showing protective [13,14], and adverse [15–17] effects as well as no association [18,19]. Endotoxin is also seen as a pro-inflammatory agent, which means that it could also be associated with worsening asthma conditions [15–17]. Indeed, it has been shown that endotoxin may aggravate asthma conditions in children with the

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List of abbreviations

ANCOVA	Analysis of covariance
ECT	Exercise Challenge Testing
FEV ₁	Forced expiratory volume in one second
FVC	Forced Vital Capacity

GM	Geometric mean
GLI	Global Lung Function Initiative
ISAAC	International Study of Asthma and Allergy in Childhood
NAEPP	National Asthma Education and Prevention Program
OR	Odds ratio

disease, in terms of increased frequency of wheezing and asthma medication use [3,17] suggesting that the indoor environment may play an important role in the management of childhood asthma. Most of the previous studies examining the relationships between asthma severity and endotoxin have focused on reports of the frequency of wheeze [17,20] and asthma medication use [17]. Additional indicators of severity, incorporating objective measures of disease severity, should be investigated for better understanding of the associations between indoor microbial exposures and asthma severity in children.

While beta-(1 → 3)-D-glucan represents a marker of both bacterial and fungal exposure [21], its role in the exacerbation of asthma is also less well investigated with previous studies focusing on endotoxin exposure as a sole marker of indoor microbial exposure [17,22]. Investigating the respiratory effects of endotoxin and beta-(1 → 3)-D-glucan exposures, in tandem, will further our knowledge of the relationships between indoor microbial exposure and asthma severity in children.

In this study, we examined the relationships between house dust endotoxin and beta-(1 → 3)-D-glucan exposure levels and asthma severity in schoolchildren with asthma. In addition, we also investigated the relationships between endotoxin and beta-(1 → 3)-D-glucan exposures and lung function in these children. Identifying specific microbial indoor exposure associated with asthma exacerbations is important as this could help guide asthma management among children and, ultimately, decrease associated morbidity and healthcare costs.

2. Materials and methods

2.1. Study population, selection and recruitment

A cross-sectional study with clinical assessments was conducted in the province of Saskatchewan, Canada from 2015 to 2016. The study population consisted of urban and rural schoolchildren (aged 7–17 years) who were part of an initial 2013 cross-sectional survey previously described [23]. Briefly, study packages, including an information letter, survey and pre-paid return envelope, were mailed to parents for self-completion in 2013. Those who consented to participate in further testing were approached in 2015. At this time, we repeated the survey in order to obtain accurate information on current respiratory symptoms that correspond to lung function values in the participants. Subsequently, clinical testing (spirometry and exercise challenge testing) as well as home dust sample collection was completed in 2015–2016.

The study was approved by the University of Saskatchewan Biomedical Research Ethics Board (Bio #: 14–162). Furthermore, all school divisions involved approved the study. 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.

2.2. Survey questionnaire

Parents completed a questionnaire based on the standardized and validated questions from the International Study of Asthma and Allergy in Childhood (ISAAC) [24], the American Thoracic Society Children's Respiratory Disease [25], and questionnaires used previously in the Saskatchewan Lung Health studies [13]. Questions about respiratory

health, general health, parental health history, environmental exposure, sociodemographic factors as well as housing characteristics were included. 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 281 (98%) further completed ECT.

During home or school visits, children performed spirometry assessment according to recommended standards [26] using the Easy-on PC spirometer (ndd Medical Technologies, Zurich, Switzerland). Measurements of forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), FEV₁/FVC ratio, and forced expiratory flow (FEF_{25%–75%}) were obtained. Predicted values were based on the all-age, multi-ethnic Global Lung Function Initiative (GLI) reference equation [27]. Children were excluded from testing if they were unable to perform the test due to existing medical conditions ($n = 3$).

ECT was also completed based on recommended protocols [28]. Briefly, children stepped up and down on a 6 inch 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 at 3 and 10 min after cessation of exercise.

2.4. Identification of children with asthma among survey participants

Asthma was identified through a combination of survey responses and results from clinical assessments (spirometry and ECT). Children were classified as having asthma if they had positive response to the questions: “Has this child ever been diagnosed as having asthma by a doctor?” and/or a positive response to: “Has this child taken prescribed asthma medication in the past 12 months?” ($n = 95$). Otherwise, they were classified as “no asthma”. We then used the validated asthma case detection algorithm developed by Gerald et al. [28] to further identify children positive for asthma ($n = 21$). This was based on an FEV₁/FVC ratio less than 80% upon spirometry testing and/or demonstrated a greater than 15% decrease in FEV₁ or 25% or greater decrease in FEF_{25%–75%} from baseline after cessation of exercise [29,30]. Overall, from this process, 116 children were identified to have asthma. The algorithm has a high sensitivity (82%) and specificity (93%) when compared to clinical assessment of asthma by a physician [28].

2.5. Classification of asthma severity

The 116 children positive for asthma were classified into one of four asthma severity groups based on the NAEPP guidelines: mild intermittent, mild persistent, moderate persistent, and severe persistent asthma.

In the NAEPP guidelines, spirometry does not distinguish between mild intermittent and mild persistent asthma (both with FEV₁ ≥ 80%), therefore these two severity groups were collapsed into one single group (mild asthma) [31]. Similarly, because it is difficult to reliably differentiate moderate persistent from severe persistent asthma based on self-reported symptom frequency alone (i.e. differentiating between “daily” and “continual” daytime symptoms or defining “frequent” nights with symptoms) [3,32], the two most severe groups (moderate

and severe persistent asthma) were also collapsed into one severity group (moderate/severe asthma). Overall, two asthma severity groups, mild asthma and moderate/severe asthma, were considered as the primary outcomes for the current study; with mild asthma used as the reference group.

2.6. Collection and analysis of dust samples to quantify endotoxin and beta-(1 → 3)-D-glucan exposure

Of the 116 children identified to have asthma, 102 (88%) consented to home dust sample collection.

Settled dust samples were vacuumed from the floor of child's play area and mattress surfaces adhering to recommended standardized protocols [33] using pre-weighed X-Cell-100 filter socks with a pore size of approximately 4.0–12.3 microns (Midwest Filtration LLC, OH, USA). Carpeted floors had 2 m² vacuumed for 4 min while completely smooth floors (e.g. hardwood, laminate, or linoleum) had 4 m² vacuumed for the same time duration. Dust collection from the mattress surfaces (with the bottom sheet on) was completed after all duvets, blankets and sheets that the child slept under were removed and the entire surface area of the mattress was then vacuumed for 2 min. Dust samples were stored in a desiccator at 4 °C until extraction and analysis.

Samples were brought to room temperature and 10 mg of sieved dust was weighed out for extraction. Dust samples were extracted with 20 mL 0.05% Tween 20 (Fisher Scientific, Mississauga, ON, Canada) in pyrogen-free water [34] (GE Healthcare Bio-Science, Mississauga, ON, Canada) and shaken at 325 revolutions per minute (RPM) for 2 h. The extracted solution was then centrifuged (Sorvall ST 16R, 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 was used to measure endotoxin in the chromogenic *Limulus* Amoebocyte Lysate (LAL) Kinetic QCL assay according to manufacturer's specifications (Lonza, Walkersville, MD, USA). The water soluble fraction of beta-(1 → 3)-D-glucan was measured in a second aliquot using the Glucatell assay kit with a beta-(1 → 3)-D-glucan-specific inhibition enzymes based on the Kinetic Onset Time protocol according to manufacturer's specifications (Associate of Cape Cod, East Falmouth, MA, USA) [35].

To quantify endotoxin and beta-(1 → 3)-D-glucan levels, the absorbance of endotoxin and beta-(1 → 3)-D-glucan was monitored at 405 nm for 2 h and 1 h, respectively, using the Biotek ELx808 plate reader (BioTek, Winooski, VT, USA). Values were compared to standard curves prepared for endotoxin (0.005 EU/mL–50 EU/mL) and beta-(1 → 3)-D-glucan (3.125 pg/mL–100 pg/mL). Endotoxin and beta-(1 → 3)-D-glucan levels were reported as concentration (per gram of sampled dust) and load (per square meter of sampled area) given that the two measures represent different aspects of indoor microbial exposure (dose and burden, respectively) [36].

2.7. Statistical analysis

All analyses were completed using 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). Categorical variables were compared between asthma severity groups 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. Mean endotoxin and beta-(1 → 3)-D-glucan levels for play area and mattress were expressed as geometric mean (GM) and compared between severity groups. Comparison of absolute values of lung function variables between severity groups were completed using the analysis of covariance (ANCOVA) adjusted for age, sex, and height.

We assessed the associations between endotoxin and beta-(1 → 3)-D-glucan levels and the dichotomous health outcome of asthma severity (mild asthma or moderate/severe asthma) using multiple logistic

regression. Similarly, multiple linear regression models were fitted to assess the associations between endotoxin and beta-(1 → 3)-D-glucan levels and lung function variables (FVC, FEV₁, FEV₁/FVC, and FEF_{25%–75%}). Additional variables included in the models were sex, age, height, parental smoking, home dampness, visible mold, asthma medication use, allergen sensitization, and location of residence. These variables were included based on statistical significance from the univariate analyses, clinical/biological importance, or the effect the removal of a variable had on the beta coefficient of other variables in the model [37,38]. Endotoxin and beta-(1 → 3)-D-glucan remained, *a priori*, in all models. Levels of endotoxin and beta-(1 → 3)-D-glucan were modelled as categorical variables in tertiles to define low (1st tertile), medium (2nd tertile), and high (3rd tertile) levels because the relationships with moderate/severe asthma did not meet the linear assumption when log-transformed (ln) endotoxin and beta-(1 → 3)-D-glucan levels were used. Low, medium, and high levels were determined based on their corresponding tertile values of the exposure distribution, separately, for play and mattress areas. The reference value was the lower endpoint of each of the tertiles. 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 beta-(1 → 3)-D-glucan (concentration and load); ii) main effects model for endotoxin; iii) main effects models for beta-(1 → 3)-D-glucan; and iv) main effects plus endotoxin and beta-(1 → 3)-D-glucan. 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 were assessed using: i) odds ratio (OR) and their 95% confidence interval (CI) for categorical outcome variables (mild asthma vs. moderate/severe asthma), and ii) beta coefficients with their standard errors for continuous outcome variables (baseline lung function measurements). Statistical significance was defined by an alpha level of 0.05.

3. Results

3.1. Characteristics of study population

The socio-demographics characteristics for the study population are presented in Table 1. Distributions for most of the characteristics, including age, were similar between the two asthma severity groups ($p > 0.05$). When comparing respiratory symptoms and asthma severity indicators between the two groups, children with moderate/severe asthma were more likely to experience night cough, wheeze during exercise, have greater than 3 episodes of asthma, speech limitations, and were more likely to miss school days compared to children with mild asthma (Table 2). Other asthma indicators were not statistically significant between the two groups.

Baseline lung function differed significantly between severity groups when assessed either as absolute or percent predicted values (Table 3). Mean FVC, FEV₁, FEV₁/FVC, and FEF_{25%–75%} were all significantly lower in children with moderate/severe asthma compared to children with mild asthma.

3.2. Mean endotoxin and beta-(1 → 3)-D-glucan exposure levels among asthma severity groups

There were no statistically significant differences in mean (geometric) endotoxin levels between the two severity groups (Table 4). However, children with moderate/severe asthma had significantly lower play area beta-(1 → 3)-D-glucan concentration and load compared to the mild asthma group.

Table 1
Demographic characteristics of study population by asthma severity group.

	Mild Asthma (n = 88)	Moderate/ Severe Asthma (n = 28)	p-value
Mean age (± SD), years	11.0 (2.7)	10.9 (2.6)	0.78
Body mass index (± SD), kg/m ²	20.4 (4.7)	20.9 (5.8)	0.62
% Overweight	14.8	16.0	0.89 [†]
% Male	61.4	75.0	0.19
Ethnic background			
% Caucasian	84.1	73.1	0.24 [†]
% Others	15.9	26.9	
Physical activity			
% Low	3.4	3.6	0.96 [†]
% Moderate	35.2	32.1	
% High	61.4	64.3	
Parental education level			
% > high school (maternal)	81.6	88.9	0.56 [†]
% > high school (paternal)	76.5	88.0	0.21
Tobacco smoke exposure			
% Parental smoking	11.8	14.3	0.75 [†]
% Environmental tobacco smoke (ETS)	10.2	3.7	0.45
Home characteristics			
% Home with air filter	50.0	53.6	0.74
% Home with humidifier	28.4	32.1	0.71
% Pet ownership	59.1	57.1	0.86
% Dampness in the home	33.3	21.8	0.26
% Home with visible mold	22.2	8.4	0.08
Atopic sensitization			
% Atopic ^a	72.4	69.6	0.79
% Mold sensitization ^b	53.9	56.5	0.83
Family history			
% Parental history of asthma	35.2	32.1	0.78
% Parental history of allergy	59.1	64.3	0.63
Location of residence			
Urban	88.6	82.1	0.35 [†]
Rural	11.4	17.9	

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

^a Children were tested for atopic sensitization using a panel of standardized allergen extracts: cat, local grass, mold (*Aspergillus*, *Alternaria*, *Cladosporium*), and house dust mite. Subjects was considered atopic if a positive reaction to at least one of the applied allergens is raised ≥ 3 mm compared to the saline control.

^b Sensitization (positive skin prick test) to any of the three tested mold allergens (*Alternaria*, *Aspergillus*, or *Cladosporium*).

3.3. Associations between endotoxin and beta-(1 → 3)-D-glucan exposure levels and asthma severity and lung function measures

Mattress endotoxin concentration levels were significantly associated with increased risk of moderate/severe asthma in a dose response manner, independent of beta-(1 → 3)-D-glucan exposure levels (Table 5). This pattern was reversed for beta-(1 → 3)-D-glucan exposure levels. Play area beta-(1 → 3)-D-glucan concentration were significantly associated with reduced risk of moderate/severe asthma independent of endotoxin exposure levels.

To further investigate indicators of severity, we looked at the associations between endotoxin and beta-(1 → 3)-D-glucan exposure levels and frequency of wheeze in the past 12 months. In contrast to overall severity assessed by the NAEPP guidelines, higher levels of mattress beta-(1 → 3)-D-glucan concentration was significantly associated with more frequent wheeze (> 3 episodes) in the past 12 months (aOR = 7.58, 95% CI: 1.17–72.76) while no significant association was observed for endotoxin.

Table 6 presents the associations between endotoxin and beta-(1 → 3)-D-glucan exposures with lung function variables. Similar to the relationships between endotoxin and moderate/severe asthma, mattress endotoxin concentration levels were significantly associated with

decreased absolute values for FVC [β = −0.32, SE = 0.12) and FEV₁ (β = −0.27, SE = 0.12) after adjusting for potential confounders, including beta-(1 → 3)-D-glucan levels. No such association was observed for beta-(1 → 3)-D-glucan levels.

4. Discussion

This study demonstrated varied associations between indoor microbial exposure and asthma severity outcomes depending on the specific microbial agent. Endotoxin exposure increased the risk of moderate/severe asthma while beta-(1 → 3)-D-glucan showed inverse associations. Also, endotoxin levels were associated with lower lung function but the association was non-existent for beta-(1 → 3)-D-glucan. However, beta-(1 → 3)-D-glucan was associated with increased wheeze frequency. These results suggest that while endotoxin may be more consistently associated with adverse lung health outcomes, beta-(1 → 3)-D-glucan has also shown associations with some indicators of asthma severity. These differential patterns may be acting through different mechanisms or may reflect different roles as a possible causal agent versus trigger.

The most recent update from the Institute of Medicine (IOM) on the review of indoor environmental exposures and asthma exacerbation showed evidence of associations between endotoxin exposure levels and asthma severity [39]. In a cross-sectional study among schoolchildren with asthma (aged 6–13 years) in the USA, Rabinovitch et al. [40] demonstrated that endotoxin levels had significant positive associations with asthma severity indices (measured as asthma symptom scores severe enough to prevent play or sleep in children). However, in this earlier study, endotoxin levels were measured from dust obtained from personal exposure monitoring of schoolchildren versus vacuumed dust sample used in the current study. In the National Survey of Lead and Allergens in Housing study, also in the USA, Thorne et al. [17] reported that endotoxin levels in bedroom floor dust were significantly associated with increased frequency in the daily need for asthma medication. We add to the previous evidence by showing that high endotoxin levels significantly increased the risk of clinically defined moderate/severe asthma, further highlighting the potential clinical importance of microbial endotoxin exposure and asthma exacerbation in children with asthma.

We also demonstrated an inverse association between high play area beta-(1 → 3)-D-glucan levels and moderate/severe asthma. A Puerto Rican study among children (aged 6–14 years) showed positive associations between high beta-(1 → 3, 1 → 6)-D-glucan exposure and asthma severity (measured as ≥ 1 visits to the emergency department [ED]/urgent care for asthma) [41]. Another study among schoolchildren with asthma in the Netherlands (aged 7–11 years) also reported a significant association between high play area beta-(1 → 3)-D-glucan and increased peak expiratory flow (PEF) variability as an indicator of asthma severity [42]. While the results of our study are in contrast to these two previous studies, one observational study in Australia found beta-(1 → 3)-D-glucan to be significantly associated with increased FEV₁ as a measure of severity indicator [43]. A separate study in the USA also showed that high beta-(1 → 3)-D-glucan exposure level was inversely associated with frequency of recurrent wheezing in infants [44]. These inconsistencies between studies could be explained by methodological discrepancies and/or differences. For example, in the Puerto Rico study, parental reports of ED visits for asthma care were used as an asthma severity indicator while we used a combination of clinical symptoms and lung function variables based on NAEPP guidelines [3]. Also, the inflammatory potency of beta-(1 → 3)-D-glucan also appears to be strongly dependent on the type and conformation of glucans in house dust. While we assayed beta-(1 → 3)-D-glucan in the current study, Blatter et al. assayed beta-(1 → 3, 1 → 6)-D-glucan which has been reported to be a stronger inducer of inflammatory responses compared to beta-(1 → 3)-D-glucan [45].

Reasons for the observed inverse association between beta-(1 → 3)-

Table 2

Profile of respiratory symptoms, asthma severity indicators, and healthcare accessibility among the study population.

	Mild Asthma (n = 88)	Moderate/Severe Asthma (n = 28)	p-value
Respiratory symptoms			
% Wheeze past 12 months	39.3	88.5	< 0.001
% Night cough past 12 months	38.6	60.7	0.04
% Wheeze during/after exercise	65.9	89.3	0.02
% Sleep disturbance due to cough past 12 months	43.2	60.7	0.11
Asthma severity indicators past 12 months			
% > 3 asthma episodes	27.3	48.1	0.04
% > 3 wheeze episodes	29.5	42.3	0.28
% Speech limit to 1–2 words	6.8	21.4	0.04
School absenteeism			
% Missed school due to breathing problem past 12 months	28.9	38.5	0.01
Mean (± SD) number of school missed days per child in the past 12 months	1.0 (2.6)	3.3 (6.0)	0.07
Median number of school missed days per child in the past 12 months	0.0	2.0	0.002[‡]
Asthma medication			
% Prescribed breathing medication past 12 months	55.7	71.4	0.14
% Taking asthma medication for at least 2 days per week	23.9	35.7	0.22
% Prescribed antibiotics for respiratory infections past 12 months	39.8	50.0	0.34
Mean (± SD) number of time prescribed antibiotic per child per year	1.6 (0.9)	2.2 (1.8)	0.11
Median number of time prescribed antibiotic per child in the past 12 months	1.0	2.0	0.10 [‡]
Healthcare accessibility			
Time travelled to access basic healthcare (± SD), minutes	15.6 (19.9)	20.7 (21.3)	0.26
Time travelled to access emergency healthcare (± SD), minutes	14.4 (14.7)	18.8 (16.7)	0.21

SD: Standard deviation.

[†]Statistical difference assessed by the Fisher's exact test due to small cell sizes (expected values < 5) [37].[‡]Test performed with Mann-Whitney *U* test.**Table 3**

Comparison of lung function values between asthma severity groups.

	Mild Asthma (n = 88)		Moderate/Severe Asthma (n = 28)	
	Absolute Value ^a Mean (SD)	% Predicted of Normal Mean (SD)	Absolute Value ^a Mean (SD)	% Predicted of Normal Mean (SD)
FVC (L)	3.15 (0.05)	102.8 (13.0)	2.83 (0.10) [‡]	92.3 (13.0) [†]
FEV ₁ (L)	2.63 (0.05)	98.8 (12.9)	2.16 (0.09) ^{‡‡}	74.0 (9.1) ^{††}
FEV ₁ /FVC	0.84 (0.10)	95.8 (7.6)	0.77 (0.02) ^{‡‡}	87.6 (10.9) [†]
FEF _{25%–75%} (L)	2.82 (0.08)	89.8 (21.8)	2.01 (0.15) ^{‡‡}	64.2 (26.5) ^{††}

SD: Standard deviation; B: Baseline; L: Litre.

^{‡‡}*p* < 0.001, [‡]*p* < 0.05 (for absolute values); ^{††}*p* = 0.001, [†]*p* < 0.001 (for percent predicted values).^a Adjusted for age, sex, and height.

D-glucan and moderate/severe asthma in the current study are unclear but could be related to the airway inflammatory pattern that may be present in our subjects (eosinophilic or neutrophilic inflammation). For example, an inhalation challenge study in guinea pigs showed that repeated exposures to beta-(1 → 3)-D-glucan induced a significant increase in eosinophil counts without an increase in neutrophils [46]. While patients with severe asthma may differ from those with mild asthma in having higher eosinophil counts in their airways [47], studies by Wenzel et al. showed that not all severe asthma patients have airway eosinophilia [48] and that neutrophil counts are also higher than normal in the airways of subjects with severe asthma [49]. Unfortunately, no measurement of eosinophil and neutrophil counts was undertaken in the current study to investigate if the observed associations between beta-(1 → 3)-D-glucan and moderate/severe asthma was related to airway inflammatory patterns.

Another possible explanation could be related to indoor mold level alteration through remediation strategies such as cleaning. A randomized controlled trial in the US assessed the effects of indoor mold reduction through moisture remediation strategies on asthma exacerbation [50]. Compared to the controlled group, remediation group had a significantly lower rate of asthma exacerbation (measured as

Table 4

Geometric mean (GSD) of endotoxin and beta-(1 → 3)-D-glucan concentration and load in house dust from play area floor and mattresses by asthma severity status (n = 102).

	Mild Asthma (n = 78)	Moderate/Severe Asthma (n = 24)	p-value
Play area			
Endotoxin concentration (EU/mg)	58.7 (2.3)	49.2 (1.9)	0.64
Endotoxin load (EU/m ²)	23102.6 (2.4)	19559.2 (2.2)	0.41
Beta-(1 → 3)-D-glucan concentration (μg/g)	9.7 (2.0)	7.1 (2.0)	0.05
Beta-(1 → 3)-D-glucan load (μg/m ²)	157.4 (5.4)	61.1 (9.3)	0.03
Mattress			
Endotoxin concentration (EU/mg)	20.8 (2.4)	20.4 (2.3)	0.93
Endotoxin load (EU/m ²)	9610.4 (2.5)	9283.6 (2.8)	0.88
Beta-(1 → 3)-D-glucan concentration (μg/g)	4.6 (2.0)	4.5 (1.7)	0.90
Beta-(1 → 3)-D-glucan load (μg/m ²)	46.7 (4.1)	40.3 (5.0)	0.67

GSD: Geometric standard deviation.

frequency of asthma symptoms in days and ≥ 2 ED visit or ≥ 1 hospitalization for asthma past 12 months). In the current study, we observed borderline significant difference in report of indoor visible mold between moderate/severe and mild asthma group (8.4% vs. 22.2%, respectively; *p* = 0.08). Since significant associations have been reported between indoor visible mold [51], moldy odor [52], and culturable mold spores [51] with increased beta-(1 → 3)-D-glucan concentration levels in house dust, it is possible that children with moderate/severe asthma in our study may be cleaning their homes more intensely with a focus on mold reduction. Such a practice might have reduced beta-(1 → 3)-D-glucan exposure producing the observed inverse association with moderate/severe asthma due to the cross-sectional nature of the study design.

Finally, beta-(1 → 3)-D-glucan is positively associated with indoor relative humidity (RH) [51] with indoor beta-(1 → 3)-D-glucan concentration levels significantly higher in summer compared to winter period [21]. Dust sample collection for this study was completed

Table 5Multiple logistic regression analyses describing the associations between endotoxin and beta-(1 → 3)-D-glucan levels[†] and moderate/severe asthma^{††} (n = 102).

	Model I OR (95% CI)	Model II ^a aOR (95% CI)	Model III ^a aOR (95% CI)	Model IV ^{a,b} aOR (95% CI)
Play area				
Endotoxin concentration (EU/mg)				
Low	1.00	1.00	–	1.00
Medium	1.61 (0.53–4.88)	2.88 (0.61–13.47)	–	4.61 (0.77–27.82)
High	1.00 (0.31–3.24)	1.11 (0.21–5.73)	–	2.88 (0.44–19.07)
Endotoxin Load (EU/m ²)				
Low	1.00	1.00	–	1.00
Medium	0.86 (0.29–2.57)	1.00 (0.24–4.14)	–	3.23 (0.54–19.52)
High	0.72 (0.23–2.22)	0.60 (0.11–3.53)	–	1.96 (0.27–14.35)
Beta-(1 → 3)-D-glucan concentration (μg/mg)				
Low	1.00	–	1.00	1.00
Medium	0.39 (0.13–1.21)	–	0.15 (0.03–0.86)[‡]	0.15 (0.03–0.89)[‡]
High	0.39 (0.13–1.21)	–	0.17 (0.03–0.87)[‡]	0.16 (0.03–0.89)[‡]
Beta-(1 → 3)-D-glucan load (μg/m ²)				
Low	1.00	–	1.00	1.00
Medium	0.45 (0.14–1.40)	–	0.27 (0.06–1.29)[‡]	0.23 (0.04–1.20)
High	0.54 (0.18–1.63)	–	0.13 (0.02–0.75)[‡]	0.10 (0.02–0.72)[‡]
Mattress				
Endotoxin concentration (EU/mg)				
Low	1.00	1.00	–	1.00
Medium	1.49 (0.46–4.86)	4.40 (0.82–23.55)	–	7.05 (1.07–46.55)[‡]
High	2.10 (0.67–6.64)	5.36 (1.01–28.67)[‡]	–	11.40 (1.45–89.43)[‡]
Endotoxin Load (EU/m ²)				
Low	1.00	1.00	–	1.00
Medium	1.68 (0.52–5.39)	3.70 (0.76–17.90)	–	4.08 (0.78–21.32)
High	1.68 (0.52–5.39)	2.13 (0.44–10.36)	–	2.41 (0.44–13.09)
Beta-(1 → 3)-D-glucan concentration (μg/mg)				
Low	1.00	–	1.00	1.00
Medium	1.94 (0.62–6.14)	–	1.40 (0.31–6.34)	1.44 (0.30–6.94)
High	1.44 (0.44–4.70)	–	1.48 (0.36–6.11)	1.48 (0.30–7.22)
Beta-(1 → 3)-D-glucan load (μg/m ²)				
Low	1.00	–	1.00	1.00
Medium	0.74 (0.25–2.18)	–	1.78 (0.44–7.24)	1.77 (0.43–7.23)
High	0.51 (0.16–1.62)	–	1.08 (0.25–4.67)	0.99 (0.22–4.42)

[†]Low, medium, and high levels for endotoxin and beta-(1 → 3)-D-glucan were determined based on their corresponding tertile values of the exposure distribution, separately, for play and mattress areas: Low (1st tertile), Medium (2nd tertile), and High (3rd tertile).

^{††}Severity category based on the NAEPP guidelines.³ Mild asthma used as reference category.

[‡]*p* < 0.05.

Model I: Model with no adjustments for moderate/severe asthma; Models II: Adjusted model for moderate/severe asthma with endotoxin as an independent variable; Model III: Adjusted model for moderate/severe asthma with beta-(1 → 3)-D-glucan as an independent variable.

aOR: Adjusted odds ratio. Models II and III were adjusted for sex, age, parental smoke, home dampness, visible mold in home, asthma medication use, allergen sensitization, and location of residence.

^a Statistical comparisons between moderate/severe asthma and mild asthma were completed using logistic regression with GEE to account for clustering within families.

^b In addition to adjusted variables in Models II and III, Model IV was mutually adjusted for endotoxin or beta-(1 → 3)-D-glucan as appropriate. That is, model with play area endotoxin concentration as an independent variable was adjusted for play area beta-(1 → 3)-D-glucan concentration. Model with play area endotoxin load as an independent variable was adjusted for play area beta-(1 → 3)-D-glucan load. Similar procedure was performed for mattress endotoxin and beta-(1 → 3)-D-glucan.

between December 2015 and April 2016. As such, beta-(1 → 3)-D-glucan levels may be lower than levels required to induce adverse respiratory outcomes in the studied population due to the relatively colder, drier, and lower RH in winter compared to summer period [53]. Alternatively, beta-(1 → 3)-D-glucan compared to endotoxin may not be as an important measure of microbial activity in relation to childhood

asthma in this region of Canada due to the low RH.

We observed an increased risk of moderate/severe asthma with high mattress endotoxin concentration in the current study. While it may be argued that the above explanations for beta-(1 → 3)-D-glucan should also be applicable to endotoxin exposures, it should be noted that endotoxin is ubiquitous in nature and represents a measure of gram-

Table 6

Multivariate linear regression analyses^a describing the associations between endotoxin and beta-(1 → 3)-D-glucan levels[†] and lung function among children with asthma (n = 102).

	FVC β(SE)	FEV ₁ β(SE)	FEV ₁ /FVC β(SE)	FEF _{25%–75%} β(SE)
Play area^b				
Endotoxin concentration (EU/mg)				
Low	Ref	Ref	Ref	Ref
Medium	−0.19 (0.11)	−0.17 (0.11)	0.01 (0.02)	−0.22 (0.22)
High	0.17 (0.12)	0.08 (0.13)	−0.01 (0.03)	0.02 (0.25)
Endotoxin Load (EU/m ²)				
Low	Ref	Ref	Ref	Ref
Medium	−0.14 (0.12)	−0.12 (0.12)	0.01 (0.02)	−0.15 (0.24)
High	0.20 (0.14)	0.19 (0.14)	0.02 (0.03)	0.20 (0.27)
Beta-(1 → 3)-D-glucan concentration (μg/mg)				
Low	Ref	Ref	Ref	Ref
Medium	0.16 (0.12)	0.19 (0.12)	0.02 (0.02)	0.28 (0.22)
High	0.13 (0.12)	0.04 (0.12)	−0.02 (0.02)	−0.03 (0.23)
Beta-(1 → 3)-D-glucan load (μg/m ²)				
Low	Ref	Ref	Ref	Ref
Medium	0.05 (0.13)	0.13 (0.13)	0.03 (0.03)	0.33 (0.24)
High	0.23 (0.14)	0.18 (0.13)	0.00 (0.03)	0.19 (0.24)
Mattress^b				
Endotoxin concentration (EU/mg)				
Low	Ref	Ref	Ref	Ref
Medium	−0.40 (0.12) [‡]	−0.32 (0.12) [‡]	0.00 (0.02)	−0.29 (0.24)
High	−0.32 (0.12) [‡]	−0.27 (0.12) [‡]	0.00 (0.02)	−0.25 (0.24)
Endotoxin Load (EU/m ²)				
Low	Ref	Ref	Ref	Ref
Medium	−0.18 (0.12)	−0.22 (0.12)	−0.02 (0.02)	−0.26 (0.22)
High	−0.14 (0.13)	−0.12 (0.12)	0.00 (0.02)	−0.12 (0.24)
Beta-(1 → 3)-D-glucan concentration (μg/mg)				
Low	Ref	Ref	Ref	Ref
Medium	0.01 (0.13)	−0.09 (0.12)	−0.03 (0.02)	−0.19 (0.23)
High	0.04 (0.13)	−0.06 (0.12)	−0.02 (0.02)	−0.12 (0.22)
Beta-(1 → 3)-D-glucan load (μg/m ²)				
Low	Ref	Ref	Ref	Ref
Medium	−0.00 (0.12)	−0.06 (0.12)	−0.02 (0.02)	−0.06 (0.22)
High	−0.08 (0.12)	−0.11 (0.12)	−0.01 (0.02)	−0.03 (0.22)

[†]Low, medium, and high levels for endotoxin and beta-(1 → 3)-D-glucan were determined based on their corresponding tertile values of the exposure distribution, separately, for play and mattress areas: Low (1st tertile), Medium (2nd tertile), and High (3rd tertile).

[‡]p < 0.05.

β(SE): Beta coefficient and standard error for the difference in lung function per levels of endotoxin or beta-(1 → 3)-D-glucan exposure.

^a Models adjusted for sex, age, height, parental smoking, home dampness, visible mold in home, allergen sensitization, asthma medication use, and location of residence.

^b In addition to adjusted variables, models were also mutually adjusted for endotoxin or beta-(1 → 3)-D-glucan as appropriate. That is, model with play area endotoxin concentration as an independent variable was adjusted for play area beta-(1 → 3)-D-glucan concentration. Model with play area endotoxin load as an independent variable was adjusted for play area beta-(1 → 3)-D-glucan load. Similar procedure was performed for mattress endotoxin and beta-(1 → 3)-D-glucan.

negative bacteria [12,52]. For these reasons, it has been suggested that endotoxin concentration in dust samples is not necessarily associated with mold exposures in contrast to beta-(1 → 3)-D-glucan which is a gram-positive cell wall of most fungi, including mold [52]. Additionally, beta-(1 → 3)-D-glucan is believed to be a less potent inducer of inflammatory reaction and respiratory symptoms than bacteria endotoxin [42]. Therefore, it is not surprising that endotoxin behaves differently than beta-(1 → 3)-D-glucan in their association with moderate/severe asthma in the current study.

Individuals with asthma appear to have a heightened response to acute pulmonary effects of endotoxin. In a controlled challenge study among adults, Kitz et al. [54] found a significant decrease in FEV₁ in those with asthma 90 min after inhalation of endotoxin extracts

compared to healthy controls. While the study showed the effects of acute exposure to endotoxin on lung function in adults, we complemented these results by looking at children with asthma in the current study. We found high mattress endotoxin levels were significantly associated with lower FVC and FEV₁ in children with asthma. These findings are also consistent with a previous study among schoolchildren (aged 6–18 years) in Canada where higher indoor endotoxin level was inversely associated with decreased FEV₁ [22] and greater diurnal PEF variability [55]. In another study in the US among schoolchildren (aged 6–13 years), personal endotoxin exposure was also associated with lower daily evening FEV₁ [40]. Results from our study further expanded the evidence in the IOM review [39] and showed that the adverse respiratory effects of endotoxin in children with asthma may be independent of other microbial exposure in the indoor environment such as beta-(1 → 3)-D-glucan. In addition, the effects may not depend on asthma severity status of a child, but that once children develop asthma, they are more likely to be sensitive to the acute inflammatory effects of endotoxin compared to those without asthma. Although we controlled for asthma medication use in our models, further studies are needed in children with poorly controlled asthma to validate the magnitude of lung function declines associated with long-term acute and/or chronic endotoxin exposure in the indoor environment.

We did not find any statistically significant associations between beta-(1 → 3)-D-glucan and lung function in the current study. However, in a separate analysis that assessed the relationships between indoor microbial exposures and asthma severity indicators, mattress beta-(1 → 3)-D-glucan concentration was significantly associated with report of more wheeze frequency (> 3 episodes) in the past 12 months. The positive association between beta-(1 → 3)-D-glucan concentration and increased episodes of wheeze (> 3 episodes) in the past year and its lack of association with lung function may suggest that, in children with asthma, beta-(1 → 3)-D-glucan exposure could be related to symptom exacerbation and not lung function impairment. Beta-(1 → 3)-D-glucan exposure might result in increased symptom severity but not enough to impair pulmonary function. Alternatively, it could be that beta-(1 → 3)-D-glucan levels in the homes of children involved in this study were lower than a level required to induce significant lung function impairment.

Limitations of our study should be considered. The participation rate experienced was low. We recruited participants from urban and rural locations and were frequently hard to reach, especially in the rural locations. This might have led to low statistical power as was seen in the case of some strong estimates found for play area endotoxin and mattress beta-(1 → 3)-D-glucan levels but lacking statistical significance in this study. Dust samples were collected at a single time-point. As such, it is possible that the current measured microbial exposure levels may not reflect the level of exposure present when the reported episode of asthma severity actually happened. However, we feel that the microbial exposure levels reported in the current study may reflect similar exposure patterns that were occurring at the time of event for two reasons. First, studies have suggested that a single dust sampling for endotoxin analysis showed little variation over time [56–58] and reflects longer-term exposure to microbial products for at least a 1 year period [59]. Second, while some studies might have reported seasonal variability in house dust levels and microbial components [21,60,61], we obtained similar results of associations between microbial exposures and asthma severity after additional adjustment for seasonality in our models. Finally, the dust extraction analysis procedure used in this study is specific in determining the water soluble (WS) fraction of beta-(1 → 3)-D-glucan [62] which may not represent the most potent fraction of beta-(1 → 3)-D-glucan compared to alkaline soluble (AS) fraction [63]. This extraction procedure may be one of the reasons beta-(1 → 3)-D-glucan levels in the current study were lower in contrast to levels obtained in studies focusing on the AS fraction of beta-(1 → 3)-D-glucan [14,42,44,64]. Furthermore, in addition to small sample size,

the lower levels of the WS fraction of beta-(1 → 3)-D-glucan may also explain the lack of association seen in the case of strong estimates found for mattress beta-(1 → 3)-D-glucan levels and asthma severity in this study. However, while the AS fraction of beta-(1 → 3)-D-glucan may represent the most potent fraction of beta-(1 → 3)-D-glucan [62] and has been the most commonly investigated fraction of beta-(1 → 3)-D-glucan [14,42,44,64], we have further demonstrated, in this study, that the WS soluble fraction of beta-(1 → 3)-D-glucan could also worsen respiratory symptoms in children with preexisting asthma conditions.

Our study also has several strengths. We used objective measures for exposure and outcome assessments and recommended guidelines to define asthma severity [3], thus limiting the possibility of bias. Furthermore, clinical data and dust samples were collected by trained technicians using standardized protocols [26,65]. Laboratory personnel were blinded to disease status of each child and to source of dust samples (play area or mattress dust). Finally, home dust collection was conducted concurrently with clinical data to eliminate possible bias in dust sampling which could have been influenced by the health status of the child.

In summary, we demonstrated that asthma severity in children is associated with indoor microbial exposure. Endotoxin increased the risk of moderate/severe asthma and worsened lung function in children with asthma while beta-(1 → 3)-D-glucan exposure was sufficient to exacerbate symptom severity but not enough to impair pulmonary function or induce moderate/severe asthma. These results are important as they help clarify the role of endotoxin and beta-(1 → 3)-D-glucan in childhood asthma morbidity, highlighting that endotoxin may have more detrimental effects on respiratory health outcome in children with asthma. This further supports the notion that asthma severity might not be associated with the same microbial exposures associated with asthma development. For examples, endotoxin [13,14,66] and beta-(1 → 3)-D-glucan [44,57,67] exposures may prevent asthma development but may also increase severity of existing asthma conditions; warranting the need for indoor microbial exposure avoidance in the management of childhood asthma.

Conflicts of interest

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

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