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House dust endotoxin, asthma and allergic sensitization through childhood into adolescence

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Abstract

Background: House dust endotoxin may have beneficial effects on allergic sensitization and asthma in children. Evidence is scarce for adolescents and most studies so far have been cross-sectional and limited to a single exposure measurement.

Objective: We assessed associations of house dust endotoxin with asthma and allergic sensitization from birth to age 17 years longitudinally taking into account exposure early in life and at primary school age.

Methods: We used data of 854 participants of the prospective Dutch PIAMA birth cohort study with house dust endotoxin measurements at 3 months and/or 5-6 years and data on asthma and/or allergic sensitization from at least one of 11 follow-ups until age 17. We assessed overall and age-specific associations of the prevalence of asthma and sensitization with mattress and living room floor dust concentrations (per gram of dust) and loads (per m^2 of sampling surface).

Results: Higher living room floor dust endotoxin concentrations at 3 months were associated with lower odds of asthma until age 4 [odds ratio (95% confidence interval) ranging from 0.70 (0.51-0.97) at age 1 to 0.76 (0.57-1.00) at age 3 per interquartile range increase], but not thereafter in children of allergic mothers. Higher living room floor dust endotoxin at 5-6 years was associated with higher odds of sensitization at 8-16 years [eg odds ratio (95% confidence interval) 1.70 (1.17-2.47) per interquartile range increase in endotoxin load].

Conclusions and clinical relevance: House dust endotoxin may have beneficial effects on asthma in preschool children of allergic mothers, which do not persist into adolescence. Beneficial associations with allergic sensitization could not be confirmed.

KEYWORDS

allergic sensitization, asthma, birth cohort, endotoxin

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1 | INTRODUCTION

Asthma is one of the most common chronic diseases in childhood. It has been estimated to affect some 235 million people worldwide.[1] The causes of asthma are not completely understood, but both genetic and environmental factors have been shown to be involved.[2,3]

Endotoxin (lipopolysaccharide [LPS]), a constituent of the outer membrane of gram-negative bacteria, has gained interest as a determinant of asthma and allergy during the past decades due to its strong immune-stimulatory and pro-inflammatory properties. Several studies reported lower risks of allergic sensitization with higher exposure to house dust endotoxin in children and young adults living in rural and urban environments.[4-11] Findings of studies assessing associations of house dust endotoxin with asthma and related symptoms are less consistent.[12,13] Higher levels of endotoxin have been reported to be associated with both increased[5,14-16] and decreased[4,10,17-20] risks of asthma (symptoms) in children and adolescents. A meta-analysis of nineteen studies of the associations of house dust endotoxin with asthma and wheeze concluded that early life exposure to endotoxin is associated with an increased risk of wheeze in infants and toddlers (odds ratio [95% confidence interval] 1.48 [1.10-1.98]), while current exposure to endotoxin is associated with a decreased risk of asthma in schoolchildren (0.68 [0.50-0.93]).[13] This suggests that associations of asthma with endotoxin are age-dependent and/or that exposure during different time periods has differential effects. The relevance of the timing of exposure in addition to the level has also been indicated in animal experiments.[21] A limitation of many previous studies, including the majority of studies that were performed within prospective cohorts, is the cross-sectional design and the lack of repeated exposure measurements, which makes it impossible to disentangle the relevance of the timing of exposure from the relevance of age.

Therefore, we assessed associations of house dust endotoxin with asthma and allergic sensitization from birth to age 17 years longitudinally taking into account exposure early in life and at primary school age. The mechanisms underlying such associations are not entirely clear, but endotoxin has strong immune-stimulatory and pro-inflammatory properties through which it can promote asthma by inducing airway inflammation on the one hand and may have protective effects against atopy development by activating type 1 T helper cell responses on the other hand.[22] Endotoxin interacts with CD14-positive cells such as monocytes and macrophages via the CD14 receptor and with CD14-negative cells such as endothelial and epithelial cells through a soluble form of CD14.[23,24] Since it has been suggested that Toll-like receptor (TLR) 4 mutations are associated with endotoxin hyperresponsiveness[25] and the association between endotoxin and allergic sensitization has been found to be modified by single-nucleotide polymorphisms (SNPs) in the CD14 receptor,[26,27] we also assessed potential modifications of associations with endotoxin by a common SNP in the CD14 receptor (rs2569190) and SNPs in the TLR4 for which effect modifications with endotoxin have been assessed previously.[28]

2 | METHODS

2.1 | Study design and study population

Details on the PIAMA birth cohort study have been published elsewhere.[29,30] In brief, pregnant women were recruited from the general population in the north, west and centre of the Netherlands in 1996-1997 through antenatal clinics. Non-allergic pregnant women were invited to participate in a "natural history" study arm. Pregnant women identified as allergic through the screening questionnaire were allocated primarily to an intervention arm with a random subset allocated to the natural history arm. The intervention involved the use of mite-impermeable mattress and pillow covers, but had no effect on the development of asthma and allergy[31] and therefore, participants of both the intervention and the natural history study were included in the present study.

The study started with 3963 newborns. Parents completed questionnaires on demographic factors, risk factors for asthma and respiratory symptoms at birth, at the child's ages 3 of months and 1 year and then annually until the age of 8 years. At ages 11, 14 and 17 years, both the parents and the participants themselves completed questionnaires. Measurements of specific immunoglobulin E (IgE) were performed at ages 4, 8, 12 and 16 in subpopulations with an over-representation of children of allergic mothers at age 4. House dust samples were collected during home visits for subsets of the population with an over-representation of children of allergic mothers during pregnancy when the children were 3 months, 1 and 5-6 years old. Subsets with house dust endotoxin levels during pregnancy and at age 1 year were too small for reliable epidemiological analyses. Therefore, we restricted the current study to participants with questionnaire data on asthma at any age from age 1 to 17 years and house dust endotoxin levels at 3 months and/or 5-6 years (n = 857, see Figure S1 for more details).

The Institutional Review Boards of the participating institutes approved the study protocol, and written informed consent was obtained from the parents or legal guardians of all participants.

2.2 | Health outcomes

Asthma was defined as a positive answer to at least two of the three following questions: (a) "Has a doctor ever diagnosed asthma in your child?," (b) "Has your child had wheezing or whistling in the chest in the last 12 months?," (c) "Has your child been prescribed asthma medication during the last 12 months?," a definition that has been developed by a panel of experts within the MeDALL consortium.[32] Those with two or more negative answers to the three questions were considered non-asthmatic. Measurements of specific IgE levels against common aero allergens were performed in blood samples collected at 1, 4, 8, 12 and 16 years (Table S1). Allergic sensitization to any (inhalant and food) allergens was defined as a specific IgE level of ≥0.35 IU/mL to one or more of the (inhalant and food) allergens tested.

2.3 | House dust collection and endotoxin measurements

House dust samples have been collected on the child's mattress and the living room floors in homes of children of allergic mothers during pregnancy and the child's first year of life and sensitized and non-sensitized children of predominantly allergic mothers at age 5-6 years as described previously[33,34] and in the online supplement. Dust including filters was weighted and extracted as described elsewhere[35] and extracts were stored frozen until analysis. Endotoxin was measured in extracts by a chromogenic kinetic Limulus amoebocyte lysate test[36] and endotoxin levels were expressed per gram of sampled dust (as a measure of concentration) and per m^2 of sampling surface area (as a measure of the total load/burden).

2.4 | Genotyping

Genotyping was done using three different platforms: Illumina Omni Express Exome (OEE) chip (n = 1377), Illumina Omni Express (OE) chip (n = 288) and Illumina Human610 (HM610) quad array (n = 404) (Illumina Inc, San Diego, CA). The *CD14*/rs2569190, *TLR4*/rs2737190 (in complete linkage disequilibrium with *TRL4*/rs1927914), *TLR4*/rs10759932, *TLR4*/rs11536889, *TLR4*/rs11536891 and *TLR4*/rs11734502 (in complete linkage disequilibrium with *TLR4*/rs4986790) SNPs were included on all platforms. More detailed information is provided in the online supplement.

2.5 | Covariates

Information on important covariates such as sex, maternal and paternal allergies (yes/no), Dutch nationality (defined as both parents being born in the Netherlands, yes/no), parental education (low/medium/high, for details see online supplement), breastfeeding at age 12 weeks (yes/no), presence of older siblings (yes/no), daycare attendance at age 2 years, maternal smoking during pregnancy (yes/no), smoking in the child's home (yes/no), mould/damp spots in the living room and/or child's bedroom (yes/no), use of gas for cooking (yes/no) and furry pets in the child's home (cats, dogs, and/or rodents, yes/no) was obtained from the parent-completed questionnaires.

2.6 | Statistical analysis

Endotoxin levels were log-normally distributed, and therefore, mean values were expressed as geometric means with geometric standard deviations. Correlations between repeated endotoxin measurements (including also measurements performed during pregnancy and at age 1 year where available for the present study population) were expressed as Pearson correlation coefficients based on natural-log (ln) transformed data.

Overall associations of endotoxin levels measured in house dust samples collected at ages 3 months and 5-6 years with asthma and allergic sensitization prevalence at subsequent ages until age 17 were analysed by generalized estimation equations (GEE) models with a compound symmetry correlation matrix taking into account correlation between repeated observations within subjects.[37] We used a logit-link for both, asthma and allergic sensitization, despite the high prevalence of sensitization and the fact that the odds ratio than overestimates the relative risk because of convergence problems for models with a log-link. Age-specific association estimates were calculated from GEE models with exposure-age interaction terms. Analyses were performed with and without adjustment for the covariates listed above; associations between endotoxin levels at age 5-6 years and asthma were additionally adjusted for any allergic sensitization at age 4 to account for the nested case-control design.[34] Associations between endotoxin levels at age 5-6 years with allergic sensitization were not additionally adjusted for any allergic sensitization at age 4 as this may be on the causal pathway between endotoxin exposure and sensitization at later ages. Time-varying covariates were selected from the questionnaire that coincided best with the exposure period. We assessed modifications of associations with endotoxin levels in 3 months dust samples (numbers were too small for 5-6 year dust samples) by *CD14*/rs2569190 and *TLR4* genotypes by adding interaction terms to the models and present genotype-specific association estimates from models with interaction terms. For SNPs with <10% of homozygous subjects for the minor allele, we combined all carriers of minor alleles. Since differential associations of house dust endotoxin have been suggested for atopic and non-atopic asthma,[4] we also performed stratified analyses by allergic sensitization at or around the time of asthma assessment (using data on asthma collected at ages 1, 4, 8, 11 and 17 years and data on sensitization collected at ages 1, 4, 8, 12 and 16 years) with endotoxin levels in 3 months dust samples (again, numbers were too small for 5-6 year dust samples). Potential confounding by study arm (natural history study, intervention study—active, intervention study—placebo) and the presence of furry pets in the child's home, which are potential determinants of the exposure and therefore could be on the causal pathway between endotoxin exposure and the health outcomes studied, was assessed as part of a sensitivity analysis, where we additionally adjusted for these two variables. Ln-transformed endotoxin levels were used as continuous exposure variables in all analyses as smoothed curves (cubic splines estimated with SAS Proc GAM using the generalized cross validation function), suggested close to linear or at least monotonous relationships in most cases (Figures S2 and S3) and associations are presented as odds ratios (with 95% confidence intervals) for an interquartile range (IQR) increase in exposure on the ln-scale.

All analyses were performed with the Statistical Analysis System (SAS 9.2, Cary, NC, USA) for Windows. Statistical significance was defined by a two-sided a-level of 5%.

3 | RESULTS

3.1 | Population characteristics

Characteristics of the study population are presented in Table 1. The population is largely a non-farming population with only 1% reporting that they had lived on a farm during the child's first year of life and at age 5, respectively (data not shown). The percentage of children with allergic mothers is high in the total study population (Table 1) and in the subset with IgE data (Table S1). Table S2 provides the characteristics of the full PIAMA cohort and the subpopulations of the current study population with endotoxin data at ages 3 months and 5-6 years. The main difference between those who have endotoxin data and the full cohort is that participants of the intervention study (who have allergic mothers) are over-represented among those with endotoxin data. In addition, participants with endotoxin data at age 5-6 years, more often have highly educated parents. Other differences are generally small. The prevalence of asthma was between 10% and 12% at most ages; roughly, between one-third and two-thirds of the population were sensitized to any of the allergens tested at the different ages, and sensitization to food allergens was more common until the age of 4 years and sensitization to inhalant allergens thereafter (Figure 1).

3.2 | House dust endotoxin levels

Endotoxin loads for the child's mattresses (mostly new at the start of the study) increased during the course of the study. Endotoxin loads for living room floors were higher at age 5-6 years than at earlier ages, because of a higher percentage of samples collected on rugs at age 5-6 years, which yield larger amounts of dust. In contrast, average endotoxin concentrations per gram of mattress or living room floor dust were similar at ages 3 months and 5-6 years (Table 2). There was no correlation between endotoxin levels in repeated mattress dust samples and no correlation between endotoxin concentrations in repeated living room floor dust samples (Table S3), no correlation between endotoxin levels in mattress and living room floor dust samples for the same participant (Table S4), but some correlation between endotoxin loads in repeated living room floor dust samples ($r \leq 0.63$, Table S3).

3.3 | Associations of house dust endotoxin with asthma and allergic sensitization

Overall, after adjustment for covariates, endotoxin loads and concentrations in 3 months and 5-6 year dust samples were not associated with the prevalence of asthma until age 17 (Table 3). Age-specific association estimates suggest lower odds of asthma with increasing endotoxin levels in living room floor dust collected at age 3 months at ages 1-4 years (OR [95% CI] ranging from 0.70 [0.51-0.97] at age 1 to

TABLE 1 Participant characteristics

Covariate	n/N	(%)
Female sex	409/854	(47.9)
Allergic father	293/852	(34.4)
Allergic mother	750/854	(87.8)
Dutch nationality	716/793	(90.3)
Parental education		
Low	91/844	(10.8)
Medium	301/844	(35.7)
High	452/844	(53.6)
Breastfeeding ≥ 12 wk	413/809	(51.1)
Older siblings	431/850	(50.7)
Daycare attendance at age 2 y	453/795	(57.0)
Mother smoking during pregnancy	120/842	(14.3)
Smoking in child's home[a]	179/845	(21.2)
Use of gas for cooking[a]	697/846	(82.4)
Mould/damp spots in living and/or bedroom[a]	74/832	(8.9)
Furry pets in the child's home[a]	499/848	(58.8)
Study arm		
Natural history	200/854	(23.4)
Intervention, active cover	345/854	(40.4)
Intervention, placebo cover	309/854	(36.2)
CD14/rs2569190 genotype		
CC	143/599	(23.9)
CT	327/599	(54.6)
TT	129/599	(21.5)
TLR4/rs2737190 genotype		
AA	282/599	(47.1)
GA	250/599	(41.7)
GG	67/599	(11.2)
TLR4/ rs10759932 genotype		
TT	461/599	(77.0)
TC	126/599	(21.0)
CC	12/599	(2.0)
TLR4/ rs11536889 genotype		
GG	458/599	(76.5)
GC	159/599	(21.5)
CC	12/599	(2.0)
TLR4/ rs11536891 genotype		
TT	421/599	(70.3)
TC	158/599	(26.4)
CC	20/599	(3.3)
TLR4/ rs11734502 genotype		
GG	539/599	(90.0)
GA	58/599	(9.7)
AA	2/599	(0.3)

[a]During the 1st year of life.

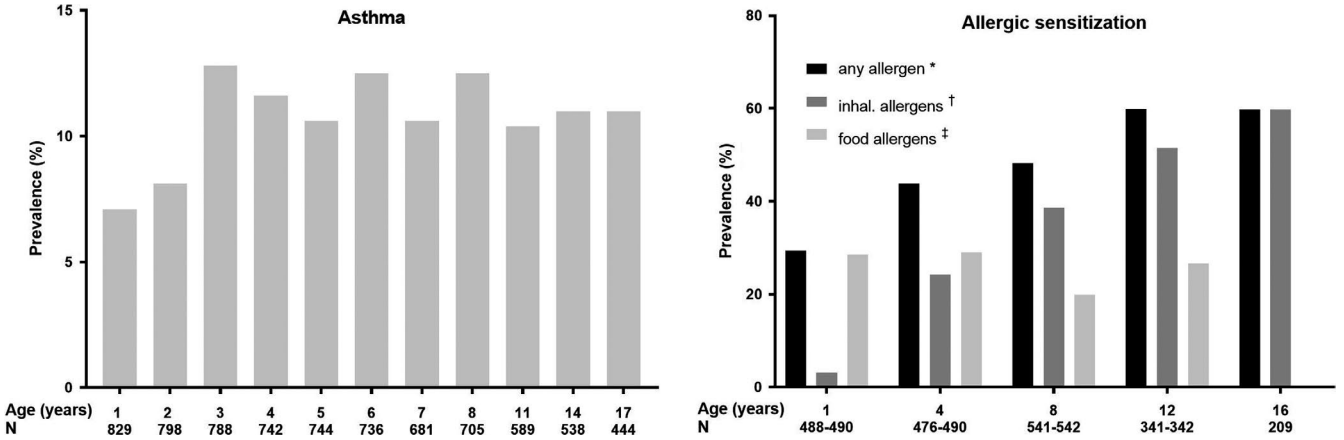

FIGURE 1 Prevalence of asthma and allergic sensitization from age 1 to 17 y

TABLE 2 Distribution of house dust endotoxin levels (in endotoxin units, EU) in mattress and living room floor dust

		Child's mattress				Living room floor[a]				
	Allergic mothers	GM (GSD)	P10	P90	N	GM (GSD)	P10	P90	N	Smooth floors
EU per m^2										
Pregnancy	100%	423 (4.8)	69	3435	190	508 (12.7)	22	17,656	129	79%
3 mo	100%	819 (3.2)	188	3547	610	495 (11.6)	18	13,248	612	80%
1 y	100%	1572 (3.4)	386	5174	91	328 (7.6)	20	5,080	124	85%
5-6 y	70%	2444 (2.5)	756	8765	344	2110 (10.0)	80	36,645	345	32%
EU per g dust										
Pregnancy	100%	11 733 (3.1)	2930	58,589	96	15 280 (4.4)	1690	143,255	87	69%
3 mo	100%	11 668 (2.7)	3627	41,308	494	14 093 (3.7)	2673	64,033	431	71%
1 y	100%	17 334 (2.7)	5350	62,625	81	12 580 (4.0)	2238	67,468	81	78%
5-6 y	70%	10 755 (2.1)	4539	28,416	345	14 569 (4.2)	3939	55,568	345	32%

[a]Wall-to-wall carpet and smooth floor otherwise until age 1; wall-to-wall carpet/rug > 4 m^2 and smooth floor otherwise at 5-6 y.

0.76 [0.57-1.00] at age 3), but not thereafter (Figure 2) and a higher risk of asthma at age 17 with higher living room floor dust endotoxin concentrations at age 5-6 years (OR [95% CI] 1.20 [0.99-1.45], Figures S4).

Also, there was no association between endotoxin levels at age 3 months and allergic sensitization until age 16 (Table 4 and Figure S5), but a positive association between endotoxin levels in living room floor dust collected at age 5-6 years and allergic sensitization to any allergen tested from age 8 onwards (Table 4). The positive associations with endotoxin levels in living room floor dust were largely driven by sensitization to inhalant allergens (Table S5), not sensitization to food allergens (Table S6), and fairly consistent across ages (Figures S6-S8).

3.4 | Effect modification

There were no statistically significant interactions between endotoxin exposure and *CD14*/rs2569190 genotype for asthma and allergic sensitization and no consistent patterns of stronger (inverse) associations in carriers of specific genotypes (Table S7). There were marginally significant ($P < .1$) interactions between endotoxin and the *TLR4*/rs2737190, *TLR4*/rs11536889 and *TLR4*/rs10759932 genotypes for asthma suggesting lower odds of asthma with higher mattress dust endotoxin levels among those with the GA *TLR4*/rs2737190 genotype (Table S8) and GG *TLR4*/rs11536889 and TC/CC *TLR4*/rs10759932 genotypes (Table S9), respectively. There were no significant interactions of *TLR4* SNPs with allergic sensitization (Tables S8 and S10).

Overall associations of house dust endotoxin in 3 months dust samples with asthma until age 17 were not significantly different between subjects with and without allergic sensitization (Figure S9, all *P*-values of interaction terms were >.23). Interactions with allergic sensitization and phenotype were not assessed for age-specific associations as numbers became too small.

3.5 | Sensitivity analyses

Estimates of the overall associations of endotoxin exposure with asthma and allergic sensitization did not change after additional

TABLE 3 Crude and adjusted overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 mo and 5-6 y with asthma from age 1 to 17

	Crude			Crude[a]			Adjusted[b]		
	N (n)[c]	OR[d]	(95% CI)	N (n)[c]	OR[d]	(95% CI)	N (n)[c]	OR[d]	(95% CI)
Dust collected at age 3 mo and asthma at age 1-17									
Child's mattress									
EU per m^2	610 (5187)	0.98	(0.81-1.18)	503 (4554)	1.00	(0.81-1.23)	503 (4554)	0.96	(0.77-1.19)
EU per g dust	494 (4202)	0.88	(0.70-1.10)	412 (3729)	0.92	(0.71-1.19)	412 (3729)	0.88	(0.67-1.16)
Living room floor									
EU per m^2	612 (5175)	0.76	(0.58-1.00)	500 (4519)	0.80	(0.58-1.10)	500 (4519)	0.84	(0.60-1.18)
EU per g dust	431 (3664)	0.83	(0.63-1.10)	351 (3180)	0.83	(0.60-1.15)	351 (3180)	0.85	(0.61-1.18)
Dust collected at age 5-6 y and asthma at age 6-17									
Child's mattress									
EU per m^2	342 (1804)	1.09	(0.84-1.43)	290 (1937)	1.07	(0.80-1.43)	290 (1937)	1.14	(0.85-1.52)
EU per g dust	343 (1807)	1.11	(0.78-1.58)	290 (1937)	1.05	(0.73-1.52)	290 (1937)	1.00	(0.69-1.44)
Living room floor									
EU per m^2	343 (1807)	0.96	(0.67-1.38)	289 (1533)	0.94	(0.63-1.39)	289 (1533)	0.88	(0.55-1.39)
EU per g dust	343 (1807)	1.02	(0.85-1.23)	289 (1533)	1.02	(0.84-1.24)	289 (1533)	1.04	(0.81-1.34)

[a]Restricted to participants with complete covariate data.

[b]Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mould/dampness in the child's home. Associations with 5-6 year dust are additionally adjusted for allergic sensitization to any allergen at age 4 to account for the nested case-control design.

[c]N = number of subjects; n = number of observations.

[d]Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 mo and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 y.

adjustment for study arm and the presence of pets in the child's home (change in odds ratio was ≤6.1%).

4 | DISCUSSION

Overall, the results of our longitudinal analysis provide no evidence for an association of endotoxin levels in mattress dust with asthma and allergic sensitization until age 17 years. However, higher endotoxin concentrations in living room floor dust at age 3 months tended to be associated with a lower odds of asthma until age 4, but not thereafter, whereas house dust endotoxin levels measured at age 5-6 years were associated with a higher odds of sensitization to inhalant allergens at ages 8 and older.

This longitudinal analysis extends earlier findings of an inverse association between endotoxin load of living room floor dust samples collected early in life and asthma at age 4 and a lack of association between endotoxin load of mattress dust samples collected at the age of 5-6 years and asthma at age 6 from a previous cross-sectional analysis within the same cohort.[33,38] The inverse associations of early life living room floor dust endotoxin exposure with asthma until age 4 (statistically significant for concentrations, marginally statistically significant ($P < .1$) for loads) are consistent with findings of previous European studies of preschool children aged 2 years[39] and 5 years,[10] albeit inconsistent with regard to the relevant exposure site (ie living room floor vs maternal or child's mattress). Studies assessing associations of house dust endotoxin with asthma in children beyond the preschool age are cross-sectional (also within cohort studies) and except one study[40] lack information on early life exposure to endotoxin.[4,6,16,17,20,38] Inverse associations between asthma and concurrent exposure to house dust endotoxin have been suggested by several studies in primary schoolchildren from the Netherlands[38] and North America,[17,40] but not from New Zealand.[16] A case-control study from Canada reported inverse associations of endotoxin with asthma in children aged 6-12 years, but not in adolescents aged 13-18 years.[19] Apart from that study, studies in adolescents are scarce and findings of these studies, which are all not limited to adolescents, are mixed.[4,6,20] A multicentre study of children aged 9-12 years from five European countries reported inverse associations between asthma and endotoxin load of living room floor dust.[20] In another European multicentre study of children aged 6-13 years from farming and non-farming households in rural areas, inverse associations of mattress dust endotoxin load were limited to atopic asthma[4] and no significant association was found between asthma and endotoxin in living room floor dust in another study of children aged 5-14 years.[6] Inverse associations have been found to be limited to atopic asthma in one study,[4] but not in the present study and another study.[41] Since differences in associations of house

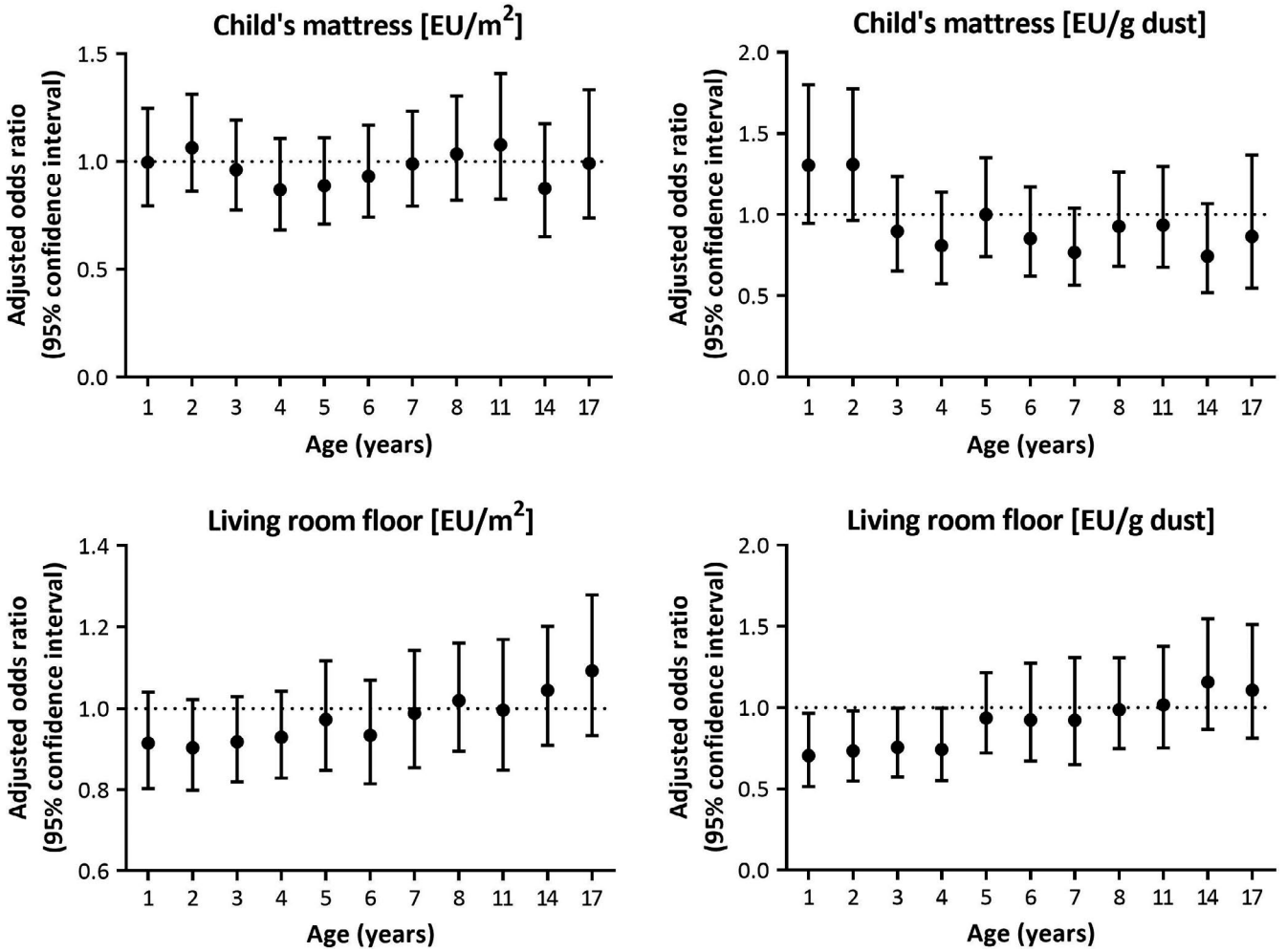

FIGURE 2 Age-specific adjusted * associations † between asthma at ages 1 to 17 y and endotoxins in mattress and living room floor dust samples collected at age 3 mo. †Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mould/dampness in the child's home. ‡Associations are presented for an interquartile range increase in exposure.

dust endotoxin with asthma between atopic and non-atopic subjects are often not taken into account, this may explain some of the heterogeneity in study findings. Taking all the evidence together, there is growing evidence for an inverse association between house dust endotoxin exposure early in life and asthma in preschool children, but not for such an association in schoolchildren and adolescents.

Studies assessing *CD14* and *TLR4* genotype-endotoxin interactions for asthma are scarce. Our findings on the interaction between endotoxin and *CD14*/rs2569190 genotype are of interest, but inconclusive. They warrant further study as numbers are small in our study and the only other (case-control) study from Barbados, West Indies that found an antagonistic interaction between endotoxin levels in the home and *CD14*/rs2569190 genotype with the TT genotype being protective for asthma among subjects with low exposure to endotoxin and associated with a higher risk of asthma among subjects with high exposure to endotoxin.[42] Moreover, we found some evidence for a modification of associations between asthma and mattress dust endotoxin levels early in life by the *TLR4*/rs2737190, *TLR4*/rs11536889 and *TLR4*/rs10759932 genotypes that are of interest. These SNPs that have been assessed as effect modifiers in farmer children previously, but no significant interactions have been observed in the earlier study.[28]

Fewer studies assessed the relationship between house dust endotoxin exposure and allergic sensitization, but findings of these studies are more consistent and suggest inverse associations in infants aged 1-2 years,[9,10] preschool[10] and schoolchildren[4,6] from Europe and the United States. Findings from a cohort from the United States were inconsistent with a lower odds of sensitization at age 7 in the second and third quartiles, but not in the fourth quartile of family room dust endotoxin concentrations measured early in life.[5] Besides in the present study, positive associations with allergic sensitization have only been reported from a European multicentre at the age of 1 year.[39] The mechanisms underlying these positive associations are not clear. Moreover, differences in study design, definitions of outcome or exposure, and statistical analysis are no likely explanations for the opposite associations as there are no systematic differences between studies showing positive and inverse associations. Exposure levels are difficult to compare between

TABLE 4 Crude and adjusted overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 mo and 5-6 y and allergic sensitization[a] from age 1 to 16

	Crude			Crude[b]			Adjusted[c]		
	N (n)[d]	OR[e]	(95% CI)	N (n)[d]	OR[e]	(95% CI)	N (n)[d]	OR[e]	(95% CI)
Dust collected at age 3 mo and sensitization at age 1-16									
Child's mattress									
EU per m^2	540 (1409)	0.89	(0.75-1.06)	457 (1226)	0.93	(0.77-1.12)	457 (1226)	0.98	(0.80-1.19)
EU per g dust	434 (1131)	0.88	(0.71-1.10)	371 (996)	0.91	(0.72-1.14)	371 (996)	0.97	(0.75-1.25)
Living room floor									
EU per m^2	546 (1424)	0.84	(0.68-1.04)	459 (1232)	0.87	(0.69-1.10)	459 (1232)	0.91	(0.71-1.16)
EU per g dust	387 (994)	1.00	(0.81-1.23)	326 (860)	1.03	(0.82-1.28)	326 (860)	1.05	(0.82-1.34)
Dust collected at age 5-6 y and sensitization at ages 8-16									
Child's mattress									
EU per m^2	306 (599)	1.05	(0.83-1.33)	271 (534)	1.14	(0.89-1.47)	271 (534)	1.13	(0.87-1.48)
EU per g dust	306 (599)	1.16	(0.88-1.53)	271 (534)	1.15	(0.86-1.53)	271 (534)	1.13	(0.84-1.52)
Living room floor									
EU per m^2	306 (599)	**1.51**	**(1.10-2.09)**	270 (533)	**1.55**	**(1.09-2.19)**	270 (533)	**1.70**	**(1.17-2.47)**
EU per g dust	306 (599)	1.15	(0.96-1.38)	270 (533)	1.19	(0.97-1.46)	270 (533)	1.24	(1.00-1.55)

$p < 0.05$ for all values that are presented in bold.

[a]Against any of the allergens tested: house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata*, *Alternaria alternata*, milk and/or egg as available at the different ages.

[b]Restricted to participants with complete covariate data.

[c]Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mould/dampness in the child's home. Associations with allergic sensitization were not additionally adjusted for allergic sensitization to any allergen at age 4 despite the nested case-control design as this may be on the causal pathway between exposure and allergic sensitization after age 4.

[d]N = number of subjects; n = number of observations.

[e]Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load) and 4.8-fold (living room floor, concentration) increases in exposure at age 3 mo and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load) and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 y.

studies because of differences in sampling location, sample collection, extractions and analysis, but are also not a likely explanation of the inconsistent effects as inverse associations have not only been shown in farming populations as described above, where exposure levels are much higher than in non-farming population. Differences with regard to the form of endotoxin may explain the inconsistency in associations with endotoxin between the present study and earlier studies. Gram-negative bacteria have several forms of endotoxin that have different effects on asthma and allergic sensitization. For example, pentaacylated endotoxin has been found to be associated with lower risks, while hexaacylated endotoxin has been found to be associated with higher risks.[43,44] However, neither in our study nor from earlier studies, information is available on different forms of endotoxin. Alternatively, co-exposure to other environmental organisms may explain inconsistencies between studies. In the GABRIELA study, it has been shown that the diversity of exposure to micro-organisms was associated with asthma; this was, however, not the case for sensitization.[45] Also, genetics may at least partly explain the contradicting findings. We could not confirm the findings of other studies suggesting an effect modification by single-nucleotide polymorphisms in the CD14 receptor,[26,27] through which endotoxin interacts with monocytes and macrophage, epithelial and endothelial cells.

Strengths of the present study include the longitudinal design with repeated assessments of health outcomes and exposures, which allowed us to assess exposure-response relationships at different ages from birth until adolescence, with exposure measurements preceding assessments of health outcomes, which has been an important limitation of previous cross-sectional studies. With the repeated exposure measurements were able to contribute to the currently limited evidence on the variability of house dust endotoxin levels over time, to show that living room floor dust endotoxin loads tend to be slightly more stable than mattress dust endotoxin levels in this age group, and to assess the relevance of exposure at different time-points. Nevertheless, we acknowledge that exposure measurements were largely limited to two time-points (3 months and 5-6 years) as the collection and analysis of dust samples is costly. Studies with more frequent endotoxin measurements are needed to assess the relevance of continuity or changes in exposure. It is currently not clear, which site is more relevant with regard to health outcomes, mattresses or living room floors, as associations have been shown for both exposure sites as discussed above. With

measurements of endotoxin levels in both mattress and living room floor dust, we demonstrated heterogeneity of endotoxin levels within homes, which means that one sample is not sufficient to characterize an individual's residential exposure. Limitations include the lack of exposure data beyond the age of 5-6 years. Several studies suggested that the effects of endotoxin on asthma and allergies are not limited to exposures during childhood, by showing associations with exposures in adulthood.[8,11,46] There is some evidence for seasonal variation of endotoxin concentrations.[47] Season of dust sampling varied between study participants, but was not associated with the health outcomes studied. Therefore, this may have introduced some random exposure measurement error and may have biased associations towards the null. Moreover, the number of subjects with exposure at age 5-6 years is small. Furthermore, endotoxin in house dust may at least partly act as a proxy for other microbial exposures such as β(1, 3) glucans[48] and other bacterial components[49] with immune-stimulatory properties. For the definition of asthma, we relied on questionnaire-reports rather than objective measures as objective measurements are not feasible in large epidemiological studies. Consequently, we cannot rule out reporting bias. Since study participants were unaware of their endotoxin exposure, outcome misclassification is most likely non-differential and (if anything) may have biased association estimates towards the null. Children of allergic mothers are over-represented in the current study sample, which is efficient as these are more prone to allergies, but may limit the generalizability of our findings to the entire cohort and the general population. Moreover, the number of children with non-allergic mothers is too small to allow separate analyses, but so far there is no evidence for potential effect modifications by parental allergy. Also, children of non-Dutch non-Western parents are under-represented and may limit the generalizability.

In conclusion, house dust endotoxin may have beneficial effects on asthma in preschool children of allergic mothers, which do not persist into adolescence. We did not find any evidence for a beneficial effect on allergic sensitization.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

The PIAMA data are not freely accessible in the public domain, because this would be in conflict with the agreement between the PIAMA study team and the PIAMA participants. However, the data underlying the findings presented in this paper are available on request. Requests can be submitted to the PIAMA Principal Investigators (see at http://piama.iras.uu.nl/index-en.php#collaboration more for information).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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House dust endotoxin, asthma and allergic sensitization through childhood into adolescence

Online supplement

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Methods

House dust collection

In brief, trained fieldworkers vacuumed the entire mattress surface area after removal of blankets and duvets, but not sheets, and 1 or 2 m^2 of the living room floor (wall-to-wall carpet or smooth floor otherwise until age 1; wall-to-wall carpet/rug > $4m^2$ and smooth floor otherwise at 5-6 years) for 2 minutes according to a standardized protocol. Dust was collected using vacuum cleaners equipped with special nozzles (ALK allergen mouthpiece; Hørsholm, Denmark) to collect the dust on paper (3 months) and glass fiber filters (5-6 years).

Genotyping

Genotyping was done using three different platforms: Illumina Omni Express Exome (OEE) chip (n=1377), Illumina Omni Express (OE) chip (n=288), and Illumina Human610 (HM610) quad array (n=404) (Illumina Inc, San Diego, CA). The *CD14*/rs2569190, *TLR4*/rs2737190 (in complete linkage disequilibrium with *TRL4*/rs1927914), *TLR4*/rs10759932, *TLR4*/rs11536889, *TLR4*/rs11536891, and *TLR4*/rs11734502 (in complete linkage disequilibrium with *TLR4*/rs4986790) SNPs were included on all platforms. Quality control inclusion measures per chip on the individuals included a missing genotype call rate <0.03, IBS <0.1875 and a heterozygosity rate deviating <4SD from the mean. Males with >1% heterozygote SNPs on chromosome X were excluded. Ethnicity was assessed using principal component analyses with HapMap CEU, CHB+JPT, and YRI reference panels, only Caucasians subject were included. QC measures per SNP included missing genotype call rate <0.05, MAF >0.05 and Hardy-Weinberg equilibrium P-value >10-6. SNPs being >1% heterozygous in males on chromosome X were excluded.

Covariates

Parental education was defined as the maximum educational level attained by the mother or father; low: primary school, lower vocational, or lower secondary education; medium: intermediate vocational education or intermediate/higher secondary education; high: higher vocational education and university.

Table S1. Overview of specific IgE measurements at ages 4 to 16 in PIAMA.

Age (years)	Subgroup	allergic mothers	Specific IgE against							
			Egg	Milk	*Dermatophagoides pteronyssinus*	Cat	Dog	Birch	*Dactylis glomerata*	*Alternaria alternata*
4	Intensive follow-up *	79%	x	x	x	x	x	x	x	x
8	All	85%	x	x	x	x	x	x	x	x
12	All	82%	x	x	x	x		x	x	
16	All	80%			x	x		x	x	

* covers all high risk children (i.e. children of allergic mothers, n=1,245) and a random sample of low-risk children (n=663)

Table S2. Participant characteristics for the full PIAMA cohort (n=3,963) and the current study population with data on endotoxin exposure at ages 3 months (n=651) and 5-6 years (n=346)

	Full PIAMA cohort		**Endotoxin data age 3 months**		**Endotoxin data age 5-6 years**	
Variable	**n/N**	**(%)**	**n/N**	**(%)**	**n/N**	**(%)**
Female sex	1,906/3,963	(48.1)	325/651	(49.9)	150/346	(43.4)
Allergic father	1,217/3,957	(30.8)	**234/649**	**(36.1)**	113/346	(32.7)
Allergic mother	1,237/3,963	(31.2)	**651/651**	**(100.0)**	**242/346**	**(69.9)**
Dutch nationality	3,327/3,684	(90.3)	536/593	(90.4)	305/334	(91.3)
Parental education						
Low	502/3,812	(13.2)	76/642	(11.8)	**30/345**	**(8.7)**
Medium	1,402/3,812	(36.8)	230/642	(35.8)	**115/345**	**(33.3)**
High	1,908/3,812	(50.1)	336/642	(52.3)	**200/345**	**(58.0)**
Breastfeeding ≥ 12 weeks	1,703/3,707	(45.9)	298/616	(48.4)	**187/330**	**(56.7)**
Older siblings	1,992/3,939	(50.6)	324/647	(50.1)	179/346	(51.7)
Daycare attendance at age 2 years	2,131/3,703	(57.5)	339/593	(57.2)	196/336	(58.3)
Mother smoking during pregnancy	703/3,920	(17.9)	**87/641**	**(13.6)**	**47/344**	**(13.7)**
Smoking in child's home [*]	957/3,815	(25.1)	143/643	(22.2)	**64/345**	**(18.6)**
Use of gas for cooking [*]	3,253/3,935	(82.7)	530/644	(82.3)	290/344	(84.3)
Mold/damp spots in living and/or bedroom [*]	312/3,698	(8.4)	59/631	(9.4)	23/342	(6.7)
Furry pets in the child's home [*†]	1,845/3,938	(46.9)	**266/645**	**(41.2)**	**141/346**	**(40.8)**
Study arm						
Natural history	394/3,963	(9.9)	**11/651**	**(1.7)**	**154/346**	**(55.5)**
Intervention, active cover	365/3,963	(9.2)	**337/651**	**(51.8)**	**82/346**	**(23.7)**
Intervention, placebo cover	3,204/3,963	(80.8)	**305/651**	**(46.9)**	**72/346**	**(20.8)**

[*] During the 1st year of life

[†] Cat and/or dog and/or rodent

[$] Participants that were randomized to the intervention study, but eventually never received any mattress cover.

Table S3. Spearman correlations between endotoxin concentrations in repeated mattress and living room dust samples.

	Pregnancy	3 months	1 year	5-6 years
Child's mattress				
EU per m^2				
Pregnancy	1.00 (n=190)	0.13 (n=179)	0.10 (n=89)	0.08 (n=44)
3 months		1.00 (n=610)	0.21 (n=90)	0.11 (n=130)
1 year			1.00 (n=91)	0.17 (n=22)
5-6 years				1.00 (n=344)
EU per g dust				
Pregnancy	1.00 (n=96)	0.14 (n=74)	0.06 (n=37)	-0.29 (n=26)
3 months		1.00 (n=494)	0.16 (n=64)	0.19 (n=102)
1 year			1.00 (n=81)	0.09 (n=20)
5-6 years				1.00 (n=345)
Living room floor *				
EU per m^2				
Pregnancy	1.00 (n=129)	0.63 (n=120)	0.35 (n=102)	0.15 (n=30)
3 months		1.00 (n=612)	0.50(n=116)	0.25 (n=137)
1 year			1.00 (n=124)	- 0.10 (n=28)
5-6 years				1.00 (n=345)
EU per g dust				
Pregnancy	1.00 (n=87)	0.26 (n=60)	0.07 (n=46)	0.00 (n=18)
3 months		1.00 (n=431)	0.30(n=54)	0.16 (n=99)
1 year			1.00 (n=81)	-0.21 (n=19)
5-6 years				1.00 (n=345)

* wall-to-wall carpet and smooth floor otherwise up to age 1; wall-to-wall carpet/rug > $4m^2$ and smooth floor otherwise

Table S4. Spearman correlations between endotoxin concentrations in mattress and living room dust samples.

	Pregnancy	**3 months**	**1 year**	**5-6 years**
EU per m^2	0.03 (n=120)	0.11 (n=571)	0.10 (n=86)	0.11 (n=343)
EU per g dust	0.05 (n=37)	0.16 (n=328)	-0.11 (n=54)	0.09 (n=344)

Table S5 Crude and adjusted overall associations between allergic sensitization to any inhalant allergen tested [*] and endotoxins in mattress and living room floor dust samples.

	Crude			Crude [†]			Adjusted [‡]		
	N (n) [§]	OR [‖]	(95% CI)	N (n) [§]	OR [‖]	(95% CI)	N (n) [§]	OR [‖]	(95% CI)
Allergic sensitization to inhalant allergens at age 1-16 and endotoxin at age 3 months									
Child's mattress									
EU per m^2	539 (1,415)	1.00	(0.79-1.26)	457 (1,232)	0.98	(0.76-1.25)	457 (1,232)	0.98	(0.75-1.28)
EU per g dust	433 (1,135)	0.92	(0.69-1.23)	371 (1,000)	0.89	(0.66-1.21)	371 (1,000)	0.92	(0.66-1.28)
Living room floor									
EU per m^2	545 (1,429)	0.93	(0.69-1.25)	459 (1,237)	0.97	(0.70-1.33)	459 (1,237)	0.97	(0.70-1.34)
EU per g dust	386 (998)	1.16	(0.88-1.52)	326 (864)	1.12	(0.84-1.50)	326 (864)	1.19	(0.88-1.61)
Allergic sensitization to inhalant allergens at age 8-16 and endotoxin at age 5-6 years									
Child's mattress									
EU per m^2	306 (599)	1.13	(0.88-1.43)	271 (534)	1.17	(0.90-1.50)	271 (534)	1.18	(0.91-1.54)
EU per g dust	306 (599)	1.27	(0.94-1.71)	271 (534)	1.26	(0.91-1.72)	271 (534)	1.26	(0.92-1.73)
Living room floor									
EU per m^2	306 (599)	**1.56**	**(1.12-2.16)**	270 (533)	**1.51**	**(1.06-2.14)**	270 (533)	**1.66**	**(1.15-2.40)**
EU per g dust	306 (599)	**1.21**	**(1.00-1.47)**	270 (533)	1.21	(0.98-1.49)	270 (533)	**1.26**	**(1.01-1.57)**

[*] House dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata* and/or *Alternaria alternate* egg as available at the different ages

[†] Restricted to participants with complete covariate data.

[‡] Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home. `

[‡] N = number of subjects; n = number of observations

[‖] Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 months and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 years.

Table S6. Crude and adjusted overall associations between allergic sensitization to any food allergen tested [*] at 1 to 16 years and endotoxins in mattress and living room floor dust samples.

	Crude			Crude [†]			Adjusted [‡]		
	N (n) [§]	OR [‖]	(95% CI)	N (n) [§]	OR [‖]	(95% CI)	N (n) [§]	OR [‖]	(95% CI)
Allergic sensitization to food allergens at age 1-16 and endotoxin at age 3 months									
Child's mattress									
EU per m^2	536 (1,275)	0.82	(0.66-1.01)	453 (1,102)	0.86	(0.69-1.07)	453 (1,102)	0.92	(0.74-1.15)
EU per g dust	430 (1,020)	0.82	(0.64-1.06)	367 (892)	0.85	(0.65-1.10)	367 (892)	0.91	(0.69-1.20)
Living room floor									
EU per m^2	542 (1,292)	0.85	(0.67-1.09)	455 (1,111)	0.87	(0.66-1.14)	455 (1,111)	0.89	(0.67-1.17)
EU per g dust	383 (905)	0.94	(0.74-1.19)	322 (780)	1.01	(0.79-1.30)	322 (780)	0.98	(0.74-1.30)
Allergic sensitization to food allergens at age 8-16 and endotoxin at age 5-6 years									
Child's mattress									
EU per m^2	301 (482)	0.88	(0.66-1.19)	266 (431)	0.91	(0.68-1.23)	266 (431)	0.90	(0.66-1.22)
EU per g dust	301 (482)	0.93	(0.67-1.28)	266 (431)	0.84	(0.61-1.15)	266 (431)	0.82	(0.58-1.14)
Living room floor									
EU per m^2	301 (482)	1.26	(0.84-1.89)	265 (430)	1.34	(0.88-2.05)	265 (430)	1.34	(0.85-2.10)
EU per g dust	301 (482)	1.01	(0.80-1.29)	265 (430)	1.07	(0.82-1.39)	265 (430)	1.06	(0.80-1.41)

[*] House dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata* and/or *Alternaria alternate* egg as available at the different ages

[†] Restricted to participants with complete covariate data.

[‡] Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home. `

[‡] N = number of subjects; n = number of observations

[‖] Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 months and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 years.

Table S7. Adjusted [*] overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 months with asthma from age 1 to 17 and allergic sensitization [†] from age 1 to 16 by CD14/rs2569190 genotype from models with endotoxin-genotype interaction terms.

	CC			CT			TT			p-value
	N (n) [‡]	OR [§]	(95% CI)	N (n) [‡]	OR [§]	(95% CI)	N (n) [‡]	OR [§]	(95% CI)	(int.)
Asthma										
Child's mattress										
EU per m^2	83 (810)	0.97	(0.57-1.65)	191 (1,877)	0.93	(0.67-1.29)	77 (755)	0.58	(0.30-1.12)	0.3915
EU per g dust	70 (683)	0.72	(0.39-1.32)	157 (1,548)	0.90	(0.63-1.30)	59 (574)	0.61	(0.31-1.20)	0.5339
Living room floor										
EU per m^2	85 (830)	1.16	(0.46-2.93)	190 (1,876)	0.73	(0.43-1.25)	76 (745)	0.95	(0.48-1.90)	0.6299
EU per g dust	64 (625)	0.69	(0.29-1.66)	134 (1,320)	0.74	(0.44-1.26)	50 (486)	1.03	(0.56-1.88)	0.6296
Allergic sensitization										
Child's mattress										
EU per m^2	83 (243)	1.17	(0.75-1.81)	189 (585)	0.87	(0.65-1.16)	77 (240)	1.12	(0.64-1.94)	0.4734
EU per g dust	70 (205)	0.87	(0.51-1.47)	155 (480)	0.96	(0.67-1.38)	59 (180)	1.04	(0.53-2.03)	0.9068
Living room floor										
EU per m^2	85 (249)	1.14	(0.65-2.01)	188 (582)	0.76	(0.53-1.08)	76 (239)	0.88	(0.49-1.57)	0.4852
EU per g dust	64 (191)	0.67	(0.40-1.14)	133 (402)	1.06	(0.77-1.46)	50 (159)	0.87	(0.43-1.76)	0.3433

[*] Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

[†] Against any of the allergens tested: house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata*, *Alternaria alternata*, milk and/or egg as available at the different ages

[‡] N = number of subjects; n = number of observations.

[§] Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 months and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 years.

Table S8. Adjusted [*] overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 months with asthma from age 1 to 17 and allergic sensitization [†] from age 1 to 16 by *TLR4*/rs2737190 genotype from models with endotoxin-genotype interaction terms.

	AA			GA			GG			p-value
	N (n) [‡]	OR [§]	(95% CI)	N (n) [‡]	OR [§]	(95% CI)	N (n) [‡]	OR [§]	(95% CI)	(int.)
Asthma										
Child's mattress										
EU per m^2	166 (1,626)	1.05	(0.67-1.62)	140 (1,384)	0.66	(0.47-0.94)	45 (423)	1.05	(0.61-1.80)	0.2057
EU per g dust	135 (1,327)	1.13	(0.72-1.79)	120 (1,180)	0.56	(0.36-0.88)	31 (298)	0.61	(0.28-1.31)	0.0814
Living room floor										
EU per m^2	168 (1,650)	0.68	(0.39-1.19)	137 (1,356)	0.98	(0.50-1.94)	46 (445)	1.53	(0.58-4.05)	0.3159
EU per g dust	113 (1,103)	0.68	(0.31-1.50)	102 (1,015)	0.85	(0.47-1.53)	33 (313)	1.16	(0.63-2.13)	0.5744
Allergic sensitization										
Child's mattress										
EU per m^2	165 (511)	1.11	(0.82-1.51)	140 (437)	0.74	(0.53-1.03)	44 (120)	1.03	(0.61-1.73)	0.1828
EU per g dust	134 (413)	1.09	(0.75-1.60)	120 (372)	0.75	(0.52-1.09)	30 (80)	1.26	(0.58-2.70)	0.2855
Living room floor										
EU per m^2	167 (520)	0.83	(0.55-1.26)	137 (428)	0.85	(0.55-1.31)	45 (122)	1.21	(0.58-2.53)	0.6740
EU per g dust	112 (335)	0.93	(0.62-1.37)	102 (326)	0.86	(0.57-1.30)	33 (91)	1.39	(0.75-2.59)	0.4452

[*] Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

[†] Against any of the allergens tested: house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata*, *Alternaria alternata*, milk and/or egg as available at the different ages

[‡] N = number of subjects; n = number of observations.

[§] Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 months and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 years.

Table S9. Adjusted [*] overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 months with asthma from age 1 to 17 by the*TLR4*/ rs10759932, *TLR4*/rs11536889, *TLR4*/rs11536891, and *TLR4*/rs11734502 genotypes (dichotomized by minor allele carriage) from models with endotoxin-genotype interaction terms.

	N (n) [‡]	OR [§]	(95% CI)	N (n) [‡]	OR [§]	(95% CI)	(int.)
			***TLR4*/rs10759932 genotype**				
		TT			**TC/CC**		
Child's mattress							
EU per m^2	271 (2,666)	0.85	(0.62-1.16)	80 (776)	0.99	(0.58-1.66)	0.6313
EU per g dust	226 (2,221)	0.91	(0.66-1.26)	60 (584)	0.46	(0.23-0.90)	0.0695
Living room floor							
EU per m^2	272 (2,681)	0.75	(0.47-1.21)	79 (770)	1.54	(0.71-3.37)	0.1217
EU per g dust	187 (1,846)	0.77	(0.47-1.28)	61 (585)	1.02	(0.59-1.78)	0.4703
			***TLR4*/rs11536889 genotype**				
		GG			**GC/CC**		
Child's mattress							
EU per m^2	273 (2,673)	0.77	(0.60-1.00)	78 (769)	1.42	(0.74-2.71)	0.0864
EU per g dust	224 (2,188)	0.72	(0.51-1.02)	62 (617)	1.22	(0.69-2.15)	0.1157
Living room floor							
EU per m^2	278 (2,734)	0.91	(0.58-1.43)	73 (717)	0.70	(0.33-1.48)	0.5490
EU per g dust	196 (2,916)	0.82	(0.54-1.24)	52 (505)	0.86	(0.28-2.63)	0.9447
			***TLR4*/rs11536891 genotype**				
		TT			**TT/TC**		
Child's mattress							
EU per m^2	248 (2,443)	0.96	(0.66-1.39)	103 (999)	0.76	(0.53-1.09)	0.3987
EU per g dust	194 (1,911)	0.88	(0.61-1.28)	92 (1,894)	0.68	(0.42-1.11)	0.4097
Living room floor							
EU per m^2	248 (2,444)	0.82	(0.54-1.25)	103 (1,007)	1.04	(0.47-2.34)	0.5829
EU per g dust	168 (1,650)	0.70	(0.43-1.14)	80 (781)	1.11	(0.57-2.16)	0.2621
			***TLR4*/rs11734502 genotype**				
		GG			**GA/AA**		
Child's mattress							
EU per m^2	315 (3,085)	0.91	(0.69-1.21)	36 (357)	0.52	(0.21-1.25)	0.2270
EU per g dust	257 (2,523)	0.86	(0.62-1.18)	29 (282)	0.42	(0.15-1.18)	0.1967
Living room floor							
EU per m^2	314 (3,078)	0.85	(0.55-1.30)	37 (373)	1.03	(0.37-2.87)	0.7259
EU per g dust	226 (2,206)	0.83	(0.54-1.27)	22 (225)	0.81	(0.41-1.62)	0.9614

[*] Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

[‡] N = number of subjects; n = number of observations.

[§] Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 months and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 years.

Table S10. Adjusted [*] overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 months with allergic sensitization [†] from age 1 to 16 by the *TLR4*/rs10759932, *TLR4*/rs11536889, *TLR4*/rs11536891, and *TLR4*/rs11734502 genotypes (dichotomized by minor allele carriage) from models with endotoxin-genotype interaction terms.

	Homozygous major allele			Minor allele			p-value
	N (n) [‡]	OR [§]	(95% CI)	N (n) [‡]	OR [§]	(95% CI)	(int.)
				***TLR4*/rs10759932 genotype**			
Child's mattress							
EU per m^2	831 (270)	0.91	(0.71-1.16)	237 (79)	1.14	(0.73-1.78)	0.3714
EU per g dust	686 (225)	0.92	(0.69-1.24)	179 (59)	0.99	(0.53-1.85)	0.8496
Living room floor							
EU per m^2	836 (271)	0.81	(0.59-1.10)	234 (78)	1.15	(0.66-2.02)	0.2888
EU per g dust	569 (186)	0.93	(0.69-1.27)	183 (61)	1.06	(0.65-1.73)	0.6799
				***TLR4*/rs11536889 genotype**			
Child's mattress							
EU per m^2	818 (272)	0.93	(0.73-1.19)	250 (77)	1.00	(0.63-1.60)	0.7945
EU per g dust	661 (223)	0.89	(0.65-1.21)	204 (61)	1.10	(0.64-1.88)	0.5003
Living room floor							
EU per m^2	832 (276)	0.89	(0.67-1.20)	238 (73)	0.79	(0.41-1.52)	0.7254
EU per g dust	584 (195)	1.02	(0.75-1.37)	168 (52)	0.77	(0.38-1.58)	0.4836
				***TLR4*/rs11536891 genotype**			
Child's mattress							
EU per m^2	768 (247)	1.00	(0.76-1.33)	300 (102)	0.83	(0.60-1.16)	0.4050
EU per g dust	601 (193)	0.95	(0.68-1.32)	264 (91)	0.91	(0.59-1.40)	0.8745
Living room floor							
EU per m^2	772 (247)	0.96	(0.69-1.34)	298 (102)	0.70	(0.44-1.13)	0.2935
EU per g dust	512 (167)	1.00	(0.72-1.40)	240 (80)	0.89	(0.59-1.34)	0.6557
				***TLR4*/rs11734502 genotype**			
Child's mattress							
EU per m^2	961 (313)	1.00	(0.81-1.25)	107 (36)	0.51	(0.23-1.16)	0.1185
EU per g dust	783 (255)	0.98	(0.75-1.29)	82 (29)	0.67	(0.29-1.51)	0.3760
Living room floor							
EU per m^2	958 (312)	0.83	(0.62-1.10)	112 (37)	1.25	(0.53-2.97)	0.3699
EU per g dust	682 (225)	0.98	(0.75-1.29)	70 (22)	0.84	(0.37-1.94)	0.7298

[*] Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

[†] Against any of the allergens tested: house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata*, *Alternaria alternata*, milk and/or egg as available at the different ages

[‡] N = number of subjects; n = number of observations.

[§] Associations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 months and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 years.)

Figure S1. House dust endotoxin and asthma: data availability.

	Pregnancy	3 months	1 year	5-6 years
Child's mattress				
/m^2	208	648	93	345
/g of dust	107	524	83	344
Living room floor				
/m^2	144	650	129	345
/g of dust	95	460	85	345
Sample *	IS	IS	IS	IS & NHS, (sensitized cases and non-sensitized controls)

Any data on endotoxin in dust at ages 3 months and/or 5-6 years:
N = 894

Information on asthma at any age up to 17 years:
N = 854

* IS = intervention study, NHS = natural history study

Figure S2. Age-adjusted smoothing splines with 95% confidence bands for the association between asthma at ages 1-17 years and house dust endotoxin at age 3 months (left column) and asthma at ages 6-17 years and house dust endotoxin at age 5-6 years (right column).

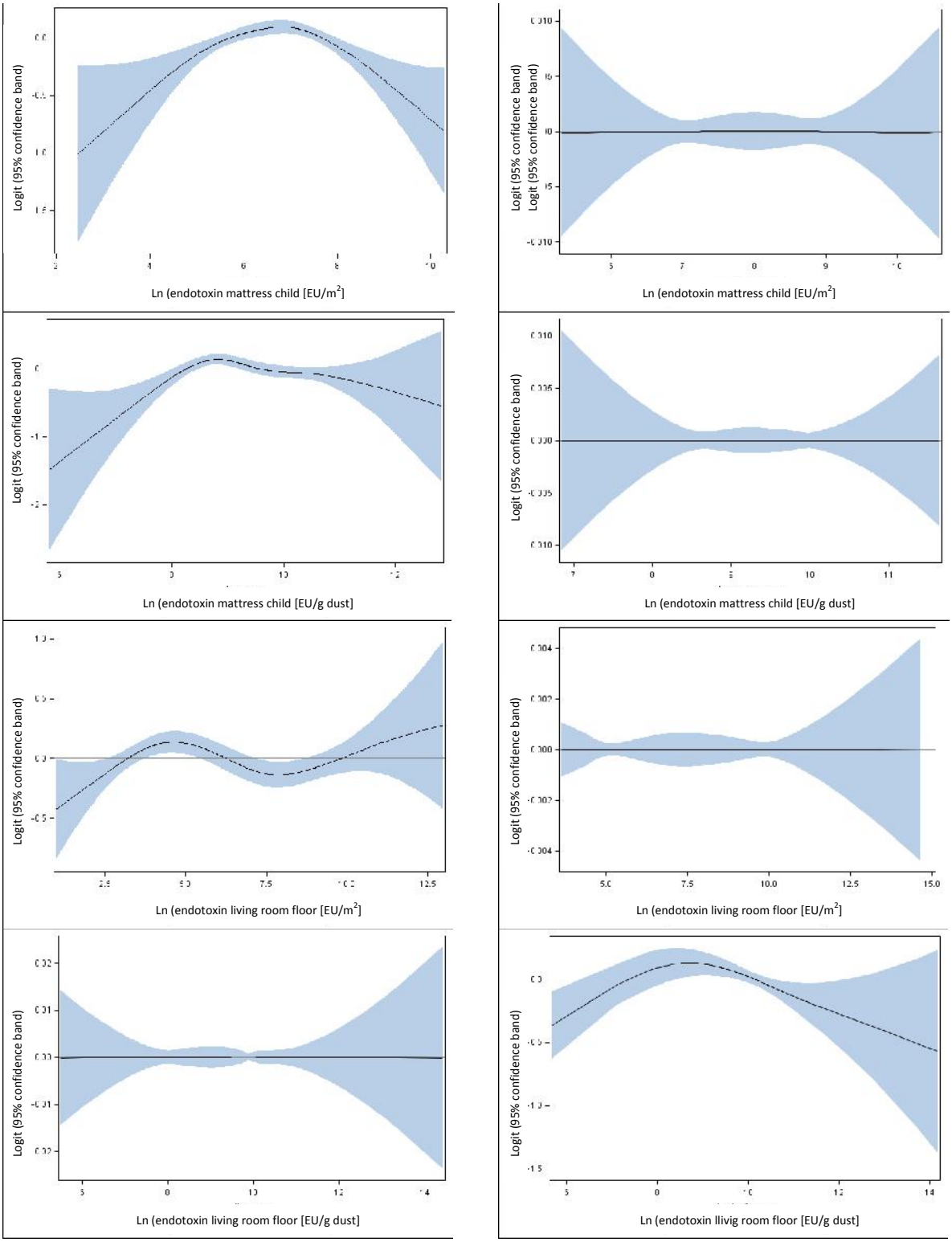


Figure S3. Age-adjusted smoothing splines with 95% confidence bands for the association between any allergic sensitization at ages 1-16 years and house dust endotoxin at age 3 months (left column) and any allergic sensitization at ages 8-16 years and house dust endotoxin at age 5-6 years (right column).

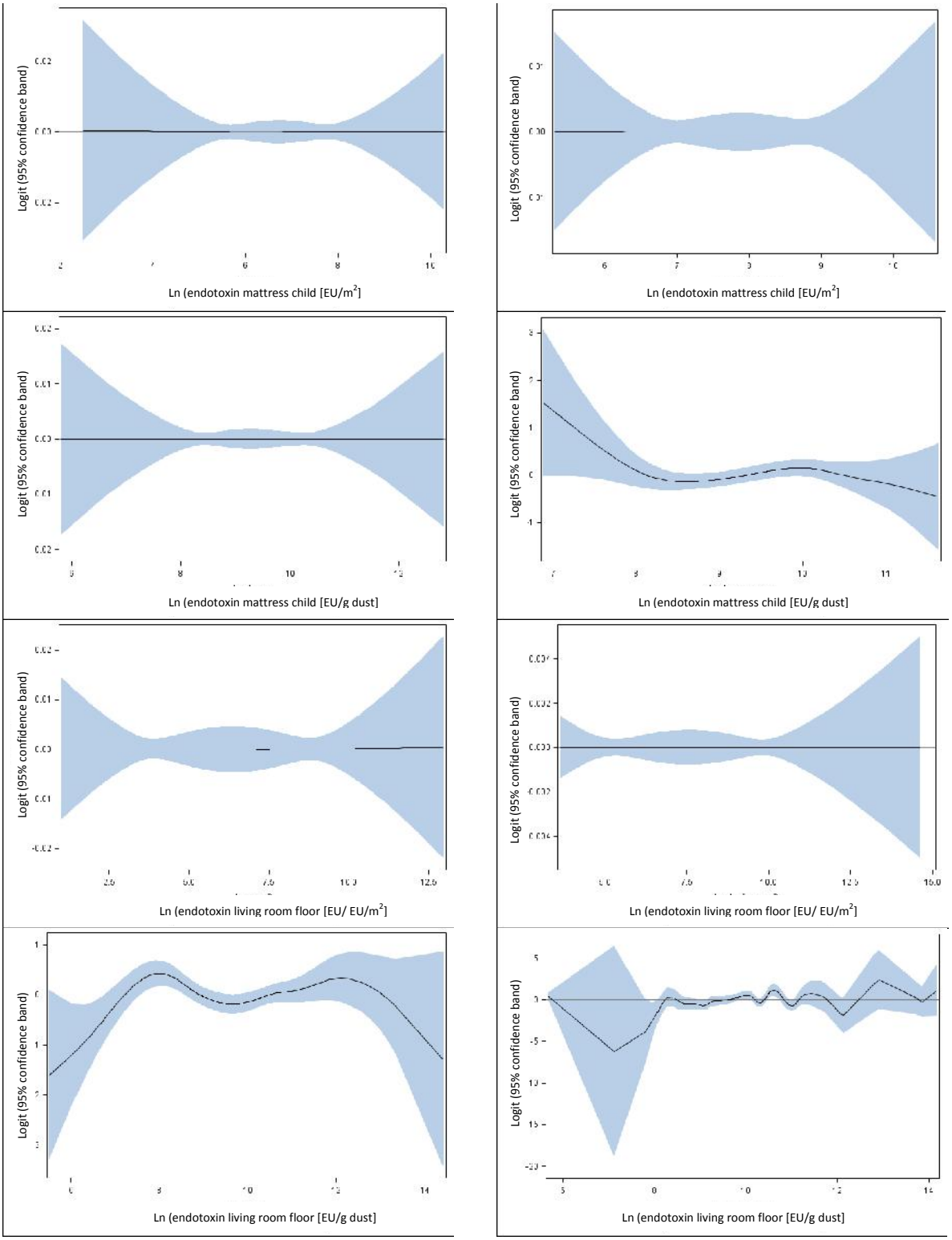
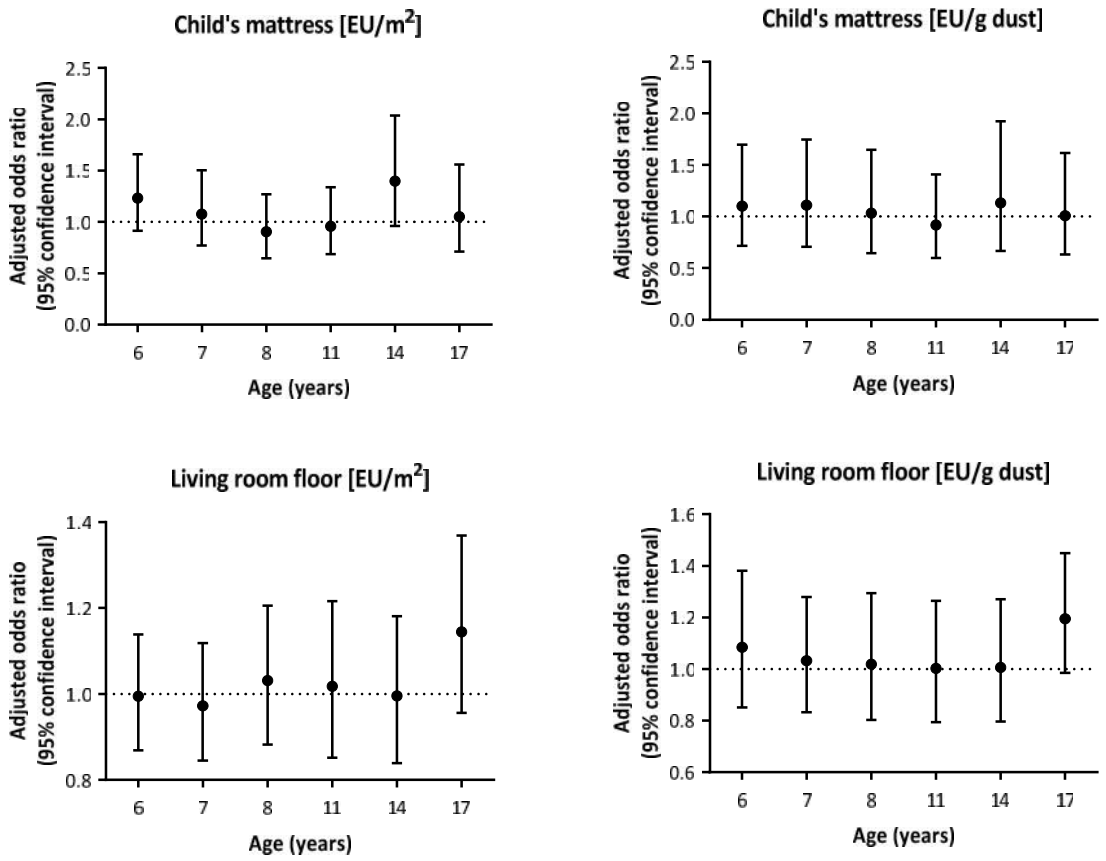


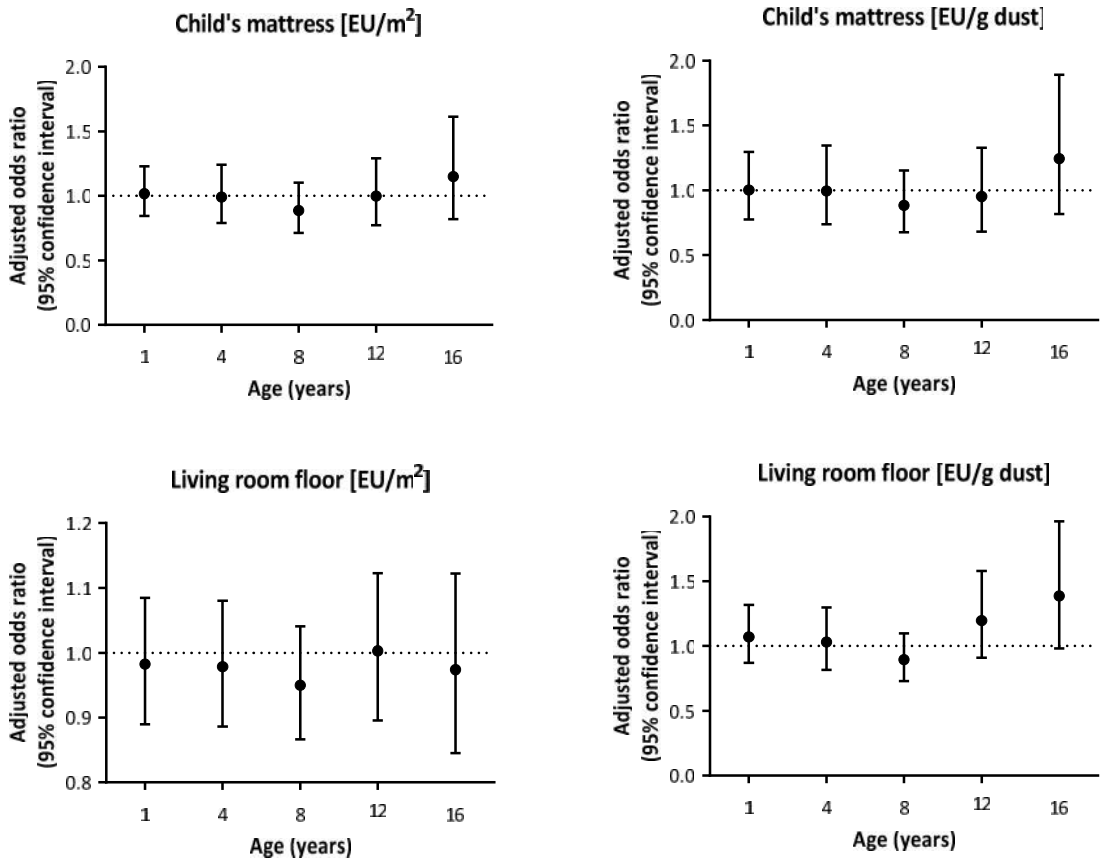
Figure S4. Age-specific adjusted * associations † between asthma at ages 6 to 17 years and endotoxins in mattress and living room floor dust samples collected at age 5-6 years



* Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home plus any sensitization at age 4 to account for the nested case-control design.

† Associations are presented for an interquartile range increase in exposure, which corresponds to 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure.

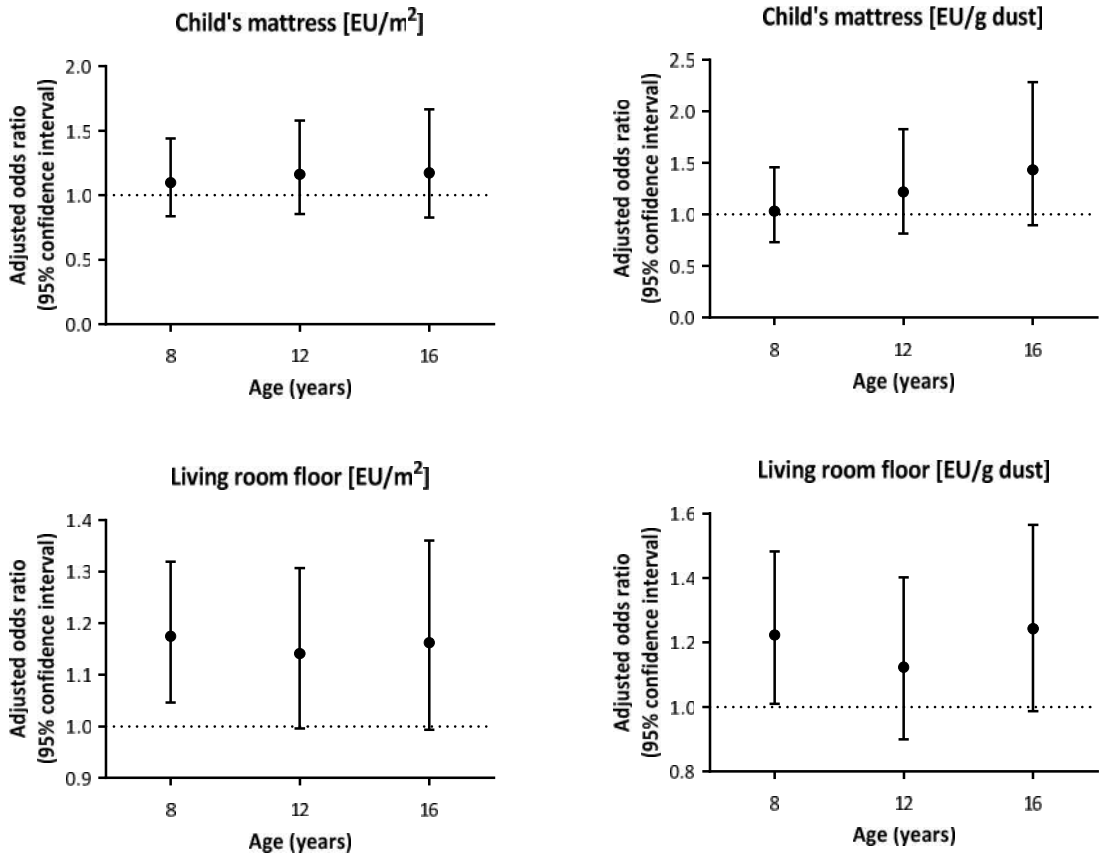
Figure S5. Age-specific adjusted * associations † between any sensitization at ages 1 to 16 years and endotoxins in mattress and living room floor dust samples collected at age 3 months.



* Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

† Associations are presented for an interquartile range increase in exposure, which corresponds to 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure..

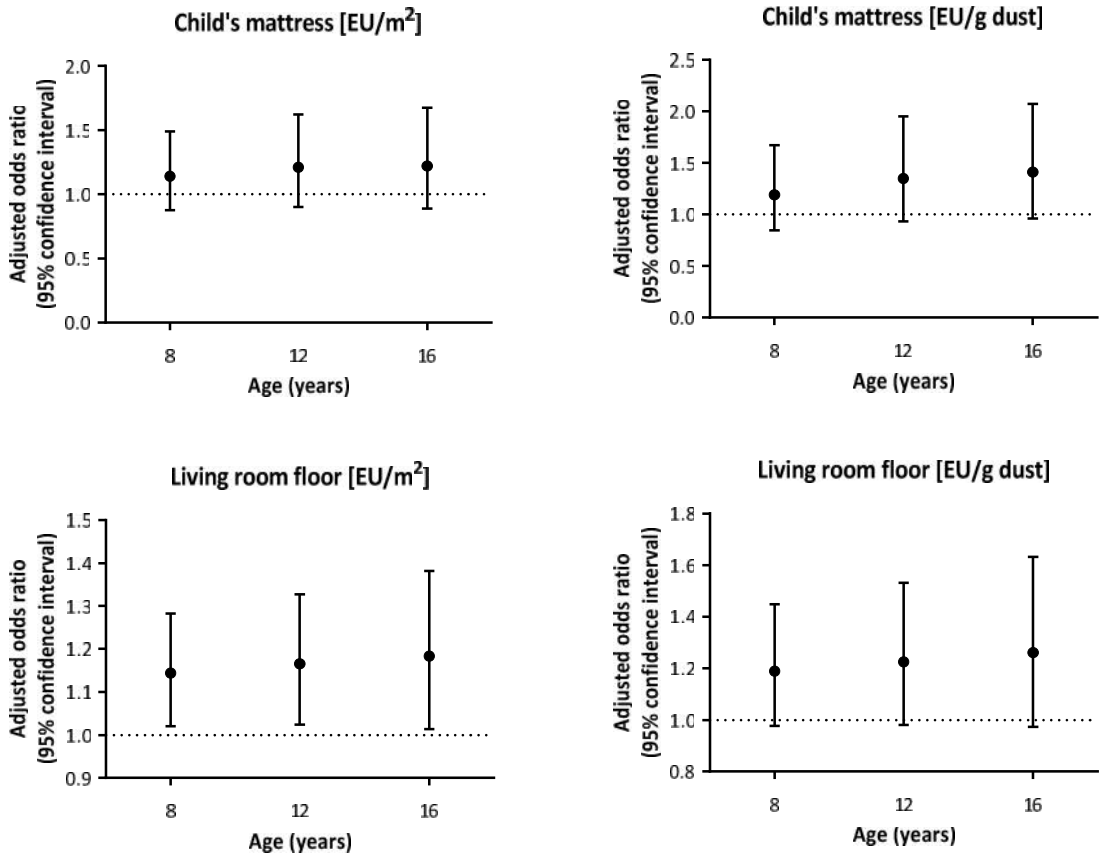
Figure S6. Age-specific adjusted * associations † between any sensitization at ages 8 to 16 years and endotoxins in mattress and living room floor dust samples collected at age 5-6 years.



* Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

† Associations are presented for an interquartile range increase in exposure, which corresponds to 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure..

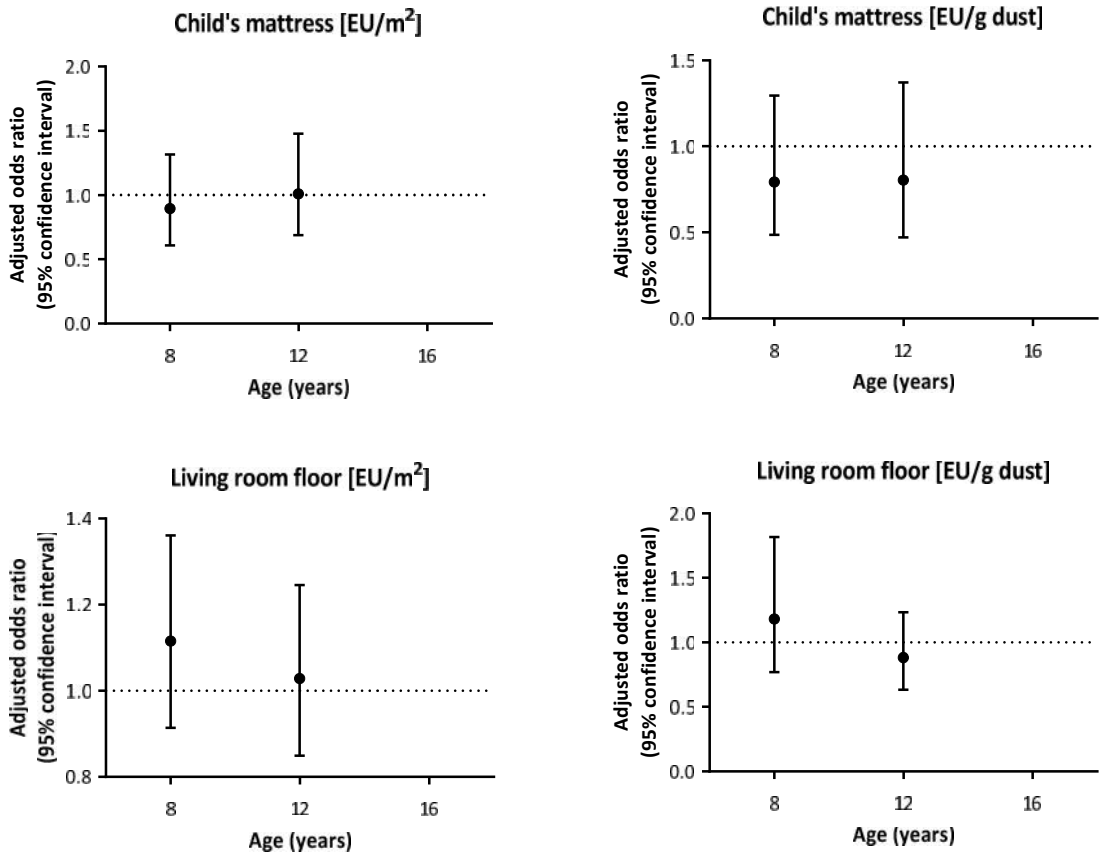
Figure S7. Age-specific adjusted * associations † between sensitization against inhalant allergens at ages 8 to 16 years and endotoxins in mattress and living room floor dust samples collected at age 5-6 years.



* Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

† Associations are presented for an interquartile range increase in exposure, which corresponds to 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure..

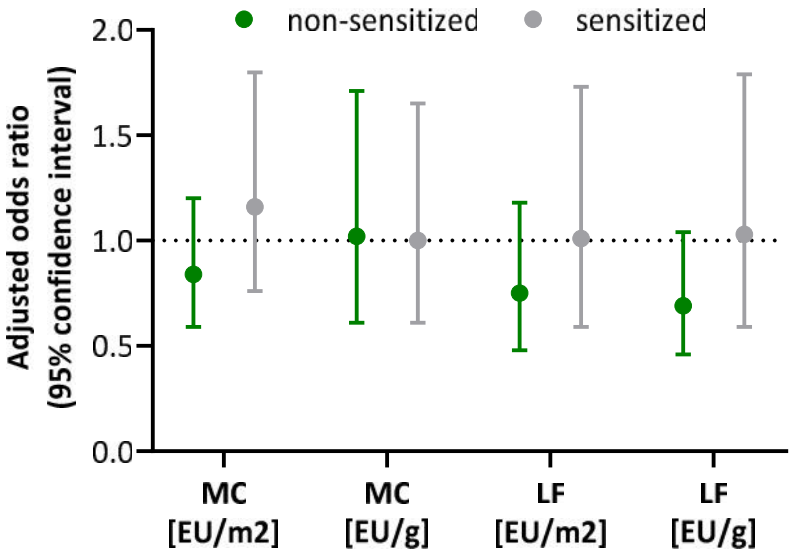
Figure S8. Age-specific adjusted * associations † between sensitization against food allergens at ages 8 and 12 years and endotoxins in mattress and living room floor dust samples collected at age 5-6 years.



* Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

† Associations are presented for an interquartile range increase in exposure, which corresponds to 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure..

Figure S9. Adjusted [*] associations [†] between asthma at ages 1 to 17 years and endotoxins in mattress (MC) and living room floor (LF) dust samples collected at age 3 months for sensitized [‡] and non-sensitized subjects separately.



[*] Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mold/dampness in the child's home.

[†] Associations are presented for an interquartile range increase in exposure ,which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure.

[‡] Against any of the allergens tested: house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata*, *Alternaria alternata*, milk and/or egg as available at the different ages