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ORIGINAL PAPER **Epidemiology of Allergic Disease**

Asthma and allergic symptoms in relation to house dust endotoxin: Phase Two of the International Study on Asthma and Allergies in Childhood (ISAAC II)

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Summary

Background Several studies have consistently reported inverse associations between exposure to endotoxin in house dust and atopy. With regard to the association between house dust endotoxin and asthma, the results are inconsistent.

Objectives To study the association between house dust endotoxin levels and respiratory symptoms and atopy in populations from largely different countries.

Methods Data were collected within the International Study on Asthma and Allergies in Childhood Phase Two, a multi-centre cross-sectional study of 840 children aged 9–12 years from six centres in the five countries of Albania, Italy, New Zealand, Sweden and the United Kingdom. Living room floor dust was collected and analysed for endotoxin. Health end-points and demographics were assessed by standardized questionnaires. Atopy was assessed by measurements of allergen-specific IgE against a panel of inhalant allergens. Associations between house dust endotoxin and health outcomes were analysed by logistic regression. Odds ratios (ORs) were presented for an overall interquartile range increase in exposure.

Results Many associations between house dust endotoxin in living room floor dust and health outcomes varied between countries. Combined across countries, endotoxin levels were inversely associated with asthma ever [adjusted OR (95% confidence interval (CI)) 0.53 (0.29–0.96) for endotoxin levels per m^2 of living room floor] and current wheeze [adjusted OR (95% CI) 0.77 (0.64–0.93) for endotoxin levels per gram of living room floor dust]. There were inverse associations between endotoxin concentrations and atopy, which were statistically significant in unadjusted analyses, but not after adjustment for gender, parental allergies, cat and house dust mite allergens. No associations were found with dust quantity and between endotoxin exposure and hayfever.

Conclusion These findings suggest an inverse association between endotoxin levels in living room floor dust and asthma in children.

Keywords allergy, asthma, children, endotoxin, house dust
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Introduction

Endotoxins are cell wall components of the outer membrane of gram-negative bacteria. They have strong immune-stimulatory and pro-inflammatory properties. In the last decade, a number of studies have consistently reported inverse associations between exposure to endotoxin in house dust and atopy in children and adults living in rural and non-rural environments [1–7]. With regard to hayfever, there is less evidence for an association with

house dust endotoxin levels. One study reported a statistically significant inverse relationship [2], while others did not find clear dose–response relationships [3, 4, 8]. The results of studies assessing the relationship between exposure to endotoxin and asthma and asthma-related symptoms are inconsistent. Two studies reported inverse associations between house dust endotoxin and (atopic) asthma [2, 9], while several other studies have shown that endotoxin in house dust is associated with an increased risk of asthma and asthma symptoms [8, 10–12] and with exacerbations of pre-existing asthma [13–15].

Geographic heterogeneity might explain part of the inconsistencies with regard to the association between endotoxin exposure and asthma in the studies mentioned above. Phase I of the International Study on Asthma and Allergies in Childhood (ISAAC) investigated the prevalence of symptoms of asthma, allergic rhinitis (AR) and eczema in 6–7- and 13–14-year-old children world-wide [16]. Phase Two of the ISAAC study [17] aims to gain further insight into the aetiology of asthma, AR and eczema by investigating potential risk factors and including objective markers. Within ISAAC Phase Two, information on respiratory symptoms including wheeze, hayfever and allergic rhinoconjunctivitis, and diagnosis of asthma was collected in children aged 8–12 years using standardized questionnaires. Measurements of allergen-specific IgE in serum were performed. In addition, in a limited number of centres, endotoxin was measured in living room floor dust samples and allergens were measured in mattress dust samples for a subset of the study population.

This gives us the opportunity to study the geographical variation of the association between endotoxin in living room floor dust and respiratory symptoms and atopy in six centres in five largely different countries including Albania and Italy, for which endotoxin levels, to our knowledge, have not been reported previously.

Materials and methods

Study design and population

The present study makes use of data that have been collected within the ISAAC Phase Two study, of which a detailed description is given elsewhere [17]. Data from six centres in five countries, for which living room floor dust samples have been collected and analysed for endotoxin, were included in the present study. These centres are: Tirana (Albania), Rome (Italy), Hastings (New Zealand), Linköping and Östersund (Sweden) and West Sussex (UK). In brief, house dust was collected from the homes of a sample of children with wheeze in the past year and a sample of children without recent wheeze selected from a general population survey of 8–12-year-old children within each study centre [17]. We aimed at $\geqslant 100$ children with wheeze and $\geqslant 100$ children without wheeze in the past year from every centre. Sampling schemes differed slightly between New Zealand and the European centres: in the European centres, all children with wheeze during the past 12 months and a random sample of children without wheeze were invited. In New Zealand, a random sample of children with wheeze and children without wheeze were invited and equal numbers of each were sampled. In all centres, one child per household was included. As a result of insufficient numbers of children with a history of wheeze in some centres and non-response, in most centres the aim of including 100 wheezers was not achieved. The actual number of participants varied from 49 (Rome) to 231 (Hastings), and the actual percentage of children with wheeze in the past year varied from 21% (Tirana) to 50% (Hastings).

Dust sampling and analysis

Dust samples were collected on filters from the participants' living room floors and mattresses according to a standardized protocol (available at http://www.iras.uu.nl, last updated on 31 March 2004) as described earlier [18]. Laboratory analysis of dust samples of the European centres took place at the Institute for Risk Assessment Sciences (Utrecht University, Utrecht, the Netherlands). Dust samples from New Zealand were analysed at the laboratory of the Wellington Asthma Research Group (Wellington School of Medicine and Health Sciences, Wellington, New Zealand). After weighing, the whole dust sample including the filter was extracted using Tween-20 and water and then analysed for endotoxin with a kinetic chromogenic Limulus amoebocyte lysate (LAL) test as described previously [18], using only one batch of LAL reagents per laboratory (Utrecht University: BioWhittaker, LAL lysate lot no. 1L676S, LPS standard lot no. 2L0090; Wellington Asthma Research Group: BioWhittaker, LAL lysate lot no. 2L130U, LPS standard lot no. 2L0510) (Walkersville, MD, USA). Endotoxin levels were expressed as endotoxin loads (endotoxin units, EU, per m^2 of living room floor) and concentrations (EU/g living room floor dust). Endotoxin samples with non-detectable endotoxin amounts ($N = 13$) were assigned a value of two-third of the lowest overall observed detectable value.

In all centres, major cat allergen (Fel d 1) and house dust mite (HDM) allergen *Dermatophagoides pteronyssinus* (Der p 1) were measured in mattress dust samples with enzyme immunoassays as described earlier [17]. Results are not discussed in this article, but we explored whether the associations between endotoxin and allergic symptoms and atopy were confounded by exposure to these allergens.

Questionnaire data

Standardized parental questionnaires on demographics, wheezing, AR and eczema identical to those used for children aged 6–7 years in ISAAC Phase One [19] were used.

Asthma ever was defined as a positive answer to the question 'Has your child ever had asthma?'. Hayfever ever was defined as a positive answer to the question 'Has your child ever had hay fever?'. As in previous analyses [20], current wheeze was defined as a positive answer to the question 'Has your child had wheezing or whistling in the chest in the past 12 months?' and current symptoms of allergic rhinoconjunctivitis were defined as positive answers to the questions: 'In the past 12 months, has your child had a problem with sneezing or a runny or blocked nose when he/she did not have a cold or the flu?' and, if yes, 'In the past 12 months, has this nose problem been accompanied by itchy watery eyes?'. Supplementary questionnaires dealt with a family history of atopic diseases.

Atopy

Subjects in the stratified sample provided a blood sample for measurement of allergen-specific IgE. Serum levels of IgE against a panel of inhalant allergens (Phadiatop; birch, timothy, mugwort and olive pollen; cat, dog, horse; HDMs *Dermatophagoides pteronyssinus* and *D. farinae*; *Cladosporium herbarum* and *Parietaria officinalis*) were analysed using the ImmunoCAP System (Phadia AB, Uppsala, Sweden) as described earlier [17] in Albania, Italy, Sweden and the United Kingdom. In New Zealand, allergen-specific IgE was measured for a panel of inhalant allergens (*D. pteronyssinus*, *D. farinae*, cat, dog, rye grass pollen and *Aspergillus fumigatus*) also using the ImmunoCAP System. For Albania, Italy, Sweden and the United Kingdom, atopy was defined as a level of at least 0.35 kU_A/L for the Phadiatop test. For New Zealand, atopy was defined as an allergen-specific IgE level of at least 0.35 kU_A/L for one or more of the allergens tested.

Statistical analysis

Distributions of amounts of sampled dust, endotoxin loads and concentrations were best described by a log-normal distribution. Therefore, means were expressed as geometric means (GM) and correlations were expressed as Pearson's correlation coefficients based on natural log-transformed data. Centre-specific associations between exposure variables and wheeze were analysed by standard logistic regression; associations between exposure and the other health end-points were analysed by a weighted logistic regression using the inverse of the selection probability as weights to account for the stratified sampling scheme by wheeze described elsewhere [21]. The analyses were performed with the SURVEYLOGISTIC procedure of the Statistical Analysis System 9.1 for Windows. Although the functional relationships between exposures and response variables (investigated by non-parametric loess smoothers [22] using the GAM function of SPlus version 6.0, Insightful Corporation, Seattle, WA, USA; data not shown) differed between centres and were not always linear, a common approach was used, allowing the combination of centre-specific estimates: amounts of dust and endotoxin loads were used as continuous exposure variables in logistic regression analyses after log transformation. Results are presented as centre-specific and combined (from meta-analyses) crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs). ORs were calculated using the same increment for all centres, defined as the overall interquartile range, which is 1.2, 1.7 and 1.4 for natural log-transformed amounts of dust, endotoxin loads and endotoxin concentrations, respectively, corresponding to factors 3.3, 5.4 and 4.2, respectively, on the original scale. A limited number of confounding variables [sex, history of parental allergic diseases, cat (Fel d 1) and HDM (Der p 1) allergen levels in mattress dust, and current smoking in the child's home] were included in the regression models. In case of heterogeneity of effects between countries ($P<0.10$), the random effects approach described in DerSimonian and Laird [23] was used to calculate combined ORs. Statistical significance was defined by a two-sided α-level of $\leqslant 5\%$.

Results

Study population

The study population consisted of a total of 840 children aged 9–12 years from six centres in five countries. The basic characteristics of the study population and frequency distributions of respiratory symptoms in relation to the study centre are presented in Table 1. In brief, in all but one centre the study population consisted of slightly more boys than girls. Frequencies of parental history of allergic disease, respiratory symptoms and atopy varied considerably between centres.

Amount of sampled dust and endotoxin levels

Amounts of sampled dust and endotoxin levels varied considerably between centres (Fig. 1). There was a 20-fold difference between the centres with the smallest and largest amounts of dust [GM 16 mg/m^2 (Rome) vs. 316 mg/m^2 (Linköping)], and a fivefold difference between the centres with the lowest and highest endotoxin loads [GM 684 EU/m^2 (Rome) vs. 3602 EU/m^2 (Östersund)] and concentrations [GM 6532 EU/g dust (Linköping) vs. 35 581 EU/g dust (Rome)]. Patterns of correlations between amounts of sampled dust, endotoxin loads and endotoxin concentrations were similar across centres (Table 2). Correlations between amount of dust and endotoxin loads and between endotoxin loads and endotoxin concentrations were moderate to high ($r=0.53$–0.80 and $r=0.60$–0.82), while there was no

Table 1. Frequency distribution of the basic characteristics of the study participants, respiratory symptoms and atopy

	Country (centre)											
	Albania (Tirana)		Italy (Rome)		New Zealand (Hastings)		Sweden (Linköping)		Sweden (Östersund)		UK (West Sussex)	
	n/*N*	(%)	*n*/*N*	(%)	*n*/*N*	(%)	*n*/*N*	(%)	*n*/*N*	(%)	*n*/*N*	(%)
Basic characteristics												
Male gender	60/120	(50.0)	26/49	(53.1)	123/231	(53.2)	61/105	(58.1)	103/188	(54.8)	83/147	(56.5)
Age in years (min–max)	9–12		9–10		10–11		10–12		9–12		10–12	
Parental history of allergies*	11/115	(9.6)	25/49	(51.0)	159/231	(68.8)	57/105	(54.3)	115/188	(61.2)	92/147	(62.6)
Current smoking at home	64/115	(55.7)	23/49	(46.9)	72/230	(31.3)	NA		NA		24/145	(16.6)
Respiratory symptoms												
Asthma ever	9/119	(7.6)	22/48	(45.8)	121/229	(52.8)	20/105	(19.0)	56/185	(30.3)	50/147	(34.0)
Current wheeze†	25/120	(20.8)	22/48	(45.8)	115/231	(49.8)	29/105	(27.6)	73/188	(38.8)	49/146	(33.6)
Hayfever ever	4/116	(3.4)	15/48	(31.3)	76/231	(32.9)	25/100	(25.0)	42/178	(23.6)	34/146	(23.3)
Current symptoms of allergic rhinoconjunctivitis	9/115	(7.8)	15/48	(31.3)	71/230	(30.9)	24/103	(23.3)	43/185	(23.2)	39/145	(26.9)
Atopy	17/82	(20.7)	25/43	(58.1)	126/223	(56.5)	31/91	(34.1)	82/178	(46.1)	56/126	(44.4)

*Asthma and/or hayfever and/or eczema.
†Current wheeze was the basis of the stratified sample selection.
NA, not available.

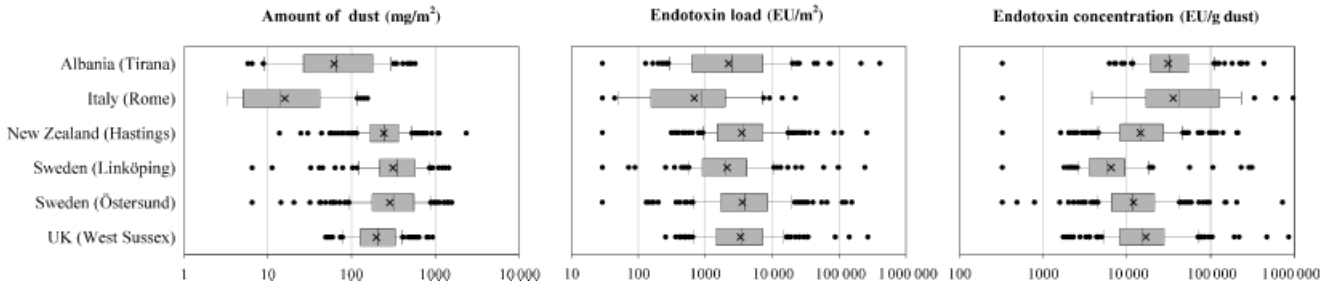

Fig. 1. Box plots of the amount of sampled dust, endotoxin load and endotoxin concentration by study centre. The box indicates the 25th and 75th percentiles of the distribution; the line within the box indicates the median. Whiskers indicate the 10th and 90th percentiles. Dots represent values outside the 10th and 90th percentiles of the distributions. Crosses represent geometric means.

correlation between amount of dust and endotoxin concentration (r = −0.11 to 0.13).

Associations between exposure to house dust and respiratory symptoms and atopy

Most centre-specific crude associations varied across centres (data not shown). Only a few associations were significant at the centre level probably due to the small sample sizes. Combined across centres, statistically significant ($P < 0.05$) inverse associations were found between the amount of dust sampled and asthma ever [OR (95% CI) 0.66 (0.47–0.93)]; between endotoxin load and asthma ever [OR (95% CI) 0.63 (0.46–0.87)]; and between endotoxin concentration and asthma ever [OR (95% CI) 0.79 (0.62–1.00)], current wheeze [OR (95% CI) 0.81 (0.70–0.93)] and atopy [OR (95% CI) 0.82 (0.69–0.96)]. Adjustment for mite (Der p 1) and cat allergens (Fel d 1) in mattress dust did not significantly alter the associations between amounts of dust sampled, endotoxin loads and concentrations and the health endpoints studied (data not shown). Changes in combined ORs

Table 2. Correlations between endotoxin load, endotoxin concentration and the amount of dust sampled

	Correlation		
Country (centre)	Amount of dust/ endotoxin load	Endotoxin load/ endotoxin concentration	Amount of dust/ endotoxin concentration
Albania (Tirana)	0.80*	0.62*	0.13
Italy (Rome)	0.71*	0.65*	−0.11
New Zealand (Hastings)	0.53*	0.82*	0.02
Sweden (Linköping)	0.72*	0.60*	−0.03
Sweden (Östersund)	0.71*	0.73*	0.10
UK (West Sussex)	0.53*	0.82*	0.00

*$P < 0.05$.

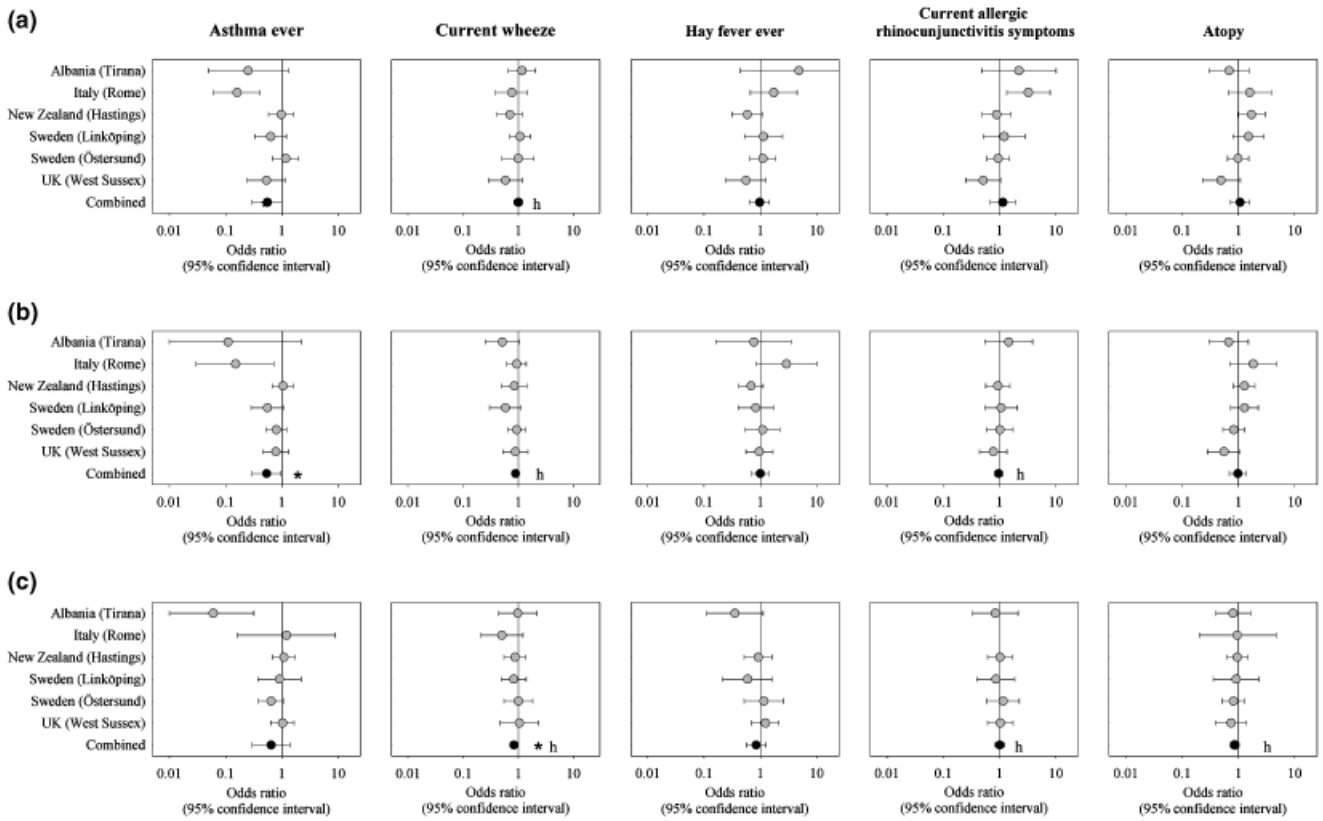

Fig. 2. Centre-specific and combined adjusted odds ratios (ORs) (95% confidence interval) for the associations between respiratory symptoms and atopy and natural log-transformed living room floor amounts of sampled dust (a), endotoxin loads (b) and endotoxin concentrations (c). All results are adjusted for gender, parental history of allergic disease, cat (Fel d 1) and house dust mite allergen (Der p 1) in mattress dust. ORs were calculated per overall interquartile range increase in exposure, which are 1.2, 1.7 and 1.4 for ln-transformed amounts of dust, endotoxin loads and endotoxin concentrations, respectively, corresponding to factors 3.3, 5.4 and 4.2, respectively, on the original scale. $^{*}P<0.05$. h, centre-specific effect estimates are homogeneous (P-value of test for heterogeneity of centre-specific estimates >0.1).

were $<10\%$ except for the associations between endotoxin loads and hayfever ever and current symptoms of allergic rhinoconjunctivitis, where the OR (95% CI) changed from 0.82 (0.66–1.02) to 1.12 (0.70–1.79) and from 1.25 (0.69–2.28) to 1.41 (0.68–2.93), respectively. The association between endotoxin concentrations and asthma ever became statistically non-significant [adjusted OR (95% CI) 0.85 (0.64–1.12)]. After additional adjustment for gender and parental allergies, the majority of the centre-specific associations still varied across centres and the combined effect estimates did not change much (Fig. 2). Some associations for hayfever and symptoms of allergic rhinoconjunctivitis were not estimable for Italy due to sample size limitations. The associations between endotoxin loads and asthma ever [adjusted OR (95% CI) 0.53 (0.29–0.96)] and between endotoxin concentrations and current wheeze [adjusted OR (95% CI) 0.77 (0.64–0.93)] remained statistically significant. The associations between the amount of sampled dust and asthma ever and between endotoxin concentrations and atopy became marginally statistically significant [adjusted OR (95% CI) 0.55 (0.30–1.00) and 0.85 (0.72–1.02), respectively, $P<0.10$] and the association between endotoxin concentrations and asthma ever became statistically non-significant [adjusted OR (95% CI) 0.64 (0.30–1.39)].

We additionally adjusted for current smoking in the child's home (data not shown), which was available for all but the Swedish centres. Changes in ORs were small ($<15\%$), except for asthma ever and current symptoms of rhinoconjunctivitis in the Albanian centre, most likely due to the small number of children with positive reports for these outcome variables, indicating little if any confounding effect of current smoking in the child's home. The combined ORs with and without adjustment for smoking for the four centres with information on smoking in the child's home are presented in Table 3.

For asthma ever, the associations with the amount of dust and endotoxin loads for the Albanian and Italian centres differed from the associations for the other centres. To evaluate the impact of the Albanian and Italian centres on the combined estimates, we re-calculated combined estimates excluding these two centres. The association between endotoxin loads and asthma ever became weaker, but remained statistically significant

Table 3. Combined adjusted odds ratios (ORs)* [95% confidence interval (CI)] for the associations between respiratory symptoms and atopy and natural log-transformed living room floor amounts of sampled dust, endotoxin loads and endotoxin concentrations with and without adjustment for smoking in the child's home

	Amount of sampled dust		Endotoxin load		Endotoxin concentration	
	OR (95% CI)†	OR (95% CI)‡	OR (95% CI)†	OR (95% CI)‡	OR (95% CI)†	OR (95% CI)‡
Asthma ever	0.41 (0.18–0.96)	0.39 (0.15–1.00)	0.40 (0.14–1.19)	0.39 (0.13–1.14)	0.56 (0.15–2.12)	0.44 (0.07–2.94)
Current wheeze	0.98 (0.72–1.32)	1.00 (0.74–1.36)	0.93 (0.76–1.15)	0.99 (0.80–1.22)	0.77 (0.60–1.00)	0.80 (0.61–1.05)
Hayfever ever	0.96 (0.45–2.04)	0.86 (0.43–1.72)	1.05 (0.57–1.94)	1.04 (0.59–1.84)	0.79 (0.41–1.53)	1.04 (0.59–1.84)
Current symptoms of allergic rhinoconjunctivitis	1.25 (0.54–2.93)	1.32 (0.51–3.38)	0.91 (0.74–1.12)	0.95 (0.77–1.18)	1.00 (0.79–1.27)	1.03 (0.81–1.30)
Atopy	0.99 (0.54–1.82)	1.05 (0.58–1.90)	0.95 (0.56–1.62)	1.00 (0.58–1.75)	0.87 (0.70–1.08)	0.88 (0.69–1.11)

*ORs were calculated per overall interquartile range increase in exposure, which are 1.2, 1.7 and 1.4 for ln-transformed amounts of dust, endotoxin loads and endotoxin concentrations, respectively, corresponding to factors 3.3, 5.4 and 4.2, respectively, on the original scale.

†Adjusted for gender, parental history of allergic disease, cat (Fel d 1) and house dust mite allergen (Der p 1) in mattress dust.

‡Additionally adjusted for smoking in the child's home.

Swedish centres were not included, because no smoking information is available.

[adjusted OR (95% CI) 0.79 (0.63–1.00)], whereas the association with the amount of dust sampled was no longer marginally statistically significant [adjusted OR (95% CI) 0.86 (0.67–1.10)].

To explore the role of atopy in the associations between endotoxin exposure, asthma ever and current wheeze, we created new outcome variables of atopic and non-atopic asthma and wheeze defined as having asthma/wheeze and being atopic and non-atopic, respectively. We found statistically significant associations with endotoxin loads for both atopic and non-atopic asthma. The effect was somewhat stronger for non-atopic asthma than for atopic asthma [combined crude OR (95% CI) 0.69 (0.49–0.96) for atopic asthma and 0.56 (0.33–0.96) for non-atopic asthma]. Likewise, the association with endotoxin concentrations was somewhat stronger for non-atopic wheeze than for atopic wheeze. However, it was statistically significant for atopic wheeze only [combined crude OR (95% CI) 0.83 (0.70–0.99) for atopic wheeze and 0.70 (0.27–1.83) for non-atopic wheeze].

Finally, we re-assessed the functional relationship between endotoxin exposure and the health outcomes studied with loess smoothers combined across centres, because centre-specific loess smoothers were inconclusive due to the relatively small numbers of participants per centre. The smoothing plots for asthma ever and current wheeze are presented in Fig. 3. Afterwards, the smoothed relationships were compared with linear fits by testing for a difference between the linear fit and the smoothed fit that includes both linear and smooth terms [24]. For none of the outcomes was this difference statistically significant. Thus, there was no indication of a non-linear association of the outcomes studied with endotoxin exposure.

Discussion

This study demonstrates statistically significant inverse associations between endotoxin levels in living room floor dust and asthma and asthma symptoms in children aged 9–12 years from six centres in five largely different countries. The associations between the amount of dust sampled and asthma ever, and between endotoxin concentrations and atopy were statistically significant in unadjusted but not in adjusted analyses. No effect of endotoxin or dust was observed for hayfever.

Adjusted associations between house dust endotoxin and asthma ever, but not between endotoxin and current wheeze, varied between centres. Differences between centres with regard to the frequency of atopy have been reported earlier [25] and might be one potential explanation for this finding. However, associations of endotoxin with asthma were also heterogeneous for the combined outcomes of atopic and non-atopic asthma and after stratification for atopy (data not shown), indicating that differences between centres are at least not entirely attributable to atopy. Another possible explanation for the heterogeneity between countries may be that house dust composition differs between centres. Microbial agents other than the ones measured in the present study, for instance extracellular polysaccharides, fungal β(1,3)-glucans and muramic acid, which have been found to be

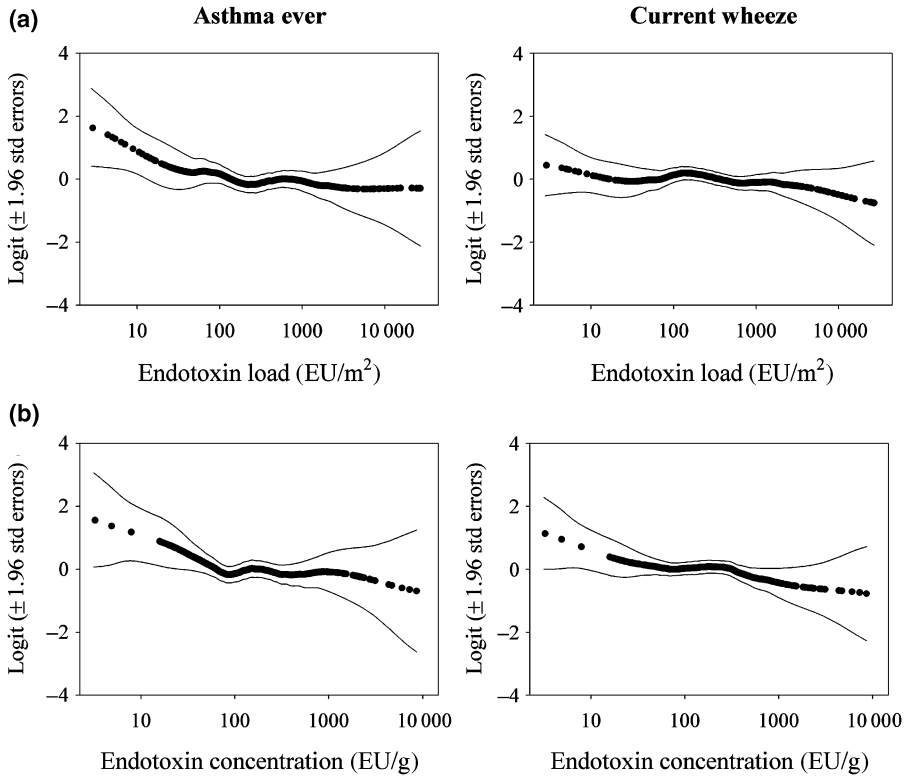

Fig 3. Adjusted loess smoothers (span = 0.6) with point-wise ±1.96 standard error bands for the association between endotoxin loads (a) and endotoxin concentrations (b) and asthma ever and current wheeze for all centres combined. Adjusted for centre, gender, parental history of allergic disease, cat (Fel d 1) and house dust mite allergen (Der p 1) in mattress dust. Results for asthma ever were additionally adjusted for current wheeze to account for the stratified sample selection.

associated with asthma [9] and (atopic) wheeze [9, 26, 27], may mediate a similar immunologic effect to endotoxin.

Our findings of an overall inverse association between exposure to house dust endotoxin and indicators of asthma are in agreement with the findings of the ALEX study [2], the PIAMA study [9] and the PARSIFAL study [26]. In the ALEX study, inverse associations were found between endotoxin loads and concentrations in mattress dust and atopic asthma of children aged 9.5±1.2 from farming and non-farming families in rural areas of Germany, Austria and Switzerland. In the Dutch PIAMA study, the lifetime prevalence of doctor-diagnosed asthma at age 4 years was significantly decreased in children with high levels of endotoxin in living room floor dust in comparison with children with low levels. In the PARSIFAL study, a protective effect of endotoxin on atopic wheeze was found in farm- and farm-reference children, but not in children attending Rudolf Steiner schools and their reference group [26]. However, after adjustment for farming, the endotoxin effect became statistically non-significant.

In contrast, several studies have shown that endotoxin in house dust is associated with an increased prevalence of asthma and exacerbations of pre-existing asthma. Thorne et al. [8] recently found that household endotoxin exposure significantly increases asthma prevalence in adults. Michel et al. [13, 14] showed that exposure to increased levels of house dust endotoxin is associated with an increased severity of allergic and non-allergic asthma (defined as increase in clinical symptoms, decrease in lung function and increase in medication use) in adults. The same was shown by Rizzo et al. [15], who found exposure to endotoxin associated with an exacerbation of asthmatic symptoms in mite allergic, asthmatic children. Several authors showed that exposure to elevated concentrations of endotoxin is associated with an increased risk of wheezing during the first year of life [10–12, 28]. Furthermore, significantly higher levels of endotoxin were found in living room floor dust (but not in bedroom floor or mattress dust) of English schoolchildren with asthma compared with matched healthy controls [29]. Beyond house dust, endotoxin has been recognized as an important factor in the aetiology of occupational lung diseases including (non-allergic) asthma caused by organic dust exposure [30].

Only a few of the studies mentioned here refer to the same age group as the present study. They refer to either young infants or adults. Results of the studies most comparable to the present one with regard to the participants' age are inconsistent. Two studies [15, 29] showed

positive associations, while the other two [2, 26] reported inverse associations between house dust endotoxin and atopic asthma/atopic wheeze. Age is therefore not likely to explain the conflicting results. Differences between studies with regard to the study population could also play a role: protective effects were mainly reported from studies including farming and non-farming children [2, 26]. However, in the ALEX study, a non-significant protective effect against atopic asthma was also present in the subgroup of non-farming children [2]. Furthermore, a protective effect was also seen in the PIAMA study [9], which does not focus on farming and non-farming children.

Differences with regard to the definition of the outcome variable are probably more important. In the ALEX study [2], the protective effect of endotoxin was limited to atopic asthma, while non-significant positive associations were reported for endotoxin and non-atopic asthma. Likewise, the PARSIFAL study [26] focused on atopic wheeze. In the present study, the number of atopic subjects with reports of asthma and wheeze and in particular the number of non-atopic subjects with reports of asthma and wheeze was small at the centre level. Analyses of atopic and non-atopic asthma and wheeze without adjustment for confounders were nevertheless possible for all centres. There was no indication of a stronger effect on atopic asthma and wheeze compared with non-atopic asthma and wheeze. Moreover, we found a statistically significant inverse association between endotoxin and atopic as well as non-atopic asthma, indicating that the association between endotoxin and asthma ever was not entirely mediated by an effect of endotoxin on atopy. However, the results should be interpreted with caution because of the small numbers of children with non-atopic asthma and wheeze.

The definition of endotoxin exposure might explain another part of the inconsistencies between the studies. Endotoxin exposure is typically expressed as endotoxin load (amount of endotoxin per m^2 of sampling surface) or endotoxin concentration (amount of endotoxin per gram of dust). Most studies presented associations with health end-points for one of the two parameters with no reference to the other. Two studies reporting associations for both parameters suggested stronger associations with endotoxin loads compared with endotoxin concentrations [2, 31]. In the present study, associations were also found to be different for endotoxin loads and concentrations. However, there was no consistent pattern of stronger associations for endotoxin loads compared with concentrations or vice versa. In some studies, the crude amount of dust has been used as another exposure parameter [9, 31]. The effect of the amount of dust could be partly attributable to endotoxin and markers of mould exposure in house dust, but could also reflect protective effects of microbial agents other than the ones measured. No significant associations with the amount of dust were found in the present study after adjustment for confounders.

Endotoxin measurements were performed in two different laboratories using the same extraction and assay protocols but with different batches of LAL reagents. Large inter-laboratory differences in measured endotoxin levels have been reported from a round-robin endotoxin study using a common extraction protocol, and part of these differences were thought to be attributable to the use of different LAL test kits [32]. Because no inter-laboratory comparison was included in the present study, we cannot rule out similar differences between the house dust endotoxin levels reported for New Zealand and those reported for the other centres. However, in recent comparison studies at the Utrecht laboratory – in which the same house dust extracts were repeatedly tested with different lot numbers of the LAL reagent and different *Escherichia coli* LPS standards – the differences between endotoxin levels measured on different days and with different LAL kits were relatively small [on average (95% CI) 1.29-fold (0.85–1.98)] compared with the range of LPS concentrations in the samples (data not shown). Because the endotoxin levels in the ISAAC study showed a wide range of values, we think that it is unlikely that our results were seriously biased by inter-laboratory differences. Nevertheless, because no inter-laboratory comparison was included in the present study, we performed additional analyses excluding New Zealand to assess the impact of inter-laboratory differences. Associations between endotoxin loads and asthma ever and between endotoxin concentrations and wheeze remained basically unchanged [OR (95% CI) 0.53 (0.29–0.96) with New Zealand and 0.46 (0.21–1.01) without New Zealand for asthma ever; 0.77 (0.64–0.93) with New Zealand and 0.75 (0.60–0.94) without New Zealand for current wheeze], indicating little if any impact of inter-laboratory differences on the observed associations. The inverse associations between exposure to endotoxin and hayfever [2] and atopy [1–3] in children reported from other studies could not be confirmed in the present study. The association between endotoxin concentration and atopy was statistically significant in unadjusted analyses and after adjustment for cat and HDM allergens in mattress dust, but became non-significant after additional adjustment for gender and parental allergies. Stronger associations between house dust endotoxin and atopy for higher cut-off values for atopy have been suggested [3, 5]. In the present study, using 0.7 k_AU/L as a cut-off value for atopy resulted in combined crude and adjusted ORs of 0.92 (0.78–1.09) and 0.92 (0.77–1.09), respectively, for endotoxin concentrations; using 3.5 k_AU/L as a cut-off value resulted in combined crude and adjusted ORs of 0.80 (0.53–1.23) and 0.82 (0.53–1.27), respectively, for endotoxin concentrations, indicating somewhat stronger effects for the highest cut-off.

A limitation of the present study might be the relatively small sample size per centre and the resulting low statistical power at the centre level. A stratified sampling approach of children with and without reports of wheeze for the last 12 months was chosen to increase statistical power with regard to this (main) outcome, but for the remaining outcomes the statistical power is limited. The number of children with reports of hayfever and symptoms of allergic rhinoconjunctivitis in Albania for instance was very low, and in the adjusted models, some associations for hayfever and symptoms of allergic rhinoconjunctivitis were not estimable for Italy due to sample size limitations. This might at least partly explain the absence of an association between exposure to house dust endotoxin and hayfever and atopy.

In conclusion, these findings suggest an inverse association between endotoxin in living room floor dust and asthma in children.

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