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Bacteria and mould components in house dust and children's allergic sensitisation

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ABSTRACT: It has been suggested that early childhood exposure to microbial agents decreases the risk of allergies in children. The current authors studied the association between microbial agents in house dust and allergic sensitisation in children aged 2–4 yrs.

Nested case-control studies were performed within ongoing birth cohort studies in Germany, the Netherlands and Sweden and ~180 sensitised and 180 nonsensitised children were selected per country. Levels of bacterial endotoxin, β(1,3)-glucans and fungal extracellular polysaccharides (EPS) were measured in dust samples from the children's mattresses and the living-room floors.

Combined across countries, higher amounts of mattress dust and higher mattress dust loads of endotoxin, β(1,3)-glucans and EPS were associated with a significantly decreased risk of sensitisation to inhalant allergens. After mutual adjustment, only the protective effect of the amount of mattress dust remained significant (odds ratio (95% confidence interval) 0.57(0.39–0.84)).

Higher amounts of mattress dust may decrease the risk of allergic sensitisation to inhalant allergens. The effect might be partly attributable to endotoxin, β(1,3)-glucans and extracellular polysaccharides, but could also reflect (additional) protective effects of (microbial) agents other than the ones measured. It is not possible to distinguish with certainty which component relates to the effect, since their levels are highly correlated.

KEYWORDS: Allergy, endotoxin, house dust, moulds, sensitisation

In the context of the hygiene hypothesis, early life exposure to microbial products is considered to play a major role in the development of asthma and allergies. However, it is not clear yet which agents account for the proposed mechanism. Of all microbial products, endotoxin has been studied most extensively. Several authors have consistently found that exposure to elevated levels of house dust endotoxin decreases the risk of allergic sensitisation in pre-school and school children [1–3].

However, endotoxin is only one of the components of house dust with immune-stimulatory properties. The advantage of endotoxin over other components is that there are well-established analytical methods available that can be applied in large epidemiological studies. Besides endotoxin, a variety of other microbial agents are known to have immune stimulatory properties. These include β(1,3)-glucans [4, 5] bacterial DNA [6] and other bacterial components [7, 8]. β(1,3)-glucans are glucose polymers present in the cell wall of most fungi and yeasts, some bacteria and vegetable materials; they have been measured as a marker of mould exposure in field studies. Moulds often grow together with different bacteria and hence levels of β(1,3)-glucan in house dust have been found to be highly correlated with endotoxin levels [9]. Thus endotoxin could be a marker for a broader range of microbial exposures.

The current authors performed a study on the effects of outdoor and indoor air pollution on the development of allergic disease in children (AIRALLERG). Levels of endotoxin and β(1,3)-glucans were measured, as were extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium spp.* as another marker for mould exposure [10], in dust samples from approximately 1,000 German, Dutch and Swedish pre-school and

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school children, and associations with the allergic sensitisation of the children aged 2–4 yrs were examined. Furthermore, the method of DOUWES *et al.* [11] was followed and the effects of the amount of sampled dust as a proxy of microbial exposure in general were examined.

MATERIALS AND METHODS

Study design and study population

The study was designed as a nested case-control study within four birth cohort studies conducted in Munich and surrounding communities, Germany (GINI (German Infant Nutritional Intervention study) [12] and LISA (influence of lifestyle-related factors on the immune system and development of allergies in childhood) [13]), in the north, centre and southwest of the Netherlands (PIAMA (Prevention and Incidence of Asthma and Mite Allergy study [14]) and the central and northern part of Stockholm, Sweden (BAMSE (Barn, Allergy, Milieu Stockholm Epidemiology) [15]). The GINI study and part of the PIAMA study were designed as intervention studies studying the effect of different hydrolysed formulas [12] and the use of mite-impermeable mattress and pillow covers [14] on the development of allergies and asthma in children with parental allergy and children with and without allergic mothers, respectively.

From each cohort (in Germany from the two cohorts combined) ~180 children aged 2–4 yrs with sensitisation to common food and inhalant allergens were selected in addition to a random sample of ~180 nonsensitised children. Since the focus of the study was on inhalant allergies and inhaled exposure, the design was to primarily select all children sensitised to inhalant allergens. If there were not enough children (which was the case in all three countries), then children sensitised to food allergens were added. The children were visited for house dust collection at ages 5 (Germany and the Netherlands) and 7 yrs (Sweden), which was 16–55, 6–30 and 14–55 months after assessment of sensitisation. In Germany and the Netherlands, the sensitised children represent all eligible sensitised children whose parents were willing to participate; in Sweden, where the number of children with sensitisation data available was larger than in the two other countries, sensitised children represent all eligible children with sensitisation to inhalant allergens plus a random sample of children sensitised to food allergens whose parents were willing to participate. This resulted in a lower proportion of children with sensitisation to food allergens in Sweden compared to the other two countries. Families should not have moved between 6 months prior to blood collection for the immunoglobulin (Ig)E measurements and the AIRALLERG home visits. However, in Germany it was not possible to strictly follow this criterion; only 76% of the German participants fulfilled the criterion of not moving home.

Definition of allergic sensitisation

Within the original cohort studies, blood samples were taken when the children were 2 (LISA), 3 (GINI) and 4 yrs old (PIAMA and BAMSE). IgE antibodies to common food and inhalant allergens were determined by a Pharmacia CAP system (Pharmacia, Uppsala, Sweden) in Germany and Sweden and by a Radio Allergo Sorbent test according to the standard operating procedures used at Sanquin Amsterdam (Amsterdam, the Netherlands). Allergen panels differed between the cohorts, but specific IgE to egg white, milk, house dust mites, cat, tree and grass pollens were measured in all cohorts (table 1). Allergic sensitisation to any allergen was defined as specific IgE antibodies of $\geqslant 0.35$ kU·L^{-1} for one of the allergens tested. Allergic sensitisation to inhalant and food allergens was defined as specific IgE antibodies of $\geqslant 0.35$ kU·L^{-1} for one of the inhalant or food allergens tested.

Dust collection

Between January 2002 and May 2003, in the months October–May, a total of 358 (Germany), 347 (the Netherlands), and 364 (Sweden) children were visited. During the home visit, two house dust samples from the child's mattress and the living-room floor were collected by vacuuming the entire mattress surface area and either 1 m^2 of rugs >4 m^2 and wall-to-wall carpets or 2 m^2 of smooth floors (if no carpets >4 m^2 were present) for 2 min according to a standardised protocol using vacuum cleaners equipped with special nozzles (ALK allergen mouthpiece; Hørsholm, Denmark) to collect the dust on glass fibre filters (ref. No. 370104; Schleicher & Schuell, Dassel, Germany). Dust samples were stored at -20°C until extraction, and sent on dry ice to Utrecht (the Netherlands) for extraction and analysis.

Dust extraction and analysis

Dust samples were not sieved. Dust, including filters, was extracted sequentially as described previously [16]. The first supernatant was used to measure endotoxin by a chromogenic kinetic Limulus Amoebocyte Lysate test [17]. The second supernatant was used to measure EPS of *Aspergillus* and *Penicillium spp.* by a sandwich enzyme immunoassay [10]. β(1,3)-glucan was measured in the third supernatant with a β(1,3)-glucan-specific inhibition enzyme immunoassay [18]. Furthermore, dust extracts were analysed for house dust mite allergens *Dermatophagoides pteronyssinus* and *D. farinae* and for cat allergen using reagents for sandwich enzyme immuno assays purchased from Indoor Biotechnologies (Cardiff, UK) as described previously [19]. Associations between sensitisation and exposure to allergens are beyond the scope of the current article and will not be discussed. However, the current authors will explore whether associations between allergic sensitisation and exposure to bacteria and mould components were confounded by allergen exposure. Exposures were expressed as both per g of sampled dust (as a measure of concentration) and per m^2 of sampling surface area (as a measure of the total burden or statistically speaking the interaction between the amount of dust and concentration of the respective biocontaminant). Samples with nondetectable amounts of endotoxin, β(1,3)-glucan, and EPS were assigned a value of two-thirds of the lowest detectable value.

Questionnaires

Questionnaire data collected within the original birth cohort studies was used to define potential confounding variables such as sex, parental allergy and parental education. Confounding variables were defined as similarly as possible given the information that was available.

Statistical analysis

Biocontaminant concentrations were log-normally distributed. Mean values were expressed as geometric means with a

TABLE 1 Number of samples below the limit of detection (LOD), geometric mean (geometric standard deviation) of the amount of dust samples, endotoxin, β(1,3)-glucan and extracellular polysaccharide in the child's mattress dust and living-room floor dust

	Germany		Netherlands	Sweden
	GINI	LISA[#]	PIAMA	BAMSE[¶]
Inhalant allergens				
Airborne allergen mix[+]				×
Animals				
Cat	×	×	×	×
Dog			×	×
Horse				×
House dust				
D. farinae	×	×		
D. pteronyssinus	×	×	×	×
House dust mix[§]		×		
Tree, grass and weed pollens				
Birch	×		×	×
Cocksfoot			×	
Timothy grass	×			×
Tree, grass and weed pollen mix[f]		×		
Mugwort				×
Moulds				
Alternaria alternate			×	
Cladosporium herbarum				×
Mould mix[##]		×		
Food allergens				
Cow's milk	×	×	×	×
Egg white	×	×	×	×
Fish				×
Food mix[¶¶]		×		×
Peanut				×
Soy bean	×			×
Wheat				×

GINI: German Infant Nutritional Intervention study; LISA: influence of lifestyle-related factors on the immune system and development of allergies in childhood study; PIAMA: Prevention and Incidence of Asthma and Mite Allergy study; BAMSE: Barn, Allergy, Milieu Stockholm Epidemiology; *D. farinae*: *Dermatophagoides farinae*; *D. pteronyssinus*: *Dermatophagoides pteronyssinus*. [#]: House dust mite specific immunoglobulin (Ig)E and food allergen specific IgE was measured only in children with positive house dust mix and food mix tests, respectively; [¶]: inhalant and food allergen specific IgE were measured only in children with positive airborne allergen mix and food mix, respectively; [+]: cat, dog, horse, birch, timothy grass, mugwort, *D. pteronyssinus*, *Cladosporium herbarum*; [§]: Hollister-Stier Labs, *D. pteronyssinus, D. farinae,* German cockroach; [f]: timothy grass, mugwort, ribwort, wall pellitory, birch; [##]: *Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Alternaria alternate*; [¶¶]: egg white, milk, fish, wheat, peanut, soy bean.

geometric standard deviation. Correlations were expressed as Pearson correlation coefficients based on natural-log (ln) transformed data. Nonparametric loess smoothers [20] were used to investigate the functional relationship between allergic sensitisation and microbial exposure. Since relationships were generally log-linear, a parametric approach with ln-transformed exposure levels was applied in subsequent analyses. First, a standard logistic regression was used to analyse the association between microbial exposure and the binary outcome of sensitisation to any allergen. The associations between microbial exposure and sensitisation to food and inhalant allergens were then analysed using polytomous logistic regression models [21] with four nominal response categories (sensitised to inhalant allergens only; sensitised to inhalant and food allergens; sensitised to food allergens only; and nonsensitised). Since there was no difference in the exposure-response relationship between children sensitised to inhalant allergens only and children sensitised to inhalant and food allergens, these two response categories were combined in the final analysis. Results are presented as adjusted country-specific and combined odds ratios (OR) from meta-analyses. OR were calculated using the same change in exposure for all countries rather than country-specific changes in exposure to ensure comparability of OR between countries. The change in exposure was defined as the average country-specific inter-quartile range increase in exposure. In case of heterogeneity of effects between countries ($p<0.10$), the random effects approach described in DER SIMONIAN and LAIRD [22] was used to calculate combined OR. Statistical significance was defined by a two-sided α-level of 5%.

TABLE 2 Description of the study population

	Germany	Netherlands	Sweden
Subjects n	356	338	358
Study design#			
Natural history	137/356 (38)	186/338 (55)	358/358 (100)
Intervention	219/356 (62)	152/338 (45)	
Males	203/356 (57)	191/338 (57)	183/358 (51)
Parental allergy¶	297/352 (84)	265/338 (78)	72/356 (20)
High parental education	282/356 (79)	196/338 (58)	213/356 (60)
Mite-impermeable mattress cover	61/355 (17)	62/310 (20)	0/356 (0)
Pets in child's home	90/355 (25)	140/329 (43)	111/358 (31)
Smoking in child's home	46/356 (13)	83/330 (25)	14/357 (4)
Breastfeeding+	177/331 (54)	202/333 (61)	264/349 (76)
Age at blood collection months	35 (22–40)	48 (46–55)	51 (40–63)
Age at dust collection months	63 (45–90)	66 (53–80)	87 (63–109)
Never moved to another house	137/356 (38)	228/338 (67)	165/358 (46)
Allergic sensitisation			
Any	164/356 (46)	152/335 (45)	184/358 (51)
Inhalant allergens	91/356 (26)	100/338 (30)	147/358 (41)
Food allergens	109/356 (31)	89/332 (27)	104/358 (29)

Data are presented as n/N (%) or median (minimum–maximum), unless otherwise stated. #: Germany: natural history study [13], intervention study [12]; ¶: defined as asthma and/or hay fever and/or eczema (Germany), asthma and/or allergy to house dust (mite) or pets, and/or hay fever (the Netherlands), asthma (Sweden); +: defined as exclusive breastfeeding during the first 4 months of life (Germany), any breastfeeding at the age of 3 months (the Netherlands) and duration of breastfeeding >6 months (Sweden).

RESULTS

Study population

The study population for the present analysis consisted of 356 German, 338 Dutch and 358 Swedish children with sensitisation status and complete information on microbial exposure. Table 2 presents characteristics of the study population along with frequency distributions for allergic sensitisation. Differences between countries are largely due to differences in the design of the original cohorts and in selection of the AIRALLERG participants. Males (Germany and Netherlands only), children from allergic parents and children from parents with high education were over-represented compared with the original cohorts due to higher sensitisation and/or participation rates among these groups (data not shown). The time lag between assessment of allergic sensitisation, dust and season of dust collection did not differ significantly between cases and controls.

Amount of dust sampled, endotoxin, β(1,3)-glucan and EPS levels

Geometric means and SDs of the amount of dust sampled and microbial agent levels in the child's mattress dust and living-room floor dust are presented in table 3. Patterns between mattress and floor differed between countries. There were weak or no correlations between amounts of mattress dust and the amounts of living-room floor dust and mattress dust and living-room floor dust biocontaminant levels, respectively ($r \leqslant 0.22$, data not shown). Endotoxin, β(1,3)-glucan and EPS loads of mattress dust were moderate-to-highly correlated with the amount of dust sampled (table 4). Weak or no correlations were found between mattress dust concentrations of endotoxin, β(1,3)-glucan, and EPS and amount of dust sampled. Mattress dust endotoxin, β(1,3)-glucant and EPS loads were moderately correlated with each other, while there were weak or no correlations between concentrations. Similar but generally stronger correlations were found for living-room floor dust samples (table 4).

Associations between exposure to microbial agents and allergic sensitisation

The current authors found negative associations between amounts of dust sampled from the children's mattresses, endotoxin and β(1,3)-glucan loads and sensitisation to any allergen (fig. 1). Effects were homogeneous ($p>0.10$) between countries and statistically significant ($p<0.05$) when combined across countries. Effects became stronger when the cut-off for sensitisation was increased (combined adjusted OR (95% confidence interval (CI)) using 0.35 kU·L^{-1}, 0.7 kU·L^{-1} and 3.5 kU·L^{-1}, respectively, as cut-off for sensitisation were 0.81 (0.68–0.95), 0.75 (0.63–0.90), and 0.64 (0.51–0.81) for the amount of dust; 0.82 (0.73–0.93), 0.76 (0.67–0.87), and 0.69 (0.58–0.81) for endotoxin loads; and 0.81 (0.71–0.93), 0.75 (0.65–0.87), and 0.67 (0.56–0.81) for β(1,3)-glucan loads). No association was seen with EPS loads and for microbial agents expressed per g of dust. When the effects of microbial agents on sensitisation to food and inhalant allergens were examined separately, the authors found effects limited to sensitisation to inhalant allergens (fig. 2). In addition, there was a negative association between EPS loads and sensitisation to inhalant allergens. No association was found for sensitisation to food allergens. Additional adjustment for use of mite-impermeable mattress covers, pet-ownership, breastfeeding and mite and cat allergen levels in mattress, dust did not change the association considerably (maximum change in OR 6%, data not shown). No adjustment was made for age since the age at blood collection is very homogeneous within cohorts except for Germany, due to combination of two cohorts where blood was collected at 2 and 3 yrs of age, respectively. To account for age and other differences between the two cohorts, models for Germany include a variable study design.

No association was found between sensitisation to inhalant allergens and amounts of dust sampled from living-room floors (combined adjusted OR (95% CI) 0.93 (0.58–1.48) per 10.6 factor increase in the amount of dust per m^2), as well as living-room floor dust endotoxin, β(1,3)-glucan and EPS loads (0.92 (0.64–1.30), 0.91 (0.56–1.47) and 0.93 (0.64–1.37) per factor 15.4, 13.0 and 79.7 increase in exposure, respectively) and concentrations (0.98 (0.87–1.10), 0.97 (0.78–1.22), and 1.00 (0.83–1.19) per factor 3.3, 1.9, and 7.5 increase in exposure, respectively).

The present authors tried to disentangle the effects of the amount of mattress dust and the mattress dust loads of the different microbial agents by first adjusting microbial agents' effects for the amount of dust only, then mutually adjusting the

TABLE 3 Number of samples below the limit of detection (LOD), endotoxin, β(1,3)-glucan and extracellular polysaccharide (EPS) levels in the child's mattress and living-room floor dust

	Germany	Netherlands	Sweden
Subjects n	356	338	358
Child's mattress dust per m^2 of surface			
Amount of dust $mg \cdot m^{-2}$	0 (240 (2.5))	0 (229 (2.1))	0 (142 (1.9))
Endotoxin $EU \cdot m^{-2}$	2 (3053 (3.3))	0 (2467 (2.5))	9 (1094 (2.8))
β(1,3)-glucan $\mu g \cdot m^{-2}$	0 (434 (2.8))	0 (357 (2.4))	0 (320 (2.5))
EPS $EPSU \cdot m^{-2}$	6 (9058 (4.7))	5 (7619 (4.6))	15 (2173 (5.3))
Child's mattress dust per gram of dust			
Endotoxin $EU \cdot g^{-1}$	2 (12 515 (2.6))	0 (10 763 (2.1))	9 (7535 (2.5))
β(1,3)-glucan $\mu g \cdot g^{-1}$	0 (1797 (1.7))	0 (1556 (1.7))	0 (2251 (1.7))
EPS $EPSU \cdot g^{-1}$	6 (36 592 (3.1))	5 (32 874 (3.2))	15 (15 120 (4.1))
Living-room floor dust per m^2 of surface			
Amount of dust $mg \cdot m^{-2}$	17 (155 (4.4))	22 (118 (5.5))	6 (292 (3.2))
Endotoxin $EU \cdot m^{-2}$	14 (2874 (6.2))	23 (2180 (9.7))	9 (4077 (5.2))
β(1,3)-glucan $\mu g \cdot m^{-2}$	0 (350 (4.9))	7 (243 (7.0))	1 (799 (3.7))
EPS $EPSU \cdot m^{-2}$	28 (4577 (13.7))	71 (1431 (30.2))	18 (9699 (9.6))
Living-room floor dust per gram of dust			
Endotoxin $EU \cdot g^{-1}$	14 (16 837 (3.2))	23 (15 124 (4.0))	9 (13 122 (3.2))
β(1,3)-glucan $\mu g \cdot g^{-1}$	0 (2225 (1.7))	7 (1948 (2.3))	1 (2689 (1.8))
EPS $EPSU \cdot g^{-1}$	28 (25 340 (6.7))	71 (8700 (13.6))	18 (30 641 (5.0))

Data are presented as n<LOD (geometric mean (geometric standard deviation)), unless otherwise stated.

effects of microbial, and finally by mutual adjustment with adjustment for dust in addition, *i.e.* by including all four exposure measures in one model. The results of the different adjustments were rather similar. Therefore, only results for the model including the four exposures are presented in figure 3. CIs became considerably larger as a result of the correlation between the different exposures. However, the effect of the amount of dust remained statistically significant and became even slightly stronger (adjusted OR 0.57 *versus* 0.70). The endotoxin effect became somewhat smaller (adjusted OR 0.88 *versus* 0.79), and the β(1,3)-glucan effects disappeared in favour of the amount of dust effect, while there was a significant

TABLE 4 Correlation between amount of dust sampled, endotoxin, β(1,3)-glucan, and extracellular polysaccharide (EPS) levels per g of dust (upper triangular matrix) and per m^2 (lower triangular matrix) for the child's mattress and the living-room floor

	Child's mattress				Living-room floor			
	Dust	Endotoxin	β(1,3)-glucan	EPS	Dust	Endotoxin	β(1,3)-glucan	EPS
Germany								
Dust	1.00	-0.09	-0.06	0.36	1.00	0.27	0.03	0.58
Endotoxin	0.66	1.00	0.26	0.24	0.87	1.00	0.28	0.47
β(1,3)-glucan	0.85	0.67	1.00	-0.05	0.94	0.87	1.00	0.20
EPS	0.79	0.64	0.67	1.00	0.88	0.85	0.87	1.00
the Netherlands								
Dust	1.00	-0.23	-0.12	0.32	1.00	0.57	0.38	0.70
Endotoxin	0.64	1.00	0.12	0.07	0.92	1.00	0.47	0.54
β(1,3)glucan	0.81	0.56	1.00	0.05	0.96	0.92	1.00	0.32
EPS	0.72	0.55	0.63	1.00	0.88	0.85	0.86	1.00
Sweden								
Dust	1.00	-0.01	0.19	0.28	1.00	0.28	0.23	0.66
Endotoxin	0.57	1.00	0.25	0.45	0.77	1.00	0.10	0.38
β(1,3)glucan	0.82	0.59	1.00	0.19	0.92	0.72	1.00	0.28
EPS	0.60	0.64	0.56	1.00	0.87	0.75	0.85	1.00

adverse effect of EPS. There was no indication of a multicollinearity problem (maximum condition index was 5.04, maximum variance inflation factor was 5.12). Mutually adjusted effects of endotoxin, β(1,3)-glucan, and EPS loads were very similar to the effects of their concentrations presented in figure 1, besides a somewhat stronger EPS effect.

DISCUSSION

The current results suggest that combined across countries, higher amounts of mattress dust and higher loads of endotoxin, β(1,3)-glucans and EPS in mattress dust are associated with a decreased risk of allergic sensitisation to inhalant allergens but not food allergens. After mutual adjustment, only the protective effect of the amount of mattress dust remained significant.

The strongest prior evidence for a protective effect of exposure to microbial agents in house dust on allergic sensitisation comes from a number of studies, which consistently showed a negative association between allergic sensitisation against inhalant allergens and exposure to house dust endotoxin in preschool and schoolchildren [1–3]. No protective effect of exposure to β(1,3)-glucan has been indicated so far. However, data on exposure to β(1,3)-glucan on sensitisation is scarce. One study reported nonsignificantly increased proportions of sensitised subjects among adults living in homes with higher β(1,3)-glucan levels compared with adults living in homes with lower β(1,3)-glucan levels [23]. In another study, no difference in prevalence of allergic sensitisation was found between students attending schools with high and low β(1,3)-glucan levels, respectively [24]. With regard to EPS there is even less data and, to the present authors' knowledge, the association between exposure to EPS and sensitisation has not yet been studied. Likewise, there are no publications on the association between sensitisation and the amount of sampled dust as a proxy for microbial exposure. Sensitisation to food allergens has not been examined in any other study. Thus, comparisons of the present results with those of other studies are limited to the association between sensitisation to inhalant allergens and endotoxin exposure.

The negative association between mattress dust endotoxin loads (without adjustment for the amount of dust sampled, β(1,3)-glucan and EPS) and allergic sensitisation to inhalant allergens in the present study is qualitatively and quantitatively in line with the findings of BRAUN-FAHRLANDER *et al.* [1] and GEHRING *et al.* [2], likewise not adjusted for the amount of dust sampled or other biocontaminants. Stronger effects for higher cut-offs for sensitisation are in agreement with previous findings [2, 25]. Associations between living-room floor dust endotoxin and sensitisation as shown by GEHRING *et al.* [2] were not found in the present study. Possible explanations for the presence of an association with mattress dust and the lack of association with living-room floor dust are that the reproducibility of repeated endotoxin measurements is greater for bed dust than for floor dust [26] and that children come into closer contact with the microbial agents while sleeping. Two different ways of adjusting for the amount of dust (*i.e.* expressing endotoxin levels per g of dust, and including the amount of dust as an additional predictor variable in the model) yielded very similar results in the present study: the endotoxin effect became somewhat weaker. In the studies of BRAUN-FAHRLANDER *et al.* [1] and GEHRING *et al.* [2], however, OR for endotoxin levels per g of dust and endotoxin levels per m^2 were very similar to each other. The reason for this is not clear, but it might be explained by different correlation patterns in the different studies. Correlations between endotoxin loads and concentrations were indeed lower in the present study than in the study by GEHRING *et al.* [2] (r=0.60–0.81 *versus* r=0.88).

When the present authors tried to disentangle the effects of the individual exposures by mutual adjustment, only the negative association with the amount of dust remained statistically significant. The endotoxin effect became somewhat weaker while the β(1,3)-glucan effect ''disappeared''. The fact that the associations between sensitisation and endotoxin and β(1,3)-glucan loads ''disappear'' after adjustment for the amount of dust indicate that the effects of biocontaminant loads are mainly due to the effect of the amount of dust and that there is no interactive effect of dust and biocontaminants in addition to the ''dust effect''. However, it is not possible to distinguish with certainty, which component relates to the effect, since their levels are highly correlated with the amount of dust sampled and with each other. Therefore, the possibility that part of the effect of the amount of dust is attributable to endotoxin, β(1,3)-glucans and EPS, cannot be ruled out. Furthermore, the effect of the amount of dust could also reflect (additional) protective effects of (microbial) agents other than the ones measured.

The present authors think that the standardised exposure assessment is one of the strengths of the present study. Associations between sensitisation to inhalant allergens and the amount of dust, endotoxin and β(1,3)-glucans were consistent between countries, despite differences in exposure pattern. Some of the associations were not statistically significant on the country level, but they became significant when they were combined in meta-analyses. A potential limitation of the nested case-control design might be that exposures were measured between 1–4 yrs after sensitisation was measured. The design was chosen because collection and analysis of house dust are time consuming and costly and therefore could not be performed for the entire birth cohorts. Collection of dust before measurement of sensitisation for a random sample of birth cohort members was not carried out due to the low prevalence of sensitisation in the age group studied, which would most likely have resulted in too small a number of sensitised children resulting in too little statistical power. However, there is some evidence that a single endotoxin measurement is a valid estimate of exposure for longer time periods [26–29]. Within-home correlation was found to be considerable for living-room floor dust samples over a period of 6 yrs (r=0.5 for endotoxin loads [29]) and somewhat higher for bed (r=0.7–0.8) [26] and bedroom floor dust samples (r=0.6) [28] over periods of up to 13 months. To the current authors' knowledge, no data is available for amounts of dust, β(1,3)-glucans and EPS. Therefore, prospective studies where exposure is measured before health outcomes are needed to confirm the present results.

Moving to another house between blood sampling for IgE measurements and collection of house dust might result in exposure misclassification. The current authors tried to solve

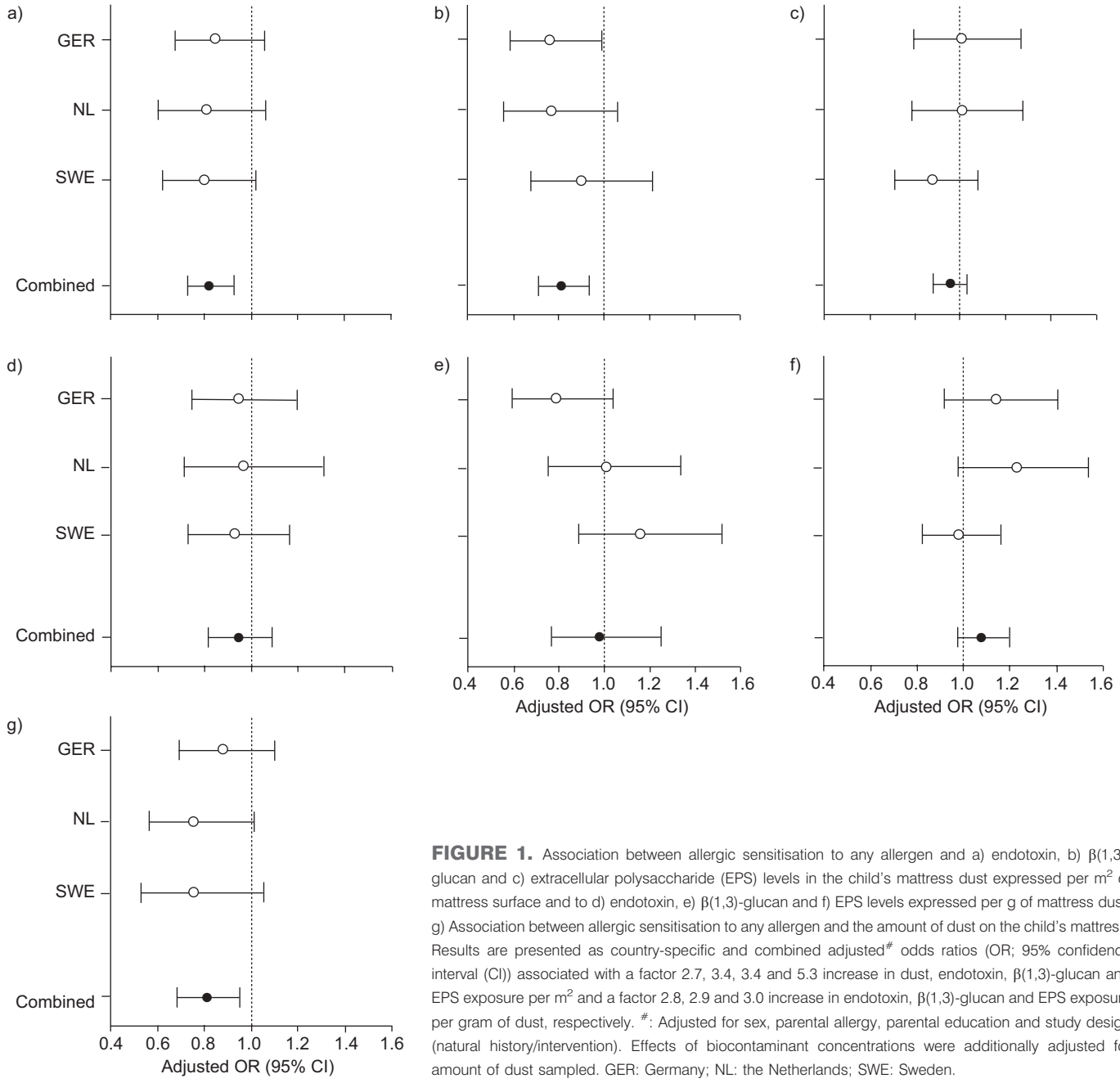

FIGURE 1. Association between allergic sensitisation to any allergen and a) endotoxin, b) β(1,3)-glucan and c) extracellular polysaccharide (EPS) levels in the child's mattress dust expressed per m^2 of mattress surface and to d) endotoxin, e) β(1,3)-glucan and f) EPS levels expressed per g of mattress dust. g) Association between allergic sensitisation to any allergen and the amount of dust on the child's mattress. Results are presented as country-specific and combined adjusted[#] odds ratios (OR; 95% confidence interval (CI)) associated with a factor 2.7, 3.4, 3.4 and 5.3 increase in dust, endotoxin, β(1,3)-glucan and EPS exposure per m^2 and a factor 2.8, 2.9 and 3.0 increase in endotoxin, β(1,3)-glucan and EPS exposure per gram of dust, respectively. [#]: Adjusted for sex, parental allergy, parental education and study design (natural history/intervention). Effects of biocontaminant concentrations were additionally adjusted for amount of dust sampled. GER: Germany; NL: the Netherlands; SWE: Sweden.

this problem by excluding children who had moved between 6 months prior to blood sampling and house dust collection from the AIRALLERG study. In Germany it was not possible to follow this criterion strictly. In total, 24% of the children moved to another house between blood sampling and house dust collection. However, excluding these children from the analysis did not change the results (data not shown).

Assuming that a single exposure measurement is valid for a period of several years, exposures measured within the AIRALLERG study could, on one hand, represent ''current'' exposures at the time (before) IgE measurements were performed, and on the other hand, represent ''early'' life exposures *e.g.* during the first year of life, which have been hypothesised to be crucial [30]. Exposures measured within the present study might yield a better estimate of early exposure in children who never moved to another house compared with children who moved to another home. Therefore children who had moved at any time during their lives were excluded from the analysis. This did not strengthen the association between exposure to microbial agents and allergic sensitisation (data not shown) indicating the relevance of current exposure over early exposure. These findings were supported by the fact that were no or low correlations between the amount of dust, endotoxin, β(1,3)-glucans and EPS levels measured in the present study and the amount of dust, endotoxin, β(1,3)-glucans

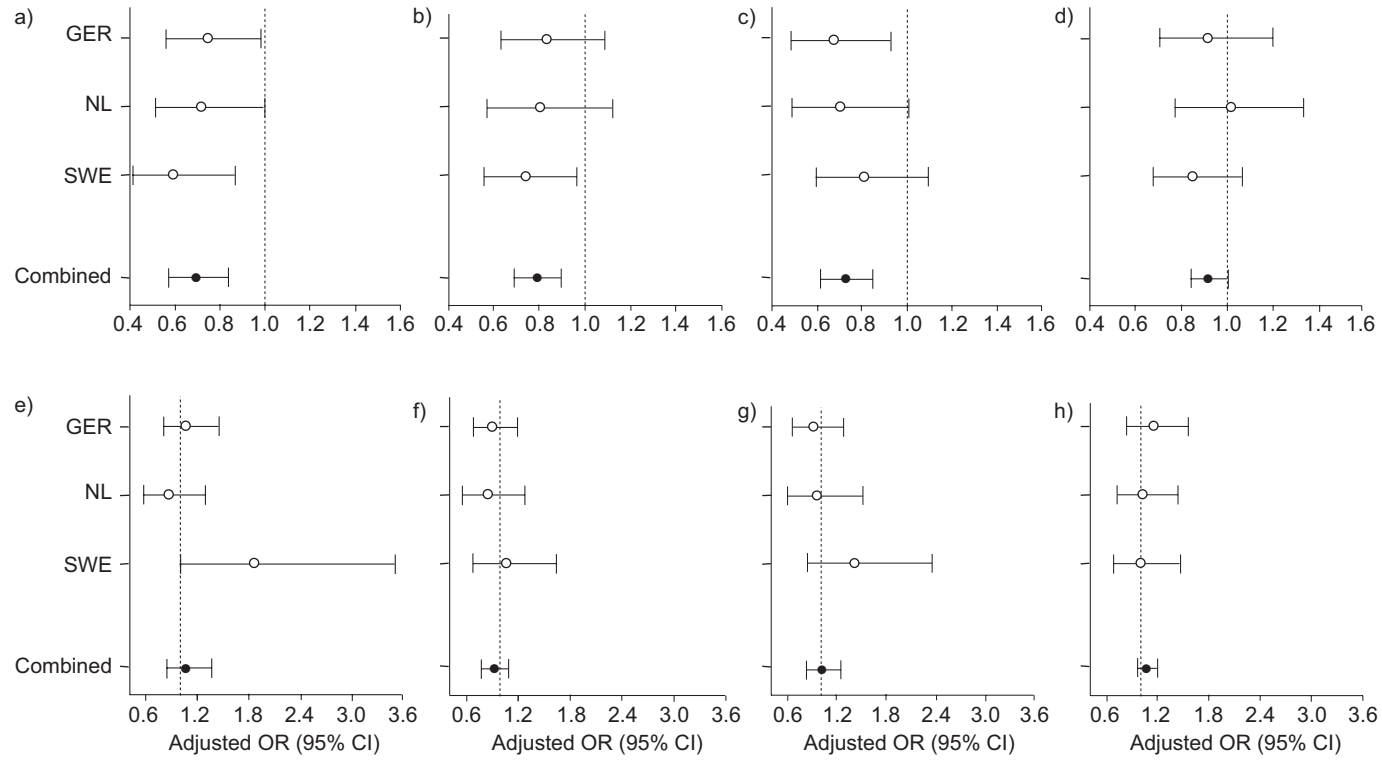

FIGURE 2. Association between allergic sensitisation to inhalant allergens (a–d) and to food allergens only (e–h) and the amount of dust (a and e), endotoxin (b and f), β(1,3)-glucan (c and g) and extracellular polysaccharide (EPS; d and h) per m^2 of the child's mattress surface. Results are presented as country-specific and combined adjusted# odds ratios (OR; 95% confidence interval (CI)) associated with a factor 2.7, 3.4, 3.4 and 5.3 increase in dust, endotoxin, β(1,3)-glucan and EPS exposure per m^2, respectively. #: Adjusted for sex, parental allergy, parental education and study design (natural history/intervention). GER: Germany; NL: the Netherlands; SWE: Sweden.

and EPS levels on the child's mattress at age 3 months (PIAMA study, n=309 for amount of dust, n≈130 for endotoxin, β(1,3)-glucans and EPS, maximum correlation 0.23; data from the German LISA study, n=135 for endotoxin, correlation 0.10), which is most likely due to the fact that children got new (bigger) mattresses between the two dust collections. Further evidence in favour of current exposure comes from a recent publication by DOUWES *et al.* [11], who found no association between sensitisation at 4 yrs and mattress dust microbial exposure at the age of 3 months in the PIAMA cohort.

Selective avoidance is another potential source of bias. Allergic parents, for instance, might tend to take measures that reduce exposure to house dust and allergens more often [19, 31], and keep their houses cleaner before the baby is born. Moreover, parents of symptomatic children might respond to their children's symptoms by keeping their houses cleaner or by changing other factors that might affect exposure. Associations between endotoxin levels and cleaning habits have been shown in one study [32], but not in others [33, 34], and the overall percentage of variability explained by cleaning habits in the former study was low. The association between the amount of house dust on the child's mattress and allergic sensitisation was rather similar for children with and without allergic parents (combined adjusted OR (95% CI) 0.74 (0.59–0.93) and 0.61 (0.43–0.86), respectively) and nonsymptomatic and symptomatic children (0.66 (0.52–0.84) and 0.65 (0.44–0.97), respectively). Thus, there is no indication of a bias due to selective avoidance measures of allergic parents or parents of symptomatic children.

The fact that the original cohorts differ with regard to inclusion criteria (GINI and PIAMA were enriched with children prone to atopy due to allergic parents), study protocols (GINI and PIAMA were at least partly designed as intervention studies), and assessment of atopy (age and set of allergens) and that selection of participants for the present study differed somewhat between countries, might be a limitation of the present study. It is not possible to assess the impact of these differences on the current study results. However, in spite of these differences, effect estimates were consistent between countries. Results were rather similar for children with and without allergic parents; adjustment for the presence of mattress covers and breastfeeding and exclusion of the German children who had moved between measurement of sensitisation and collection of dust did not change the results. Therefore, the present authors believe that combining country-specific estimates by meta-analysis can be justified and results in valid effect estimates. Nevertheless, the possibility that differences between study populations have an impact on the current results cannot be completely ruled out.

Conclusion

Higher amounts of mattress dust might decrease the risk of allergic sensitisation to inhalant allergens. The effect might be

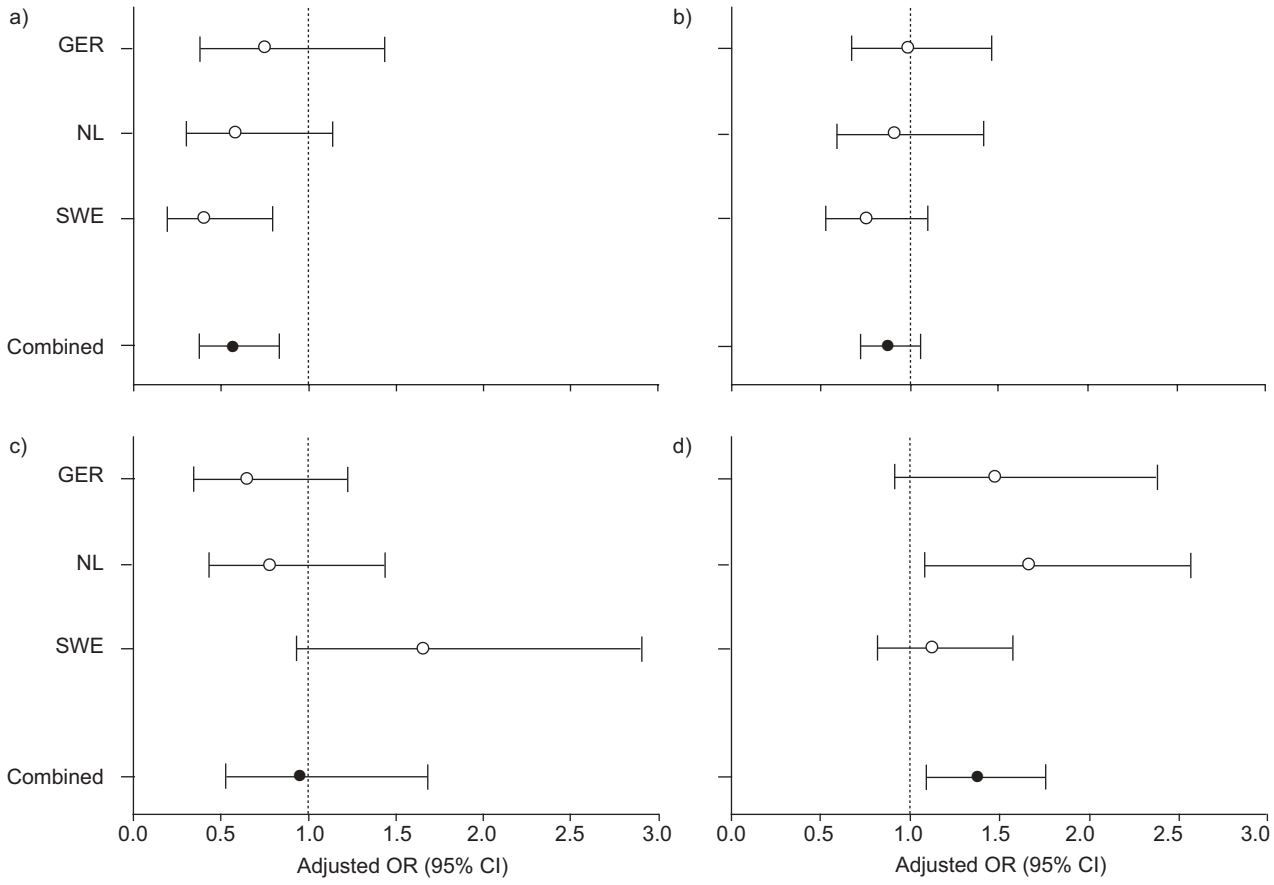

FIGURE 3. Mutually adjusted association between allergic sensitisation to inhalant allergens and a) the amount of dust sampled on the child's mattress, b) endotoxin, c) β(1,3)-glucan and d) extracellular polysaccharide (EPS) levels in the child's mattress dust expressed per m^2 of mattress surface. Results are presented as country-specific and combined odds ratios (OR; 95% confidence interval (CI)) associated with a factor 2.7, 3.4, 3.4 and 5.3 increase in dust, endotoxin, β(1,3)-glucan and EPS exposure per m^2, respectively, and were additionally adjusted for sex, parental allergy, parental education and study design (natural history/intervention).

partly attributable to endotoxin β(1,3)-glucans and extracellular polysaccharides, but could also reflect (additional) protective effects of (microbial) agents other than the ones measured. It is not possible to distinguish with certainty which component relates to the effect, since their levels are highly correlated with the amount of dust sampled and with each other.

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