

Determinants of house dust, endotoxin, and β -(1→3)-D-glucan in homes of Danish children

Abstract Little is known about the geographic variation and determinants of bacterial endotoxin and β -(1,3)-D-glucan in Danish house dust. In a population of 317 children, we: (i) described loads and concentrations of floor dust, endotoxin, and β -(1→3)-D-glucan and (ii) their correlations and (iii) assessed their determinants; (iv) Finally, we compared our findings with previous European studies. Bedroom floor dust was analyzed for endotoxin content by the kinetic limulus amoebocyte lysate assay and for β -(1→3)-D-glucan by the inhibition enzyme immunoassay. The parents answered questions regarding potential determinants. We found: geometric means (geometric standard deviations) 186 mg/m² (4.3) for dust; 5.46×10^3 EU/m² (8.0) and 31.1×10^3 EU/g (2.6) for endotoxin; and 142 μ g/m² (14.3) and 0.71×10^3 μ g/g (7.3) for β -(1→3)-D-glucan. High correlations ($r > 0.75$) were found between floor dust and endotoxin and β -(1→3)-D-glucan loads, while endotoxin and β -(1→3)-D-glucan concentrations were moderately correlated ($r = 0.36$ – 0.41) with the dust load. Having a carpet was positively associated with dust load and with endotoxin and β -(1→3)-D-glucan concentrations. Pet keeping, dwelling type, and dwelling location were determinants of endotoxin concentrations. No other determinants were associated with β -(1→3)-D-glucan concentrations. Compared with other European studies, we found lower β -(1→3)-D-glucan loads and concentrations but higher endotoxin loads and concentrations suggesting a geographically determined different composition of Danish floor dust compared with other European regions.

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Practical implications

The type of flooring (hard-surface or textile) was the strongest determinant for loads of floor dust and concentrations of endotoxin and β -(1→3)-D-glucan. Endotoxin concentrations in Danish house dust might be higher, and β -(1→3)-D-glucan might be lower than concentrations seen in other Scandinavian countries. In future studies, this should be taken into account when comparing the health effects of domestic endotoxin and β -(1→3)-D-glucan concentrations across countries.

Introduction

In recent decades, an increasing prevalence of asthma and allergy in the Western world has been observed (Asher et al., 2006; Eder et al., 2006). Therefore, there has been an interest in potential indoor exposures related to these conditions. Special attention has been

paid to the potential effects of house-dust-associated endotoxin and β -(1→3)-D-glucan as relevant markers of indoor exposure. Endotoxin is a lipopolysaccharide, which is part of the outer membrane of mainly Gram-negative bacteria. β -(1→3)-D-glucan is part of most fungal cell walls, some bacteria, most higher plants, and many lower plants (awniczek-Waczyk and Górny,

2010). Both endotoxin and β -(1 \rightarrow 3)-D-glucan are ubiquitous in the indoor and outdoor environment and have been found to induce manifold effects on human health in terms of both adverse and beneficial health effects (Douwes, 2005; Liebers et al., 2008; Rylander, 1999). A positive association has been found between both the respiratory and general health of a group of pupils attending a school where high β -(1 \rightarrow 3)-D-glucan loads were present, as compared to a school with low loads (Rylander et al., 1998). Moreover, high β -(1 \rightarrow 3)-D-glucan loads have been associated with an increase in PEF variability in asthmatic children (Douwes et al., 2000) and persistent atopic asthma and new-onset bronchial hyper-responsiveness (Maheswaran et al., 2014). Further, it has been proposed that endotoxin increases the risk of wheezing in younger children, but may be a protective factor for asthma in older children (Litonjua et al., 2002; Mendy et al., 2011). Furthermore, endotoxin protects against hay fever, atopic asthma, and atopic sensitization (Braun-Fahrlander et al., 2002; Carlsten et al., 2011; Gehring et al., 2002, 2008). Still, other studies do not find any associations between endotoxin and β -(1 \rightarrow 3)-D-glucan and respiratory symptoms (Bertelsen et al., 2010; Rennie et al., 2008). The overall inconsistency in study findings raises uncertainty regarding the true human health effect of house-dust-associated endotoxin and β -(1 \rightarrow 3)-D-glucan. It should be noted though that this inconsistency may be due to differences between studies in the type of population or the timing of the dust measurement. However, the potentially adverse or beneficial health effects induced by endotoxin and β -(1 \rightarrow 3)-D-glucan emphasize the value of investigating the various associations of house dust components and the factors which may influence the levels of these components. This is important to achieve control of respiratory and allergic symptoms or, ideally, to prevent symptoms from occurring.

Previous studies have identified several determinants as being associated with both endotoxin and β -(1 \rightarrow 3)-D-glucan in domestic settings including: number of occupants in the home, flooring type, farming status, season, and heating system (Casas et al., 2013; Douwes et al., 1998; Giovannangelo et al., 2007b; Schram et al., 2005). Further, lack of cleanliness and presence of pets have been associated with higher endotoxin loads and concentrations (Chen et al., 2012; Dassonville et al., 2008; Gehring et al., 2004; Thorne et al., 2009; Wickens et al., 2003). Recently, attention has also been drawn to geographic variation in levels and determinants of domestic endotoxin and β -(1 \rightarrow 3)-D-glucan (Casas et al., 2013; Chen et al., 2012; Thorne et al., 2009). Southern European countries like Italy appeared to have higher concentrations of endotoxin in house dust than Northern European countries like Sweden and Iceland (Chen et al., 2012), while β -(1 \rightarrow 3)-D-glucan showed higher concentrations in houses of

a Finnish cohort as compared to the German and Dutch cohorts (Casas et al., 2013). This suggests that climatic conditions, such as temperature and relative humidity, building materials, or cultural differences in occupant behavior may be essential determinants of house-dust-associated domestic endotoxin and β -(1 \rightarrow 3)-D-glucan.

To our knowledge, only one study has been conducted in Denmark on domestic levels and determinants of endotoxin (Frankel et al., 2012). Based on daytime airborne dust sampling in five Danish homes, Frankel et al. found no associations between season, temperature, relative humidity, air exchange rate, and endotoxin loads. The study did not include β -(1 \rightarrow 3)-D-glucan and included only a few determinants. Therefore, to contribute to the European perspective on the subject, our study was designed to focus on house-dust-associated endotoxin and β -(1 \rightarrow 3)-D-glucan in homes of Danish children. The objectives of this study were: (i) to assess levels of house dust, endotoxin, and β -(1 \rightarrow 3)-D-glucan per m²-sampling area (load) and per gram of dust (concentration), (ii) to assess correlations between house dust, endotoxin, and β -(1 \rightarrow 3)-D-glucan loads and concentrations, (iii) to investigate determinants for loads and concentrations of house dust, endotoxin, and β -(1 \rightarrow 3)-D-glucan, and (iv) to compare our findings with those from previous European studies in different geographic regions based on a literature review.

Materials and methods

Study design and selection of homes

The present cross-sectional study was part of the Danish Mold in Buildings research program focusing on indoor environmental exposures and health effects in Danish school children. The selection of schools and pupils are described in detail by Ebbehøj et al. (2005). A total of 417 pupils aged 6–10 year (first to third grade) were invited to participate in the study, and 330 pupils were included (response rate 79%). The overall study was designed to examine both the school environment and dust from the children's home environment. Our study focused only on the latter. The study was approved by the Funen and Vejle Ethical Committees in Denmark.

Dust sampling

House dust from the children's bedroom floor was collected by the children's parents in February 1999 and from December 1999 through March 2000. Sampling instructions were given to the parents, in both verbally and written form, and sampling equipment was sent to the parent by ordinary mail. Settled house dust was sampled with the family's own

vacuum cleaner using sampling nozzles and filter boxes from ALK (Allergologisk Laboratorium, Copenhagen, Denmark). The parents were instructed to vacuum for 1 min in a 1 m² area from a rug or a wall-to-wall carpet preferentially. If the area of the rug was <1 m² or if no wall-to-wall carpet was present in the child's bedroom, the dust sample was taken from a smooth floor. After dust sampling, the parents closed the sample filter and nozzle box with a plastic lid and sealed it in a plastic bag to minimize contamination during transport. The filter was then returned directly to this study's examiners at a planned meeting. The response rate for returning the dust samples was 96% (317/330).

Gravimetric analysis

Filters were kept at constant temperature (22°C) and relative humidity (50%) for at least 16 h before being weighed at the National Research Center for the Working Environment (NRCWE, Copenhagen, Denmark). All visible objects like hair pins, pieces of paper or plastic, tiny screws or nails, etc. were removed from the dust before weighing. As filters had not been individually pre-weighed, the net amount of sampled dust was determined by subtracting the average weight of 50 non-used filters, which was 26.7 mg (s.d. = 0.04). Thus, possible errors introduced by this procedure were small compared with the mean (186 mg) and range of the 10th–99th percentile (20–1734 mg) in dust loads in the samples (see Results).

Dust extraction

House dust extraction was performed at NRCWE. Filters were transferred to 5–300 ml [volume depending on the total net dust weight, to reach a 0.2–3% (w/v) dust suspension] extraction fluid (pyrogen-free water with 0.05% Tween-20) and extracted at room temperature for 1 h as recommended by Douwes et al. (1995). After centrifuging (1000 g) for 15 min, 2 \times 1 ml of the supernatant was harvested and stored at –80°C until endotoxin analysis could be performed. Extraction tubes with the non-dissolved pellets and the remaining supernatant fluid were also frozen at 20°C.

Endotoxin analysis

The supernatant was analyzed in duplicate for endotoxin using a chromogenic kinetic Limulus Ameboecyte Lysate test (Lot number 9L1890; BioWhittaker, Inc., Walkersville, MD, USA) at NRCWE. A standard curve obtained from an *Escherichia coli* O55:B5 reference endotoxin was used to determine the concentration in terms of endotoxin units (EU) from which values for EU/sample, EU per m² sampled surface (endotoxin load), and EU/gram of dust (endotoxin concentration)

were calculated. All samples were above the detection level (endotoxin LOD = 0.005 EU/ml; corresponding with approximately 0.25–2.5 EU/g dust).

β -(1 \rightarrow 3)-D-glucan analysis

The frozen residues and remaining supernatants were sent on dry ice to the Institute for Risk Assessment Sciences (IRAS, Utrecht, the Netherlands) for further processing and β -(1 \rightarrow 3)-D-glucan analysis as described by Douwes et al. (1996). The dust residue pellets in the thawed tubes were dissolved by vigorous vortexing for 30–60 s, after which extraction was performed at 120°C. After cooling down to room temperature, tubes were centrifuged (15 min 2000 g) and supernatants harvested and frozen in aliquots at –20°C. The enzyme immunoassay (EIA) β -(1 \rightarrow 3)-D-glucan was assayed with the inhibition enzyme immunoassay with laminarin (Fluka Chemie AG, Buchs, Switzerland) as the calibration standard (Douwes et al., 1996). Although less sensitive than the commercially available and more expensive β -(1 \rightarrow 3)-D-glucan-specific LAL assay, this inhibition EIA has been shown to be sufficiently sensitive to detect and quantitate β -(1 \rightarrow 3)-D-glucan in the large majority of mattress and floor dust samples in several previous house dust analysis series from various European countries (Gehring et al., 2001; Giovannangelo et al., 2007b; Schram et al., 2005).

The second supernatant produced and tested thus contained a mixture of agents released during the first (endotoxin) extraction at room temperature and components like β -(1 \rightarrow 3)-D-glucans that are only solubilized by more aggressive treatment. Control experiments at IRAS have confirmed that the room temperature extracts are indeed devoid of β -(1 \rightarrow 3)-D-glucan immunoreactivity, while on the other hand, the presence of other soluble components released during the first step does not interfere with the β -(1 \rightarrow 3)-D-glucan reactivity in the inhibition EIA (data not shown).

The β -(1 \rightarrow 3)-D-glucan LODs were 0.8 μ g/ml, which given the dust suspension concentration of 0.2–3%, means a LOD of 4 μ g/m² for the β -(1 \rightarrow 3)-D-glucan load, and approximately 40–50 μ g/g dust for the β -(1 \rightarrow 3)-D-glucan concentration. For the 8% of β -(1 \rightarrow 3)-D-glucan-levels below the LOD, we assigned two out of three of the β -(1 \rightarrow 3)-D-glucan-LOD-value (Croghan and Egeghy, 2003).

Potential determinants

The children's parents reported the potential determinants of endotoxin and β -(1 \rightarrow 3)-D-glucan in their homes to the study's examiners who filled in a standardized questionnaire at a face-to-face meeting. Potential determinants were identified from the literature and consisted of the following characteristics: (i) *Occupant and animal characteristics*: number of

people in the household, crowding (people per square meter), pet keeping (yes/no), specific pets (cat, dog, rabbit, guinea pig, or other pets); (ii) *Dwelling characteristics*: type of dwelling (1 floor, 2–3 floors, apartment), residential area (50–100, 101–150, 151–200, >200 m²), location of dwelling (urban area, rural area, farm, or other than farm), heating (community, natural gas coal and oil, electricity, wood or other biomass, solar thermal, and soil), carpet or rug (yes/no); (iii) *Other characteristics*: indoor smoking (yes/no), number of cigarettes smoked per day (0, 1–9, 10–19, and ≥20 cigarettes), season of dust sampling (winter or spring); and (iv) *Home mold and dampness characteristics*: moisture damage within the last year (yes/no), action on elimination of moisture damage (yes/no), visible mold in the child's bedroom (yes/no), and inner-side window moisture in the child's bedroom (yes/no).

Statistical analyses

Levels of bedroom floor dust, endotoxin, and β -(1→3)-D-glucan were best described by a log-normal distribution [natural-log (ln)]. Levels of house-dust-associated endotoxin and β -(1→3)-D-glucan were described in terms of load (per m²-sampling area) and concentration (per gram of sampled dust) and were presented as geometric means (GM) with geometric standard deviations (GSD). Pearson's correlation coefficients were used to describe associations between endotoxin, β -(1→3)-glucan, and bedroom floor dust loads and concentrations. ANOVAs and *t*-tests were performed to describe associations with occupants, animals, housing, and home dampness characteristics in order to determine their individual effect as expressed in geometric values. All variables associated with floor dust, endotoxin, or β -(1→3)-D-glucan in the unadjusted analyses were included in the multivariate linear regression analyses based on a significance level of $P < 0.05$. Additionally, the number of people in the household, the presence of visible mold and dampness, and the type of heating system were included as potential determinants, as these factors have been identified as important determinants of dust and dust component levels in previous studies. Thus, we used the following model for the multivariate linear regression analyses: y (=floor dust ln(mg/m²) or endotoxin ln(EU/g) or β -(1,3)-D-glucan ln(μ g/g)) = α (intercept) + $\beta_1 \times$ (type of dwelling) + $\beta_2 \times$ (location of the dwelling) + $\beta_3 \times$ (carpet) + $\beta_4 \times$ (visible mold) + $\beta_5 \times$ (season) + $\beta_6 \times$ (number of people) + $\beta_7 \times$ (pet keeping) + $\beta_8 \times$ (indoor smoking) + $\beta_9 \times$ (community heating).

Multivariate results were presented as β -coefficients and 95% confidence intervals. The β -coefficients represent the estimated difference between mean ln-transformed floor dust load, endotoxin or β -(1→3)-D-glucan concentration values in the presence vs. absence of the specific determinant. A tendency was defined by a

P -value <0.1. Statistical analysis was conducted using Stata version 13 (StataCorp LP, College Station, TX, USA), and figures were developed using GraphPad Prism version 6 (GraphPad Software, Inc., San Diego, CA, USA).

Literature search

To allow comparisons of our findings with previous study findings, we performed a literature search through the MEDLINE database in October 2013. All papers containing the following terms in the title or abstract were identified: 'Determinants' or 'Predictors' or 'Housing characteristics' or 'Home characteristics' or 'Indoor' or 'Domestic' AND 'Endotoxin' or ' β -(1→3)-D-glucan' or 'Microbial agent' or 'Microbial component' or 'Microbial marker' or 'Dust'. This literature search identified 6854 publications. Further selection of articles was based on the following inclusion criteria: endotoxin or β -(1→3)-D-glucan in house dust, with a primary focus on determinants of endotoxin or β -(1→3)-D-glucan, European population studies, English language papers, and publications from the last 25 years. Thus, a review on the basis of 6 multicenter studies and 11 single-country studies was completed with synthesis of primarily statistically significant results of multivariate models.

Results

Loads, concentrations, and correlations of floor dust, endotoxin, and β -(1→3)-D-glucan

Table 1 shows the levels of floor dust, endotoxin, and β -(1→3)-D-glucan expressed in load (per m²) and concentration (per gram). The GM was 186 mg per m² (GSD = 4.3) for dust; 5.46×10^3 EU/m² (GSD = 8.0) and 31.1×10^3 EU/g of dust (GSD = 2.6) for endotoxin; and $142 \mu\text{g}/\text{m}^2$ (GSD = 14.3) and $0.71 \times 10^3 \mu\text{g}/\text{g}$ of dust (GSD = 7.3) for β -(1→3)-D-glucan. High positive correlations were found between loads of dust and both endotoxin and β -(1→3)-D-glucan ($r = 0.85$ and $r = 0.78$), while concentrations of endotoxin and glucan were moderately correlated with the dust load ($r = 0.36$ and $r = 0.41$). Endotoxin and β -(1→3)-D-glucan were also significantly correlated: moderate for concentrations ($r = 0.43$, $P < 0.001$) and high for load ($r = 0.79$, $P < 0.001$) (data not shown). To evaluate whether having a carpet or rug in the child's bedroom could potentially bias the result, we carried out an additional correlation analysis in separate strata which showed that having a carpet or a rug had no evident influence on the correlations (data not shown).

Determinants for levels of floor dust, endotoxin, and β -(1→3)-D-glucan

Unadjusted analyses. Results of the unadjusted analyses of potential determinants for levels of floor dust,

Table 1 Load, concentration, and correlations of endotoxin, β -(1,3)-D-glucan, and floor dust

	<i>n</i>									Detectable (%)
Floor dust	317									—
Endotoxin	317									100
β -(1,3)-D-glucan	292									92 ^a
Load (per m ²)										
	Percentile									
	GM	(GSD)	10th	25th	50th	75th	90th	95th	99th	<i>r</i>
Floor dust, mg	186	4.3	20	70	275	540	890	1335	1734	} -0.85*
Endotoxin, 10 ³ EU	5.46	8.0	0.23	1.54	8.74	23.3	45.8	93.4	159	
β -(1,3)-D-glucan, μ g	142	143	6	40	296	860	1903	2682	5234	
Concentration (per gram)										
	Percentile									
	GM	(GSD)	10th	25th	50th	75th	90th	95th	99th	<i>r</i>
Floor dust, mg	—	—	—	—	—	—	—	—	—	} -0.36**
Endotoxin, 10 ³ EU	31.1	2.6	0.10	33.2	68.8	144	280	329	366	
β -(1,3)-D-glucan, 10 ³ μ g	0.71	7.3	0.09	0.50	1.30	2.10	3.70	5.17	6.77	

n, numbers; GM, geometric mean; GSD, geometric standard deviation; 10th, 25th, 50th, 75th, 90th and 95th percentiles in geometric values.

r denotes Pearson's correlation coefficient for correlations between the amount of floor dust in mg and microbial markers per m² and per gram of dust.

*For β -(1→3)-D-glucan-levels below the LOD, we used two out of three of the β -(1→3)-D-glucanLOD-value.

***P*-value <0.01.

endotoxin, and β -(1→3)-D-glucan can be seen in Tables 2 and 3. Higher dust loads were measured in bedrooms with a carpet and in winter sampling. On the other hand, a lower dust load was observed in homes where more than 20 cigarettes were smoked per day, compared to homes with no presence of cigarette smoking.

Significantly higher endotoxin concentrations were seen in homes with pets. The subanalyses showed that keeping a dog or a guinea pig was associated with higher endotoxin concentrations. Moreover, higher endotoxin concentrations were associated with living in an apartment or dwelling with 2–3 floors and with living on a farm. In addition, higher endotoxin loads were associated with indoor smoking of 1–9 cigarettes per day, and both higher endotoxin loads and concentrations were associated with having a carpet and winter sampling.

Higher β -(1→3)-D-glucan loads and concentrations were found in the presence of a carpet or a rug, and β -(1→3)-D-glucan loads were higher when dust was sampled during winter. Loads and concentrations of β -(1→3)-D-glucan were lower in homes which had experienced a moisture damage the last year and the point at which action had been taken to eliminate the moisture damage. Additionally, an expected high correlation was observed between having had moisture damage in the home within the last year and when action had been taken on eliminating the moisture damage ($\rho = 0.77$, $P < 0.00$).

Multivariate analyses. The results of the multivariate linear regression analyses are shown in Figure 1. Overall, the percentage of variability explained by the determinants was low: floor dust ($R^2_{\text{adj}} = 11.0\%$), endotoxin ($R^2_{\text{adj}} = 13.4\%$), and β -(1→3)-D-glucan ($R^2_{\text{adj}} = 4.3\%$). We tried to optimize the models by a forward inclusion of determinants. However, this only resulted in a minor improvement of the model performance. Further, the determinants of floor dust, endotoxin and β -(1→3)-D-glucan levels and the percentages of explained variability were very similar regardless of whether loads or concentrations were analyzed (data not shown). Concentration results may be considered more valid than load results in that the load may be more sensitive to the total amount of dust collected and the accuracy of the collection area. Therefore, concentration results are only presented.

Overall, the multivariate analyses showed the same significant associations between determinants and both dust and endotoxin concentrations as in the unadjusted analyses, with the exception of season of dust sampling and indoor smoking, which had only borderline significance for these two outcomes. Having a carpet or a rug was the only determinant which was significantly and positively associated with both floor dust loads and endotoxin and β -(1→3)-D-glucan concentrations with a ln- β -coefficient (95%CI) for floor dust load = 0.77 (0.42–1.12); ln- β -coefficient (95%CI) for β -(1→3)-D-glucan concentration = 0.45 (0.23–0.68); and ln- β -coefficient (95%CI) for endotoxin concentration = 0.63

Table 2 Descriptions of floor dust, endotoxin, and β -(1,3)-glucan in relation to the potential determinants: occupant, animal, housing, mold, and dampness characteristics

		Floor dust		Endotoxin				β-(1,3)-glucan			
		GM	GSD	GM	GSD	GM	GSD	GM	GSD	GM	GSD
n (%)		mg/m ²		10 ³ EU/m ²		10 ³ EU/g		μg/m ²		10 ³ μg/g	
Occupant and animal characteristics											
Number of people in the household ^a											
2 people (ref)	14 (4)	215	4.3	8.82	6.5	41.0	1.9	272	3.7	1.30	2.6
3 people	33 (10)	152	5.1	4.70	9.8	30.8	2.7	146	10.6	0.82	6.0
4 people	161 (52)	179	4.0	4.82	8.2	29.6	2.7	131	13.9	0.67	7.8
>4 people	109 (34)	205	4.4	6.49	7.4	33.9	2.6	146	18.6	0.69	7.5
Pet keeping											
Any pets ^b											
No (ref)	87 (27)	174	5.0	4.58	10.7	26.3	2.8	154	17.6	0.74	9.5
Yes	230 (73)	191	4.0	5.84	7.1	33.9	2.5*	137	13.3	0.70	6.5
Type of pet ^{b,c}											
Dog	99 (31)	174	3.9	6.24	6.4	38.5	2.4*	132	13.3	0.71	6.7
Cat	107 (34)	200	3.9	6.74	6.8	37.7	2.4	153	12.7	0.72	6.8
Rabbit	55 (17)	208	3.9	5.19	8.8	29.6	2.9	136	12.2	0.66	6.2
Guinea pig	27 (9)	226	3.6	10.5	5.4	49.3	2.3*	183	2.4	0.78	4.9
Other pets	95 (30)	194	4.3	6.73	6.8	37.0	2.5	153	12.9	0.75	6.1
Housing characteristics											
Type of dwelling ^a											
1 floor (ref)	147 (46)	190	3.9	4.5	8.3	26.5	2.8	131	14.9	0.67	7.7
2–3 floors	123 (39)	166	4.8	5.83	7.5	36.8	2.2**	126	17.6	0.67	8.4
Apartment	47 (15)	232	3.9	8.45	8.0	36.4	2.8*	247	6.2	1.02	3.4
Residential area ^a											
50–100 m ² (ref)	42 (13)	229	4.2	8.72	7.6	38.0	2.3	223	8.6	0.87	5.1
101–150 m ²	124 (39)	163	4.4	4.15	8.9	27.7	2.9	113	14.5	0.63	7.4
151–200 m ²	90 (29)	219	3.8	7.00	6.2	32.0	2.7	181	15.3	0.86	6.8
>200 m ²	61 (19)	162	4.7	4.77	8.8	35.9	2.2	114	17.2	0.60	9.4
Location ^b											
Urban (ref)	251 (76)	183	4.3	4.93	8.6	28.9	2.6	173	6.0	0.68	7.5
Rural (non-farm)	55 (17)	227	3.8	7.42	5.9	33.2	2.5	227	4.5	0.70	7.4
Farm	24 (7)	135	4.8	7.51	6.7	55.5	2.2**	179	7.2	1.04	4.6
Heating ^{b,c}											
Community	180 (57)	201	4.2	5.95	7.7	31.3	2.6	159	12.2	0.71	6.5
Natural gas, coal, and oil	93 (29)	158	4.4	4.57	8.6	30.2	2.9	119	19.0	0.74	8.9
Electricity	14 (4)	268	3.5	9.53	4.8	37.3	2.1	287	7.3	1.07	3.1
Wood or other biomass	93 (29)	165	4.7	4.61	8.9	35.0	2.2	124	14.1	0.70	7.9
Solar thermal and soil	3 (1)	115	1.8	4.13	2.7	36.0	1.5	128	5.9	1.12	3.4
Carpet or rug ^b											
No (ref)	93 (30)	105	4.4	2.33	8.2	23.2	3.1	55	15.3	0.46	9.2
Yes	223 (70)	234	3.9**	7.74	7.2**	36.0	2.4**	210	12.7**	0.85	6.4*
Home mold and dampness											
Moisture damage (within the last year) ^b											
No (ref)	285 (90)	194	4.0	5.78	7.9	32.3	2.6	156	13.1	0.78	6.5
Yes	31 (10)	117	6.2	3.07	8.7	26.2	2.4	52	25.5*	0.30	14.4*
Action on elimination of moisture damage ^b											
No (ref)	296 (93)	191	4.1	5.65	7.8	32.0	2.6	154	12.9	0.78	6.4
Yes	21 (7)	128	7.4	3.42	10.1	26.7	2.5	42	41.1*	0.18	19.5*
Visible mould in child's bedroom ^b											
No (ref)	295 (93)	185	4.3	5.46	8.2	32.0	2.6	142	14.8	0.72	7.4
Yes	22 (7)	233	3.6	5.51	5.9	29.3	2.2	139	9.7	0.62	5.4
Inner-side window moisture on child's bedroom ^b											
No (ref)	225 (71)	175	4.5	4.94	8.9	30.9	2.8	126	14.8	0.70	7.8
Yes	92 (29)	220	3.8	7.03	5.8	33.6	2.2	189	13.0	0.80	6.1

n, numbers; GM, geometric mean; GSD, geometric standard deviation; ref, reference group.

^aANOVA test for differences in ordinal categorical variables in levels of floor dust and microbial markers.

^bChi-squared test for differences in binary categorical variables in levels of floor dust and microbial markers.

^cDifferences in levels of the subgroup answering yes and the reference group answering no were tested. Answering no did not foreclose answering yes to the other subcategories.

P*-value <0.05; *P*-value <0.01 compared with reference group.

Table 3 Descriptions of floor dust, endotoxin, and β -(1,3)-glucan in relation to the potential determinants: indoor smoking and season

		Floor dust		Endotoxin				β-(1,3)-glucan			
		GM	GSD	GM	GSD	GM	GSD	GM	GSD	GM	GSD
<i>n</i> (%)		mg/m ²		10 ³ EU/m ²		10 ³ EU/g		μg/m ²		10 ³ μg/g	
<i>Indoor smoking</i>											
<i>Any^a</i>											
No (ref)	196 (62)	165	4.2	4.79	7.4	29.2	2.6	116	13.7	0.64	6.9
Yes	121 (38)	226	4.3	6.75	8.9	36.1	2.6	195	15.1	0.83	7.7
<i>Mother smokes^a</i>											
No (ref)	228 (72)	176	4.3	5.24	7.8	30.8	2.6	127	14.2	0.64	7.1
Yes	79 (25)	201	4.3	6.09	8.7	32.6	2.6	190	14.5	0.92	7.5
<i>Father smokes^a</i>											
No (ref)	239 (75)	180	4.2	5.21	7.8	30.2	2.6	130	14.5	0.68	7.2
Yes	78 (25)	207	4.6	6.33	8.6	36.9	2.7	183	13.9	0.82	7.3
<i>Amount of cigarettes^b</i>											
0 cigarettes (ref)	200 (63)	167	4.2	4.83	7.7	29.6	2.6	114	14.8	0.62	7.5
1–9 cigarettes	50 (16)	272	3.9	11.7	5.1*	38.9	1.9	339	8.9	1.26	2.9
10–19 cigarettes	48 (15)	232	4.4	5.02	13.1	31.3	3.3	151	18.8	0.62	10.4
≥20 cigarettes	19 (6)	102	4.9*	3.21	6.3	35.7	2.3	120	11.9	0.98	9.9
<i>Other characteristics</i>											
<i>Season of examination^a</i>											
Winter	203 (64)	215	4.0**	7.14	6.8*	35.2	2.4*	181	12.5*	0.80	5.7
Spring (ref)	114 (36)	144	4.6	3.42	9.8	26.1	2.9	92	17.3	0.58	10.3

n, numbers; GM, geometric mean; GSD, geometric standard deviation; ref, reference group.

^aChi-squared test for differences in binary categorical variables in levels of floor dust and microbial markers.

^bANOVA test for differences in ordinal categorical variables in levels of floor dust and microbial markers.

P*-value <0.05; *P*-value <0.01 compared with reference group.

(0.14–1.12). Furthermore, the percentage of variability explained by carpeting was 6.4% for floor dust load, 1.7% for β -(1→3)-D-glucan concentration, and 4.2% for endotoxin concentration. Neither the number of people living in the home, crowding, community heating, number of cigarettes smoked, visible moisture, and mold nor other mold-related determinants were significantly associated with dust load and endotoxin and β -(1→3)-D-glucan concentrations. Furthermore, keeping pets in the home was not associated with neither dust load nor β -(1→3)-D-glucan concentrations, but was significantly associated with higher endotoxin concentrations. Although the associations were not significant, a tendency toward higher floor dust load and β -(1→3)-D-glucan concentration was found in homes with indoor smoking and in apartments when compared to one-floored dwellings. Further, a tendency was observed for higher β -(1→3)-D-glucan concentrations at farms compared with rural or urban non-farm homes, and a tendency of lower dust loads and endotoxin and β -(1→3)-D-glucan concentrations was seen for homes with more than two occupants.

European population studies on levels and determinants of endotoxin and β -(1→3)-D-glucan house dust levels

Tables 4 and 5 show results from European epidemiological studies on levels and determinants for loads and concentrations of endotoxin and β -(1→3)-D-glucan in house dust. Most studies focused on

endotoxin (*n* = 15), and only five studies investigated β -(1→3)-D-glucan. Among the studies, differences in loads and concentrations were large, especially for endotoxin with concentrations ranging from 0.89 to 81.8×10^3 EU/g and β -(1→3)-D-glucan concentrations ranging from 0.18 to 3.00×10^3 μg/g. Studies in rural areas, including farms, found higher endotoxin concentrations than in urban populations (Schram et al., 2005; Waser et al., 2004). In general, the sampling location in the home influenced the loads and concentrations of endotoxin and β -(1→3)-D-glucan, with the lowest levels found in mattress dust, higher levels found in bedroom floor dust, and the highest levels found in living room floor dust. German, French, and Swedish studies analyzed bedroom floor dust collected using a vacuum sampling method similar to that used for our study (Barnig et al., 2012; Douwes et al., 1998; Moniruzzaman et al., 2012). The German and Swedish studies found lower endotoxin loads and concentrations, but higher β -(1→3)-D-glucan loads and concentrations than in our study (Douwes et al., 1998; Moniruzzaman et al., 2012). The endotoxin concentrations in our study were found to be more than fourfold higher than in these other studies (Barnig et al., 2012; Douwes et al., 1998; Moniruzzaman et al., 2012). The β -(1→3)-D-glucan concentrations were as much as two- to threefold lower in our study compared with results from multicenter studies that used a similar sampling method, but a dif-

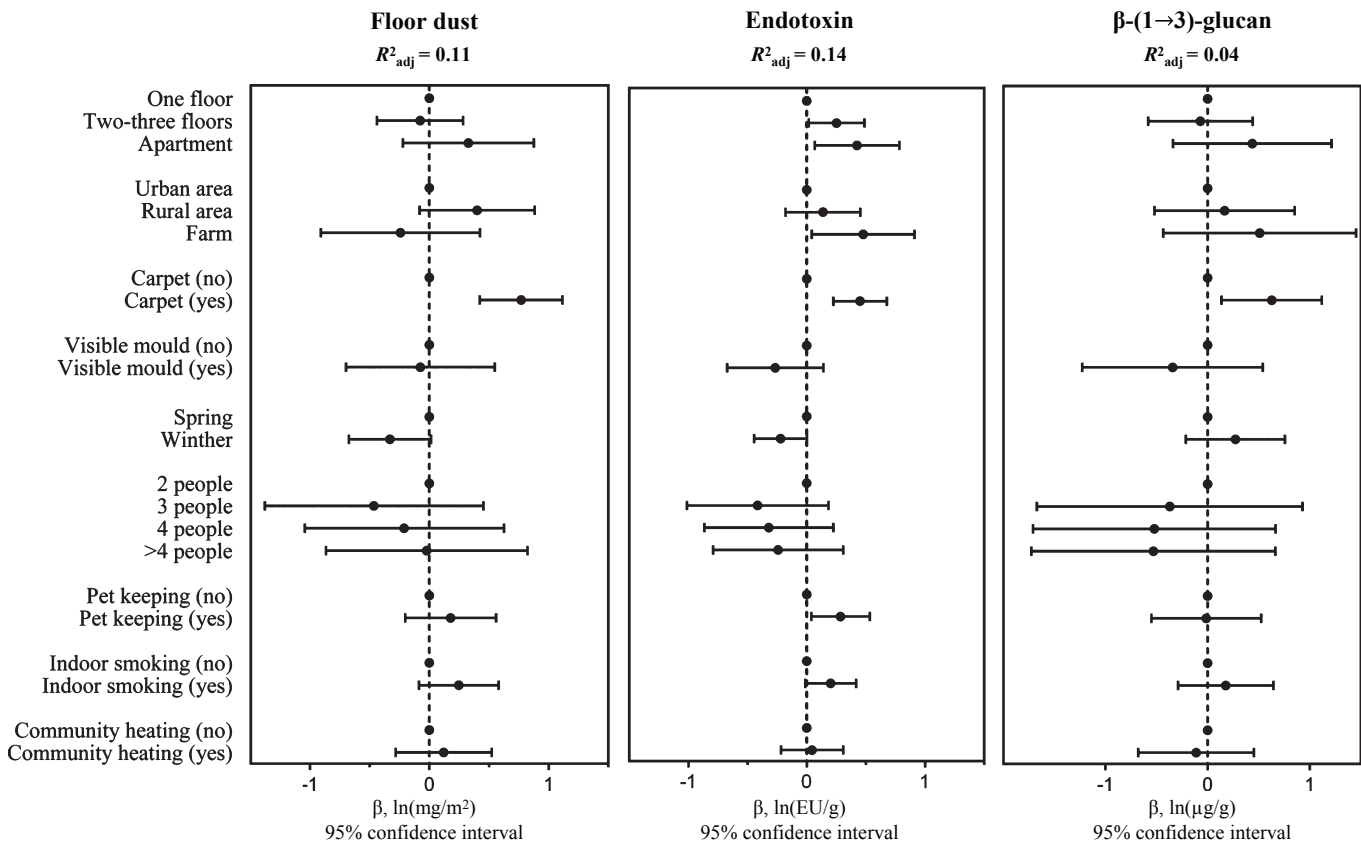


Fig. 1 Multiple linear regression results on the association between potential determinants and loads of floor dust, and concentrations of endotoxin and β -(1,3)-D-glucan. Analyses are adjusted for: type of dwelling, location of the dwelling, presence of carpet, visible mold, season, number of people, pet keeping, indoor smoking, and community heating. Association estimates are presented as β -coefficients and 95% confidence intervals in ln-transformed scales [floor dust; ln(mg/m²), endotoxin; ln(EU/g), and β -(1,3)-D-glucan; ln(μ g/g). Dot equal to 0 and represent the β -coefficient of each determinant's reference category

ferent sampling location (Casas et al., 2013; Giovannangelo et al., 2007b; Schram et al., 2005).

As in our study, only a small fraction of the variation in levels of endotoxin and β -(1→3)-D-glucan could be explained by the investigated determinants. The most frequently reported significant determinants for loads and concentrations of endotoxin and β -(1→3)-D-glucan by the European studies were pet keeping, number of occupants, season, farming, carpeting, lack of cleanliness, and visible dampness. The identification of these determinants was independent of whether the studies were either multicenter or single-center in nature, focused on children's or adult's house dust exposure, or the selected dust sampling location (i.e. either mattress or living room floor). In line with the above-listed determinants identified by previous studies, our own study revealed carpeting as the strongest determinant for both endotoxin and β -(1→3)-D-glucan concentrations. Moreover, when considering studies investigating bedroom floor dust, we found similar determinants of increased endotoxin concentrations where pet keeping and farming occurred; however, we found no significant association between the type of

heating system and endotoxin concentration. Season of dust sampling, number of occupants, visible mold, and dampness in the dwelling were also identified as essential determinants of endotoxin and β -(1→3)-D-glucan loads and concentrations by previous studies; this, however, was not the case in our study.

Discussion

Main findings

To our knowledge, this is the first study to report loads and concentrations of endotoxin and glucan, and their determinants in homes occupied by Danish children. On one hand, we found higher endotoxin loads and concentrations compared with previous European studies (Chen et al., 2012; Douwes et al., 1998; Moniruzzaman et al., 2012). On the other hand, we also found lower floor dust loads (Douwes et al., 1998) and β -(1→3)-D-glucan loads and concentrations compared with previous studies (Casas et al., 2013; Douwes et al., 1998; Giovannangelo et al., 2007b; Schram et al., 2005). The presence of carpet or a rug in the

Table 4 Epidemiological studies on loads and concentrations and determinants of endotoxin and β -(1→3)-D-glucan: an European overview of multicenter studies

References	Country	Population (M)	Sampling site and sampling method	Agents	Levels	Determinants
This study	Denmark	330 children	Sampling site: Child's bedroom floor Sampling method: VCs with ALK filters. BR: 1 min/m ²	Endotoxin β -(1→3)-D-glucan	BR (GM): 5.46 × 10 ³ EU/m ² 31.1 × 10 ³ EU/g BR (GM): 0.14 × 10 ³ µg/m ² 0.71 × 10 ³ µg/g	Textile flooring (rug or carpet) Type of dwelling Location of dwelling Pet keeping
Multicenter studies Waser et al. (2004)	Austria Germany Switzerland	812 children The ALEX study	Sampling site: Child's mattress Living room floor Sampling method: New VCs with ALK filters. CM: 2 min/m ² LR: 4 min/2–4 m ²	Endotoxin	CM (GM): 22.8–37.8 × 10 ³ EU/g LR (GM): 44.9–81.8 × 10 ³ EU/g	Farming Contact with farm animals Pet keeping Floor cleaning
Schram et al. (2005)	Austria Germany the Netherlands Sweden Switzerland	478 children The PARSIFAL study	Sampling site: Child's mattress Living room floor Sampling method: VCs with ALK glass fibre filters. CM: 2 min/whole M LR: 2–4 min/1–2 m ²	Endotoxin β -(1→3)-D-glucan	CM (median) ^a : 8.00–15.0 × 10 ³ EU/g LR (median) ^a : 15.0–48.0 × 10 ³ EU/g CM (median) ^a : 2.00–3.00 × 10 ³ µg/g LR (median) ^a : 3.00 × 10 ³ µg/g	Farming
Giovannangelo et al. (2007a)	Sweden Germany The Netherlands	1065 homes The AIRALLERG study of birth cohorts: BAMSE, LISA, PIAMA	Sampling site: Child's mattress Living room floor Sampling method: VCs with ALK glass fibre filters. CM: 2 min/whole M LR: 2–4 min/1–2 m ²	Endotoxin	CM (median) ^a : 1.10–3.00 × 10 ³ EU/m ² 8.00–12.0 × 10 ³ EU/g LR (median) ^a : 2.00–7.00 × 10 ³ EU/m ² 12.0–20.0 × 10 ³ EU/g	Pet keeping Occupants (>4 persons) Type of flooring (carpets) Window opening
Giovannangelo et al. (2007b)	Sweden Germany The Netherlands	1065 homes The AIRALLERG study of birth cohorts: BAMSE, LISA, PIAMA	Sampling site: Child's mattress Living room floor Sampling method: VCs with ALK glass fibre filters. CM: 2 min/whole M LR: 2–4 min/1–2 m ²	β -(1→3)-D-glucan	CM (median) ^a : 0.20–0.45 × 10 ³ µg/m ² 1.80–2.10 × 10 ³ µg/g LR (median) ^a : 0.18–1.10 × 10 ³ µg/m ² 2.00–2.80 × 10 ³ µg/g	Type of flooring (carpets)
Chen et al. (2012)	10 EU-countries	974 homes Cohort: ECRHS II	Sampling site: Mattress Sampling method: VCs with ALK filters. M: 2 min/m ²	Endotoxin	M (GM): 0.82–4.81 × 10 ³ EU/g	Outdoor temperature Cat or dog keeping Household crowding index Visible damp patches Season (Spring comp. to Winter) Dog keeping Dampness Occupants
Casas et al. (2013)	Germany The Netherlands Spain Finland	1572 homes Birth cohorts: HITEA (incl.: LISA, PAMA, INMA, LUKAS2)	Sampling site: Living room floor Sampling method: VCs with ALK paper filters (LISA, PIAMA and (INMA). Nylon socks (LUKAS2)	Endotoxin	LR (GM) (LISA, PIAMA and LUKAS2) and LR sofa (GM)(INMA): 3.20–23.0 × 10 ³ EU/g LR (GM): 0.90–2.40 × 10 ³ µg/g	

Study findings are presented according to: First author, country/countries, population/number of homes/study, sampling location, microbial markers, levels, and determinants.

CM, child's mattress; MM, mother's mattress; LR, living room; BR, bedroom; M, mattress; VC, vacuum cleaner; AM, airborne measurement; EU, endotoxin units; GM, geometric mean.

^aThe exact levels are not presented in the paper. Thus, the given numbers are approximates derived from the original paper's figures.

Table 5 Epidemiological studies on loads and concentrations and determinants of endotoxin and β -(1→3)-D-glucan: an European overview of single country studies

References	Country	Population (N)	Sampling site and sampling method	Agents	Levels	Determinants
Single country studies						
Douwes et al. (1998)	Germany	25 homes	Sampling site: Mattress Bedroom floor Living room floor Sampling method: VCs with ALK paper filters. M: 2 min/m ² BR&LR 8 min/4 m ²	Endotoxin β -(1→3)-D-glucan	BR (GM): 4.00×10^3 EU/m ² 7.30×10^3 EU/g M (GM): 2.60×10^3 EU/m ² 2.70×10^3 EU/g BR (GM): 0.70×10^3 μ g/m ² 1.29×10^3 μ g/g M (GM): 0.71×10^3 μ g/m ² 0.76×10^3 μ g/g LR (GM): 0.56×10^3 μ g/m ² 1.29×10^3 μ g/g	Building age Heating system
Gehring et al. (2001)	Germany	395 homes	Sampling site: Living room floor Sampling method: New VCs with ALK paper filters. LR: 2 min/m ²	β -(1→3)-D-glucan	LR (GM): 1.20×10^3 μ g/m ² 1.71×10^3 μ g/g	Flooring (Carpets) Dog keeping Occupants (>4 persons)
Heinrich et al. (2001)	Germany	454 homes	Sampling site: Living room floor Sampling method: VCs with ALK paper filters. LR: 2 min/m ²	Endotoxin	LR (GM): 1.81×10^3 ng/m ² 1.97×10^3 ng/g	Cat and dog keeping Cockroach
Bischof et al. (2002)	Germany	405 homes The INGA study	Sampling site: Living room floors Sampling method: New VCs with ALK paper filters. LR: 2 min/m ²	Endotoxin	LR (GM): $1.91\text{--}2.71 \times 10^3$ ng/g	Building age Cat and dog keeping Lower story Cleaning frequency Occupancy during dust sampling
Heinrich et al. (2003)	Germany	745 homes The INGA study	Sampling site: Living room floor Sampling method: VCs with ALK cellulose filters. LR: 2 min/m ²	Endotoxin	LR (median): 27.8×10^3 EU/g	Season (January–March)
Gehring et al. (2004)	Germany	2157 children 2108 adults	Sampling site: Child's mattress Mother's mattress Sampling method: New VCs with ALK paper filters. CM/MM: 2 min/m ²	Endotoxin	CM (GM) ^a : 1.00×10^3 EU/m ² $5.30\text{--}7.00 \times 10^3$ EU/g MM (GM) ^a : 2.10×10^3 EU/m ² 2.80×10^3 EU/g	Contact with animals Pet keeping Season (Summer) Number of occupants
Chen et al. (2007)	Germany	2166 homes	Sampling site: Child's mattress Mother's mattress Sampling method: New VCs with ALK paper filters. CM/MM: 2 min/m ²	Endotoxin	CM (median) ^a : 1.02×10^3 EU/m ² 5.87×10^3 EU/g MM (median) ^a : 2.07×10^3 EU/m ² 3.01×10^3 EU/g	Social factors not related to endotoxin levels
Dassonville et al. (2008)	France	162 newborn children	Sampling site: Airborne measurement Sampling method: Inhalable-dust samplers with glass fiber filters. Height: 1.2–1.5 m above floor level. Airflow: pump 3.5 l/min. Time: 24 h	Airborne endotoxin	AM (GM): 0.51 EU/m ³	Occupants per square meter Cold season Visible cockroaches Frequency of cleaning Use of floor cloths
Barnig et al. (2012)	France	150 homes	Mattress Bedroom floor Air measurement Sampling method: New VCs. M: 10–15 min BF: 2 min/m ² AM: Gilair pump	Endotoxin	M (mean) ^a : $0.80\text{--}3.00 \times 10^3$ ng/g BR (mean) ^a : $3.75\text{--}5.00 \times 10^3$ ng/g AM (mean) ^a : $0.10\text{--}0.20$ ng/m ³	Farming

Table 5 Continued

References	Country	Population (M)	Sampling site and sampling method	Agents	Levels	Determinants
Moniruzzaman et al. (2012)	Sweden	383 homes 400 children	Sampling site: Child's bedroom floor Living room Sampling method: VCs with cellulose filters. BR&LR: 0.5–2.5 m ²	Endotoxin	BR (GM): 5.73 × 10 ³ EU/g LR (GM): 6.22 × 10 ³ EU/g	Pet keeping Agricultural activities
Frankel et al. (2012)	Denmark	5 homes	Sampling site: Living room Sampling method: Gesamtstaub-pronahme inhalable dust samplers with teflon filters. Height: 1.5 m above floor level. Airflow: 3.5 l/min. Time: 6 h	Airborne endotoxin	AM (GM): 0.88 (Fall) EU/m ³ 1.48 (Summer) EU/m ³	No associations between season, temperature, relative humidity, air exchange rate, and endotoxin loads

CM, child's mattress; MM, mother's mattress; LR, living room; BR, bedroom; M, mattress; VC, vacuum cleaner; AM, airborne measurement; EU, endotoxin units; GM, geometric mean.

^aThe exact levels are not presented in the paper. Thus, the given numbers are approximates derived from the original paper's figures. Study findings are presented according to: First author, country/countries, population/number of homes/study, sampling location, microbial markers, levels, and determinants.

child's bedroom was the strongest determinant for higher loads of total floor dust and concentrations of endotoxin and β -(1→3)-D-glucan. No other determinants were significantly associated with floor dust and β -(1→3)-D-glucan concentrations. Apartments or dwellings with 2–3 floors, rural or farm settings, and presence of a pet were all associated with higher endotoxin concentration. Overall, these findings were similar to those of previous European studies (Gehring et al., 2001; Schram et al., 2005; Waser et al., 2004).

Loads, concentrations, and correlations of floor dust, endotoxin, and β -(1→3)-D-glucan

Our study confirms that the amount of dust sampled is strongly correlated not only with the loads of endotoxin and β -(1→3)-D-glucan but also (although moderately) with their concentrations as observed in previous studies (Douwes et al., 1998; Gehring et al., 2001; Giovannangelo et al., 2007b). Comparison between studies may be difficult, however, due to differences in the characteristics of study populations and their sample sizes, as well as methodological issues, such as sampling location, sampling method, dust storage, extraction, and choice of assays for marker detection. All of these factors have been shown to affect reported levels of endotoxin and β -(1→3)-D-glucan (Brooks et al., 2013; Douwes, 2005; Ownby et al., 2010; Spaan et al., 2008). To minimize variation, the parents of the children participating in our study were given standardized instructions for dust sampling and handling. However, we did not systematically check compliance. The dust sampling location (e.g. bedroom, mattress, or living room) is a known determinant for levels of dust, as well as dust components (Abraham et al., 2005; Leung et al., 2010; Park et al., 2000). Our results are based on bedroom floor dust samples. Only three

previous European studies investigated endotoxin loads and/or concentrations in bedroom floor dust (Barnig et al., 2012; Douwes et al., 1998; Moniruzzaman et al., 2012), and only one study (Douwes et al., 1998) also reported dust loads. The dust load reported by Douwes et al. (1998) (GM 543 mg/m²) was more than twice as high as the load measured in our study (overall GM 186 and 234 mg/m² for carpeted floors). However, the results by Douwes et al. (1998) were observed in a small, selected group of 25 German houses having wall-to-wall carpet. Other studies focused on the allergen content of bedroom floor dust and measured similar dust loads (Michel et al., 1991) or lower dust loads (Park et al., 2000) than found in our study. Therefore, due to the different methodologies used across studies and the limited number of studies against which we might compare our findings, it would be useful to reinvestigate domestic house dust and its associated endotoxin and β -(1→3)-D-glucan in Danish homes in order to validate our findings.

Multicenter studies may provide a better framework for comparing loads and concentrations of floor dust, endotoxin, and β -(1→3)-D-glucan, if sampling and analysis methods are standardized and a large population studied. Significant differences in concentrations of endotoxin and β -(1→3)-D-glucan were noted on a comparison of studies conducted by two European multicenters (Casas et al., 2013; Chen et al., 2012). Endotoxin concentrations were reported as being low in Scandinavia, higher in the South- to Mid- and Alpine Europe, and highest in the Mediterranean areas. β -(1→3)-D-glucan concentrations, on the other hand, were high in both Sweden and Finland when compared to mid-European regions (Casas et al., 2013; Giovannangelo et al., 2007b; Schram et al., 2005). The high endotoxin concentrations and the low β -(1→3)-D-glucan concentrations detected

in house dust for our population seem to contradict the suggested North–South gradient of the multicenter studies. However, it should be noted that contrary to other Scandinavian countries, Denmark has a coastal climate, which makes it comparable to countries with more humid climates, such as those in north-western Europe (e.g. the Netherlands and the British Isles) or mid-European regions. This is also indicated by the similarities of our β -(1→3)-D-glucan concentrations to those reported for the German study population by Casas et al. (2013) and between our β -(1→3)-D-glucan loads and those reported for the Dutch study population by Giovannangelo et al. (2007b). Furthermore, similar concentrations of endotoxin have been found in the United States by Thorne et al. (2009), with a GM of approximately 37.0×10^3 EU/g in 588 bedroom floor dust samples and in New Zealand by Wickens et al. (2003), with a reported GM of 22.7×10^3 EU/g in living room samples. The relatively high endotoxin and low β -(1→3)-D-glucan loads and concentrations in our study are of significance when comparing their health effects between countries and should be considered in the interpretation of future findings.

Determinants of bedroom floor dust, endotoxin, and β -(1→3)-D-glucan

Overall, our study did not disclose determinants which were not previously identified in the European studies. The literature review suggested that the only recurring characteristics for loads and concentrations of endotoxin and β -(1→3)-D-glucan were the inclusion of farming or rural populations in the studies (Barnig et al., 2012; Moniruzzaman et al., 2012; Schram et al., 2005; Waser et al., 2004) and the sampling location in the home. Moreover, significant determinants in the European studies were largely the same as observed outside Europe (Abraham et al., 2005; Gereda et al., 2001; Ownby et al., 2010; Park et al., 2000; Rennie et al., 2008; Singh et al., 2011; Sordillo et al., 2011; Thorne et al., 2009; Wickens et al., 2003). Nevertheless, while the determinants of significance recur in several studies, there is heterogeneity in their effects on microbial concentrations when compared across countries (Casas et al., 2013).

In our study, carpeting was identified as the strongest determinant for loads of floor dust and concentrations of endotoxin and β -(1→3)-D-glucan. This aligns with previous study results, which have shown that carpeted floors revealed up to tenfold higher β -(1→3)-D-glucan concentrations (Douwes et al., 2000; Giovannangelo et al., 2007b) and twofold higher endotoxin concentrations (Gehring et al., 2001; Giovannangelo et al., 2007a) when compared to hard-surfaced floors. Carpets serve as a reservoir of house dust and microorganisms due to a higher surface and a lower resuspension of particles when compared to hard-surface

flooring (Buttner et al., 2002). This might be one possible explanation for our finding of higher endotoxin and β -(1→3)-D-glucan concentrations in the carpeted bedrooms.

Surprisingly, we found β -(1→3)-D-glucan to be lower in homes that had experienced moisture damage within the preceding year. At the same time, we found lower β -(1→3)-D-glucan concentrations when action had been taken to eliminate the moisture damage, with a high correlation between these last two mentioned variables. Because of the cross-sectional design in which markers of exposure are measured at the same time as the determinants, we are unaware of the timely sequence of these scenarios. Therefore, the lower β -(1→3)-D-glucan concentrations observed in homes with moisture damage may be due to the fact that action had been taken to eliminate this damage.

Additionally, the literature review emphasized the presence of both pets and human occupancy of the home as commonly found determinants for concentrations of both endotoxin and β -(1→3)-D-glucan (Casas et al., 2013; Gehring et al., 2001; Waser et al., 2004). Our own analyses revealed the presence of pets, as well as the home being a farm home or an apartment or a dwelling with 2–3 floors, as determinants of high endotoxin concentrations. Yet, when comparing findings between studies on determinants of floor dust, endotoxin, and β -(1→3)-D-glucan, it is important to keep in mind that each study used its own set of determinants, and thus a comparison between existing studies cannot be fully comprehensive.

The main strength of our study is that it is one of the first to investigate domestic loads, concentrations, and determinants of endotoxin and β -(1→3)-D-glucan in Danish homes. This study was based on a large sample size and with a broad range of determinants. Further, it demonstrates that the Scandinavian profile of endotoxin as a marker of house-dust-associated exposure may be different from the Danish housing stock exposure levels and that the Danish profile may more resemble that of north-western Europe.

Although several determinants were included in our study, they could only predict loads of dust and concentrations of dust components to a limited extent. The percentage of explained variability in our study was less than in other studies for floor dust ($R^2 = 25$ – 73% , Giovannangelo et al., 2007b) and endotoxin ($R^2 = 22\%$, Bischof et al., 2002), and similar for β -(1→3)-D-glucan ($R^2 = 7$ – 11% , Giovannangelo et al., 2007b). The variability that remains unexplained may be attributed to factors we did not take into account for in our analyses because our study was designed to investigate a limited set of parameters. These factors might include: dwelling age, insulation, ventilation, cleaning patterns, cleanliness, heating details, temperature, relative humidity, and occupancy of pets and people in the sampling room. Furthermore, the age and

type of vacuum cleaner used for sampling is known to have an effect on the dust and endotoxin concentrations measured (Wickens et al., 2003). Unfortunately, information regarding the family's vacuum cleaner was not available in this study. Another important limitation of the study is a lack of information regarding the effect of random temporal changes on endotoxin and β -(1 \rightarrow 3)-D-glucan loads and concentrations. Only one dust sample was taken from each home on an arbitrary day. Although few studies exist which have conducted repeat dust sampling and analyses for the same homes, data from comparison studies (Heinrich et al., 2003; Schram-Bijkerk et al., 2006) indicate that random temporal variations are a significant factor in the low percentage of explained variability of determinants.

Conclusion

In conclusion, we found lower β -(1 \rightarrow 3)-D-glucan loads and concentrations, but higher endotoxin loads and

concentrations compared with previous European studies. Our findings suggest that the Danish floor dust composition may have a different geographically determined composition compared with other European regions, including other Scandinavian countries. This should be taken into account in future studies which compare the health effects of especially domestic endotoxin concentrations across different countries. Further, in line with previous studies, the amount of dust sampled and the presence of carpeting were identified as significant determinants of endotoxin and β -(1 \rightarrow 3)-D-glucan concentrations.

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