

ORIGINAL ARTICLE

EPIDEMIOLOGY AND GENETICS

The 8-year follow-up of the PIAMA intervention study assessing the effect of mite-impermeable mattress covers

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Abstract

Background: Exposure to high levels of house dust mite (HDM) allergens is associated with the development of allergic sensitization to HDM, a risk factor for the development of asthma, rhinitis, and allergic dermatitis. We studied the effect of an early intervention with mite-impermeable mattress covers on HDM allergen levels and the development of asthma and mite allergy throughout the first 8 years of life.

Methods: High-risk children (allergic mother) were prenatally recruited and randomly allocated to two groups receiving mite allergen-impermeable ($n = 416$) and placebo mattress covers ($n = 394$) or no intervention ($n = 472$). Asthma and allergies were assessed yearly by questionnaire. Specific immunoglobulin E and bronchial hyper-responsiveness were measured at the age of 8 years. Mattress dust samples collected at different time points were analyzed for HDM allergens.

Results: At the age of 8 years, levels of HDM allergen *Der f1* but not *Der p1* were lower in the active than the placebo mattress cover group. In repeated measures analyses, we found a temporary decreased risk of asthma symptoms at the age of 2 years in the intervention group compared to the placebo group and a temporary association between higher HDM allergen exposure at the age of 3 months and more asthma symptoms.

Conclusion: Early intervention with mite-impermeable mattress covers is successful in reducing exposure to *Der f1*; it only temporarily reduces the risk of asthma symptoms and does not reduce the risk of hay fever, eczema, and allergic sensitization.

Some 20 years ago, house mite allergens especially from the species *Dermatophagoides pteronyssinus* (*Der p*) and *Dermatophagoides farinae* (*Der f*) were considered to be an important cause of asthma (1, 2). Prospective cohort studies reported that exposure to high levels of house dust mite (HDM) allergens early in life was associated with the development of allergic sensitization to HDM (3, 4). The association between mite allergen exposure and the development of asthma, however, was less consistent. Asthma patients experience clinical benefits from allergen avoidance measures (5, 6). Therefore, it was suggested that allergen avoidance should be an important part of asthma management in sensitized patients (2).

Using mite-impermeable mattress encasings was considered promising (7). In the 1990s, a number of prospective cohort studies including this study were started to assess the effect of allergen prevention on the development of allergic sensitization and allergic disease (8–13). House dust mite intervention measures were consistently associated with a reduction in HDM allergen levels in these studies (14–18). The effects of these interventions on the development of asthma and allergies, however, were not consistent (11, 16, 17, 19–21). Within the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study, we examined the effect of mite allergen-impermeable mattress covers on HDM aller-

gen levels and the development of sensitization and allergic symptoms during the first 8 years of life with annual follow-ups. We now present the longitudinal analyses of the effect of the intervention on the development of asthma and allergies. Moreover, we studied the direct association between early life exposure to HDM allergen and the development of asthma and allergies during the first 8 years of life.

Methods

Study design and study population

The PIAMA study is a prospective birth cohort study (22). Between March 1996 and May 1997, women were recruited during their second trimester of pregnancy from a series of communities in the North, West, and Center of the Netherlands. Nonallergic pregnant women were invited to participate in a 'natural history' study (NHS). Pregnant women identified as allergic through a validated screening questionnaire (23) were primarily allocated to a randomized, double-blind intervention study (IS, $n = 855$) with a random subset ($n = 472$) allocated to the NHS. The intervention consisted of mite-impermeable polyester-cotton mattress and pillow covers for the parental bed(s) and the child's bed (ACb; Allergy Control Products, Saratoga Springs, NY, USA). The placebo group received cotton placebo covers. Only after the 8-year follow-up, participants were told whether they had received active or placebo mattress covers. The institutional review boards of the participating institutes approved the study protocol, and written informed consent was obtained from all participants. We restricted this analysis to all participants of the IS ($n = 721$) and high risk (HR) participants of the NHS with allergic mothers (NHS-HR, $n = 449$) with information on at least one of the health endpoints studied. Participants of the NHS will serve as a second control group.

Health outcomes

Information on allergic symptoms of the child was asked in yearly questionnaires that were completed by the parents approximately at the time of the child's birthday until the age of 8 years. From these questionnaires, the health outcomes were selected to describe asthma, allergies, and related symptoms. The exact definitions of the health outcomes studied are given in the Data S1.

At the age of 8 years, all children were invited for a medical examination including determination of BHR and collection of blood samples performed by trained investigators. BHR was defined as a decrease in forced expiratory volume in 1 s of 20% or more at a cumulative dose of ≤ 0.61 mg methacholine bromide. Specific immunoglobulin (Ig) E to common inhalant and food allergens were measured as described in the online Data S1. Allergic sensitization was defined by an IgE titer ≥ 0.35 IU/ml.

The medical ethics committees of the participating institutes approved the study protocol, and all parents gave written informed consent.

House dust samples

When the children were approximately 3 months and 8 years old, trained fieldworkers took dust samples from the children's mattresses. All dust samples were collected on paper filters with vacuum cleaners equipped with filter holders according to a standardized protocol that has been described in detail elsewhere (24). Collection and analysis of dust are described in more detail in the Data S1. Levels of *Der p1* and *Der f1* and the sum of the two allergen levels were used as exposure variables.

Statistical analysis

Mite allergen levels were log-transformed and geometric means (GM) with geometric standard deviations presented. Linear regression was used to compare allergen levels between study groups. Results are presented as GM ratios with 95% confidence intervals (CI). Longitudinal associations of yearly questionnaire reports of binary health outcomes until age 8 years with study group and HDM allergen levels were analyzed by generalized estimation equations with a log link (25). Associations of binary outcomes assessed at the age of 8 years only with study group and HDM allergen levels were analyzed by generalized linear models with a log link. We investigated the effect of the intervention on asthma-related health outcomes at the age of 8 years by allergic sensitization at the ages of 1 and 8 years, respectively, in models with sensitization-intervention interaction terms. Allergen levels were categorized using the detection limit and tertiles (in analyses with the sum of *Der p1* and *Der f1*) and medians (in separate analyses of *Der p1* and *Der f1*) of the levels above the detection limit as cutoffs. Associations were presented as relative risks with 95% CI.

The statistical significance was defined by a two-sided alpha-level of 5%. Calculations were performed using the Statistical Analysis System (SAS 9.2; Cary, NC, USA).

Results

Study population

A flowchart of the data availability is presented in Fig. S1. Characteristics of the participants are presented in Table 1. Children of the three study groups differed with regard to sex, maternal asthma and HDM allergy, and pet-ownership. More than half of the participants still used the mattress covers at the age of 8 years. Compliance with the intervention was slightly higher for active covers (Table 1) and significantly higher in families with fathers allergic to HDM; none of the other characteristics was significantly associated with compliance (data not shown). Frequency distributions of health outcomes are presented in Fig. 1 and Table 1, respectively.

Effect of the intervention on HDM allergen levels at the age of 8 years

In the intention-to-treat analysis, no difference was found between active and placebo groups with regard to mattress

Table 1 Characteristics of the study population

	Intervention				Natural history	
	Placebo cover		Active cover		High risk	
	n/N	(%)	n/N	(%)	n/N	(%)
Covariates						
Allergies mother†						
Asthma ever	98/337	(29)	97/382	(25)	100/447	(22)*
Allergic to HDM	224/332	(67)	248/378	(66)	248/445	(56)*
Allergic to pets	189/336	(56)	207/379	(55)	226/445	(51)
Hay fever	238/329	(72)	259/376	(69)	311/446	(70)
Allergies father†						
Asthma ever	34/337	(10)	32/368	(9)	29/444	(7)
Allergic to HDM	60/336	(18)	67/368	(18)	72/441	(16)
Allergic to pets	41/334	(12)	51/368	(14)	60/443	(14)
Hay fever	75/334	(22)	95/368	(26)	101/445	(23)
Allergies older siblings†						
Asthma	25/331	(8)	25/375	(7)	30/447	(7)
Eczema	57/331	(17)	81/375	(22)	87/447	(19)
Hay fever	10/331	(3)	18/375	(5)	15/447	(3)
Female gender	148/338	(44)	210/383	(55)*	207/449	(46)
Mother smoked during pregnancy	47/332	(14)	46/371	(12)	83/443	(19)
ETS exposure at home ≥ once/week‡	85/332	(26)	88/373	(24)	114/442	(26)
Born in winter§	62/338	(18)	67/383	(17)	105/449	(23)
Preterm birth¶	22/316	(7)	15/363	(4)	22/437	(5)
Breastfeeding at 12 weeks of age	178/331	(54)	181/379	(48)	214/443	(48)
Other children <12 years in household**	161/330	(49)	192/367	(52)	208/438	(47)
Day care center ≥ 8 h/week†	86/331	(26)	85/373	(23)	98/442	(22)
Pet (cat/dog/rodent/bird) in the home‡	123/329	(37)	182/373	(49)*	204/446	(46)*
Damp-/mold spots‡, **	41/325	(13)	52/366	(14)	53/429	(12)
Mother low educational level	73/332	(22)	86/371	(23)	101/443	(23)
Father low educational level	77/321	(24)	97/366	(27)	100/437	(23)
Compliance with intervention						
Mattress cover use at the age of 8 years††						
Yes	146/273	(53)	181/311	(58)	NA	
No	96/273	(35)	85/311	(27)	NA	
Unknown	31/273	(11)	45/311	(15)	NA	
Health endpoints (age 8 years)						
Bronchial hyper-responsiveness	52/191	(27)	64/226	(28)	57/228	(25)
Allergic sensitization	73/188	(39)	90/226	(40)	89/228	(39)
Sensitization to HDM	83/183	(45)	101/200	(51)	95/221	(43)

NA, not applicable; HDM, house dust mite; ETS, environmental tobacco smoke.

†By questionnaire.

‡At the age of 1 year.

§December 21, 1996–March 20, 1996.

¶<37 weeks of gestation.

**In living room and/or child's or parents' bedrooms.

††Only children who were still in the study at the age of 8 years.

*Significant difference (P -value χ^2 test < 0.05) with placebo group.

dust HDM allergen levels measured at the age of 8 years (Table S1). In the adherent-to-protocol analysis, *Der f1* but not *Der p1* was significantly reduced in the active compared to the placebo cover group (Table 2). No difference was found between placebo covers and NHS-HR.

Effect of the intervention on asthma and allergies during the first 8 years of life

We found a temporary decrease in asthma symptoms at the age of 2 years in the intervention group, and no indication of

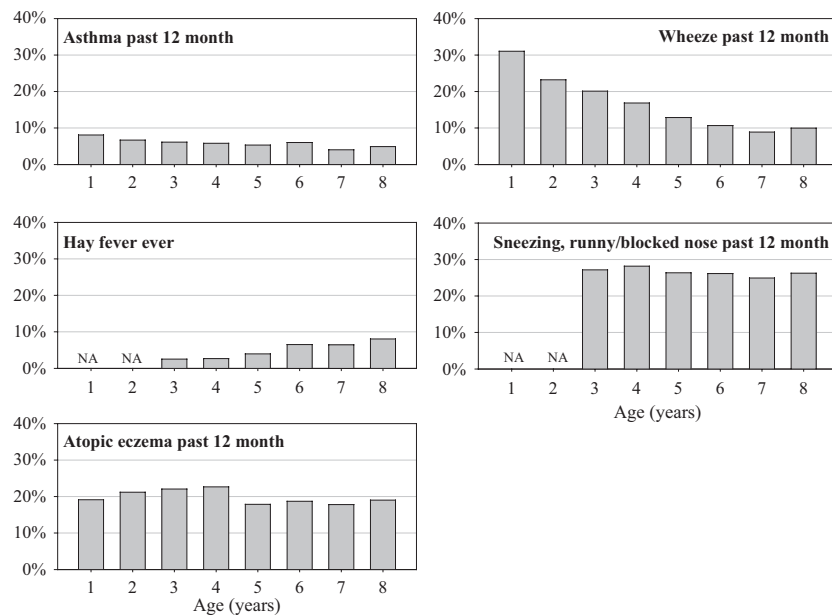


Figure 1 Frequency distributions of health outcomes at the ages of 1–8 years.

Table 2 House dust mite allergen levels in the child's mattress dust at the age of 8 years for the three study groups expressed as geometric means (GM) with geometric standard deviations (GSD), and geometric means ratios (GMR) with 95% confidence intervals (CI) for participants who still used the mattress covers at the age of 8 years

	N	<LOD†	GM (GSD)	GMR (95% CI)‡
<i>Der f1</i> (ng/g)				
Placebo cover	19	1	2849 (5.7)	1
Active cover	18	1	886 (3.5)	0.31 (0.11–0.88)
NHS-HR	30	1	3001 (5.6)	1.05 (0.42–2.66)
<i>Der p1</i> (ng/g)				
Placebo cover	19	4	410 (4.7)	1
Active cover	18	5	397 (5.9)	1.00 (0.29–3.47)
NHS-HR	30	7	392 (5.1)	0.80 (0.26–2.45)

†Number of samples below the limit of detection.

‡Placebo cover is reference group.

a decreased risk of asthma and allergies in the active cover group compared to the placebo cover group during the first 8 years of life (Fig. 2 and Table 3). At the age of 8 years, the risk of atopic eczema was significantly increased in the active cover group compared to the placebo group. Overall, participants of the active group of the IS more often reported atopic eczema during the first 8 years of life than participants of the NHS; no other differences existed (Fig. S2). After excluding participants of the IS who did not use the mattress covers anymore at the age of 8 years, there was still no protective effect of active mattress covers (Table 3). We found no modification in the effect of the intervention on asthma-related

health outcomes by allergic sensitization at the ages of 1 and 8 years (Table S2).

Associations of asthma and allergies until age 8 years with early exposure to HDM

House dust mite allergen levels at the age of 3 months are presented in Table S3. We found an increased risk of asthma among children with the highest total HDM exposure, but no dose–response relationship (Fig. 3). None of the other health outcomes including allergic sensitization to HDMs and BHR (Table S4) was associated with HDM exposure. No association was found between the separate levels of *Der p1* and *Der f1* and any of the health outcomes studied (Figs S3 and S4 and Table S5).

Discussion

Significantly lower levels of *Der f1* but not *Der p1* were found in the active cover group when performing an adherence-to-protocol analysis. Besides a temporary decrease in asthma symptoms at the age of 2 years in the active group, we found no indication of a decreased risk of asthma and allergies in the active compared to the placebo cover group and a significant increase in atopic eczema in the active cover group compared to the placebo cover and NHS-HR groups. We found a temporary positive association between HDM allergen exposure at the age of 3 months and later asthma symptoms, but no increased risk of asthma and allergies for children with the highest exposure to HDM allergens.

The present findings extend results from previous analyses within the same cohort up to the age of 4 years (26, 27). The intervention was successful in reducing *Der f1*, but not *Der*

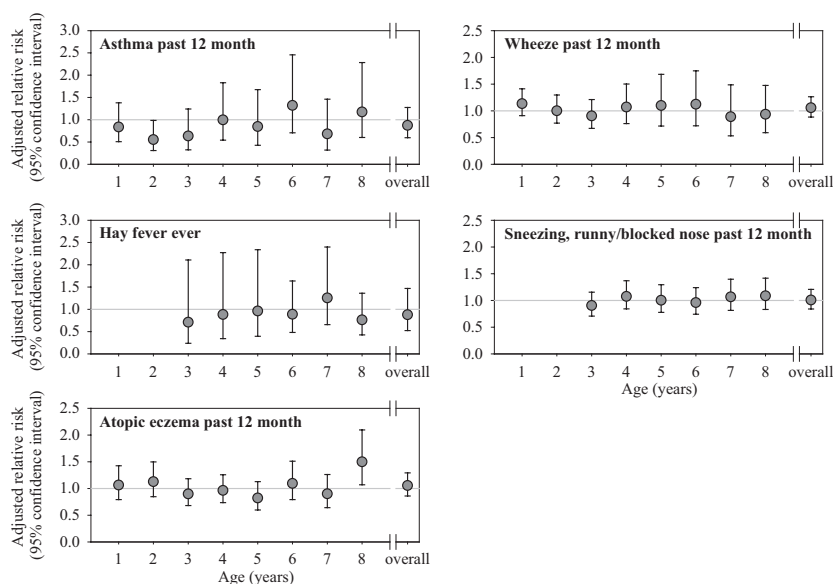


Figure 2 Adjusted age-specific and overall associations between the intervention (active covers vs placebo covers) and asthma, hay fever, eczema, and related symptoms during the first 8 years of life. (Adjusted for all variables from Table 1 that were statistically significantly ($P < 0.05$) different between active cover group and

placebo group or natural history high-risk group and placebo group, respectively, that is, mother asthma, mother allergic to house dust mite, gender, and pets at home. Associations are presented as relative risks with 95% confidence intervals).

Table 3 Adjusted[†] associations[‡] between the intervention and health outcomes during the first 8 years of life in all participants of the intervention study and those who still used the mattress covers at the age of 8 years

	All				Mattress covers used at the age of 8			
	Active cover vs placebo cover		NHS-HR vs placebo cover		Active cover vs placebo cover		NHS-HR vs placebo cover	
	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
During the first 8 years of life								
Asthma [§]	0.87	(0.60–1.28)	0.97	(0.68–1.39)	1.12	(0.62–2.03)	1.13	(0.68–1.88)
Wheeze [§]	1.06	(0.88–1.26)	0.97	(0.81–1.17)	1.14	(0.85–1.52)	1.09	(0.84–1.42)
Hay fever [¶]	0.88	(0.52–1.47)	1.20	(0.75–1.91)	0.98	(0.44–2.18)	1.51	(0.76–2.98)
Sneezing, runny/blocked nose [§]	1.01	(0.84–1.21)	0.88	(0.74–1.05)	1.24	(0.94–1.63)	1.05	(0.82–1.34)
Atopic eczema [§]	1.05	(0.86–1.29)	0.84	(0.68–1.03)	1.16	(0.85–1.58)	0.91	(0.69–1.21)
At the age of 8 years								
Bronchial hyper-responsiveness	1.07	(0.86–1.33)	0.91	(0.73–1.16)	1.22	(0.90–2.02)	1.03	(0.77–1.39)
Allergic sensitization	1.10	(0.87–1.40)	1.04	(0.82–1.32)	1.16	(0.82–1.65)	1.14	(0.83–1.55)
Sensitization to HDM	1.12	(0.82–1.52)	0.98	(0.71–1.35)	1.26	(0.78–1.66)	1.20	(0.78–1.85)

[†]Adjusted for all variables from Table 1 that were statistically significantly ($P < 0.05$) different between active cover group and placebo group or natural history high-risk group and placebo group, respectively, that is, mother asthma, mother allergic to house dust mite (HDM), gender, and pets at home.

[‡]Associations are presented as relative risks (RR) with 95% confidence intervals (CI).

[§]During past 12 months.

[¶]Ever.

p1 levels. The reason for this is not clear. A German study found determinants of *Der p1* and *Der f1* to be different; among others *Der p1*, but not *Der f1*, was positively associated with additional humidity (28). This might have played a

role in our findings. Differences between the intention-to-treat and the adherence-to-protocol analyses with regard to the effect of the intervention on mite allergen levels are consistent with reports from another cohort study, where

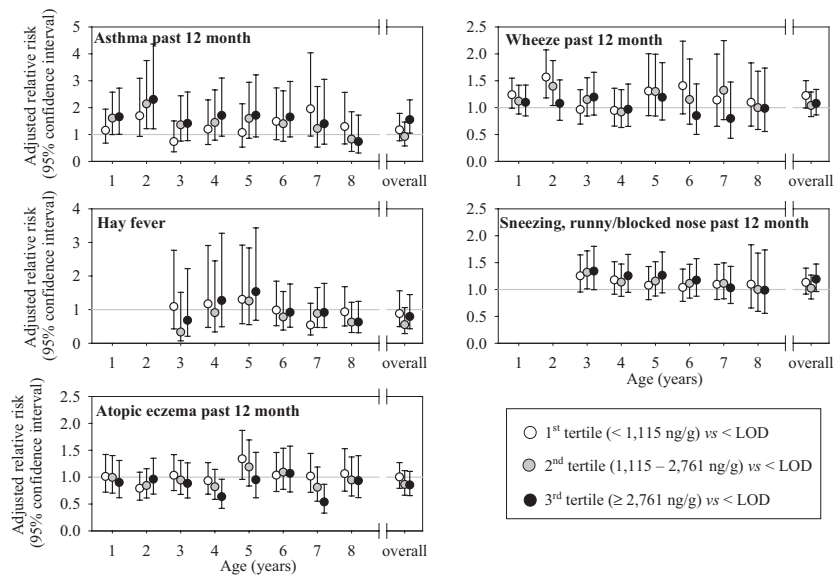


Figure 3 Adjusted age-specific and overall associations between house dust mite (HDM) allergen levels (sum of Der p1 and Der f1) at the age of 3 month and asthma, hay fever, eczema, and related symptoms during the first 8 years of life. (Adjusted for all variables from Table 1 that were statistically significantly ($P < 0.05$) different

between active cover group and placebo group or natural history high-risk group and placebo group, respectively, that is, mother asthma, mother allergic to HDM, gender, and pets at home. Associations are presented as relative risks with 95% confidence intervals).

differences between randomization groups in HDM allergens disappeared after cessation of the interventions (29). Our HDM intervention was not successful in preventing asthma and allergies, despite a significant reduction in *Der f1* levels. As reported previously, the PIAMA intervention study by coincidence recruited its participants in years with two consecutive very cold winters, which significantly reduced mite allergens in all arms of the study, so that the effect of the intervention was considerably smaller than it could have been (30). However, our findings are consistent with the findings of two other prospective birth cohort studies investigating the independent effects of HDM allergen reduction on the development of asthma and allergies. These studies reported that the interventions were successful in reducing HDM allergen levels (15, 17), but not symptoms (17, 29, 31). Up to now, significant reductions in asthma, allergies, and related symptoms have only been reported in studies where HDM allergen interventions were part of a multifaceted intervention program including additional intervention measures such as avoidance of food and pet allergens or passive smoking (11, 17, 19, 20). Together, this suggests that interventions with reduction in HDM allergen exposure only are probably insufficient. A limitation of multifaceted intervention studies is the inability to identify the components of the intervention program that are primarily responsible for the effect. Much larger studies are needed to test all possible combinations of interventions. Another limitation of multiple interventions is that blinding of study participants and placebo-controlled experiments are impossible. In light of these limitations, alternative approaches for developing future preventive strategies have to be considered. Careful joint analyses of observational

‘natural history’ birth cohort studies including many combinations of risk factors could be a promising alternative to intervention studies.

A surprising finding was the increase in atopic eczema in the active cover group. The CAPS study also showed that the risk of eczema was increased in the active compared to the control group (17, 31). It has been hypothesized that this may be due to the removal of other microbial agents such as endotoxin in addition to HDM allergens by the intervention (17). In the present study, however, after adjustment for endotoxin in mattress dust at the age of 3 months (available for 473 participants of the IS), the risk of eczema at the age of 8 years remained increased in the active cover group [$RR_{\text{active vs placebo}}$ (95% CI) 1.53 (1.01–2.29) after adjustment for endotoxin]. Increased sweating because of the active (polyester-cotton) mattress covers, which was reported back to us by several study participants, might be another explanation for the increase in atopic eczema in that group (32). However, we cannot test this hypothesis as we did not assess this systematically.

Publications from prospective studies on HDM exposure and asthma development were sparse until roughly 10 years ago. An early small prospective study showed a significantly increased risk of asthma at the age of 11 in children exposed to high levels of HDM (4), which could not be confirmed by another cohort study published 3 years later (33). During the last decade, several prospective studies have been published on the association between exposure to HDM allergens and asthma and allergies in children. Three cohort studies found no association of HDM allergen levels with symptoms of asthma and allergic sensitization (34–37). In two cohort

studies, HDM exposure was positively associated with allergic sensitization, and sensitization to HDM was associated with asthma and related symptoms at school age, but no direct association was found between HDM allergen levels and asthma (38,39). This means that there is some evidence for the association between HDM exposure and allergic sensitization to HDM, but very little evidence for the association between HDM allergen exposure and asthma. Apart from a transient association between total HDM exposure and asthma symptoms, we found no associations of health outcomes during the first years of life with HDM allergen levels in dust samples collected at 3 months. A positive association between HDM exposure at 3 months and allergic sensitization to HDM was found in previous analyses at 4 years (40), but not in the present analysis at 8 years. As the prevalence of HDM sensitization almost doubled between 4 and 8 years (14% vs 27%), and basically all children who were sensitized at 4 years were still sensitized at 8 years, this means that HDM sensitization that starts after the age of 4 years was not related to early HDM exposure.

In the present study, we used standardized questionnaires including core questions from the International study of asthma and allergies in childhood (ISAAC) (41) to define asthma. The use of questionnaire data generally presents some limitations such as the lack of clinical details. In addition, the diagnosis of asthma in young children is particularly challenging. The diagnosis in young children is often based on the presence of characteristic but nonspecific symptoms such as recurrent wheeze, persistent cough, breathing difficulties, and chest tightness (42). However, the prevalence of respiratory symptoms changes rapidly during childhood, and only a minority of children with reports of symptoms in early childhood develop asthma at school age (43).

Repeated house dust samples at the age of 8 years were available only for a subset of our study population. Children were randomly selected from all children who did not change address from birth until the age of 8 years; whose homes were visited for collection of house dust at earlier ages; and who were living in the middle or the western part of the Netherlands (44). We believe that the observed effect of the blinded intervention on HDM allergen levels in mattress dust at the age of 8 years has not been influenced by our selection criteria. However, we cannot rule out selection bias completely.

Conclusion

In the PIAMA cohort at the age of 8 years, the intervention with mite-impermeable mattress covers, started at birth, was still associated with a reduced exposure to one of the HDMs (*Der f1*), but did only temporarily reduce the risk of asthma and did not reduce the risk of hay fever or eczema symptoms and allergic sensitization to HDMs or BHR.

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Author contributions

Bert Brunekreef, Johan C de Jongste, Jorrit Gerritsen, and Henriette A. Smit were responsible for the conception and design of the study. Marjan Kerkhof, Marieke Oldenwening, Robert T. van Strien, Alet H. Wijga, and Ada Wolse contributed to the data collection and data management. Ulrike Gehring and Saskia Willers contributed to the data analysis. All authors contributed to the interpretation of data. Ulrike Gehring wrote the manuscript; all other authors critically reviewed the manuscript and approved the final version of the manuscript for submission.

Conflict of interest

None of the authors has a conflict of interest related to the work presented in this article.

Supporting information

Additional Supporting Information may be found in the online version of this article found at: <http://www.wileyonlinelibrary.com>

Figure S1. Flow chart of data availability in the PIAMA intervention study.

Figure S2. Adjusted¹ age-specific and overall associations between the intervention (active covers vs natural history high risk group) and asthma, hay fever, eczema, and related symptoms during the first 8 years of life (¹Adjusted for all variables from Table 1 that were statistically significantly ($P < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, and presence of pets. Associations are presented as relative risks with 95% confidence intervals).

Figure S3. Adjusted¹ age-specific and overall associations between *Der p1* levels at the age of 3 month and asthma, hay fever, eczema, and related symptoms during the first 8 years of life (¹Adjusted for all variables from Table 1 that were statistically significantly ($P < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, and pets at home. Associations are presented as relative risks with 95% confidence intervals).

Figure S4. Adjusted¹ age-specific and overall associations between *Der f1* levels at the age of 3 month and asthma, hay fever, eczema, and related symptoms during the first 8 years of life (¹Adjusted for all variables from Table 1 that were statistically significantly ($P < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, and pets at home.

Associations are presented as relative risks with 95% confidence intervals).

Table S1. House dust mite allergen levels in the child's mattress dust at age 8 years for the three study groups expressed as geometric means (GM) with geometric standard deviations (GSD), and geometric means ratios (GMR) with 95% confidence intervals (CI) for all participants.

Table S2. Adjusted¹ associations² between the intervention and asthma-related health outcomes at age 8 years by allergic sensitization at ages 1 and 8 years (¹Adjusted for all variables from Table 1 that were significantly ($P < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, pets at home. ²Associations are presented as relative risks (RR) with 95% confidence intervals (CI)).

Table S3. Distribution of house dust mite allergen levels in house dust samples collected on the child's mattress at age 3 months.

Table S4. Adjusted¹ associations² between house dust mite allergen levels (*Der p1* + *Der f1*) per gram of mattress dust collected at age 3 months and health outcomes at age 8 years in participants of the intervention study and high-risk participants of the natural history study. Children with non-detectable allergen levels served as reference category (¹Adjusted for all variables from Table 1 that were statistically significant

cantly ($P < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, pets in the home. ²Associations are presented as relative risks (RR) with 95% confidence intervals (CI)).

Table S5. Adjusted¹ associations² between levels of house dust mite allergens *Der p1* and *Der f1* per gram of mattress dust collected at age 3 months and health outcomes at age 8 years in participants of the intervention study and high-risk participants of the natural history study. Children with non-detectable allergen levels served as reference category (¹Adjusted for all variables from Table 1 that were statistically significantly ($P < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, pets in the home. ²Associations are presented as relative risks (RR) with 95% confidence intervals (CI)).

Data S1. Supplementary material.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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The 8 year follow-up of the PIAMA intervention study assessing the effect of mite-impermeable mattress covers

Supplementary Material

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Materials and Methods

Questionnaire-based health outcomes

Information on allergic symptoms of the child was asked in yearly questionnaires that were completed by the parents approximately at the time of the child's birthday until the age of 8 years. These questionnaires included core questions from the International study of asthma and allergies in childhood (ISAAC)(1). Health outcomes were defined by the following questions: Asthma during the past 12 months – “Did a doctor ever diagnose asthma in your child? If yes, has your child had asthma in the past 12 months?”; wheeze during the past 12 months – “Has your child had wheezing or whistling in the chest in the last 12 months?”; sneezing, runny/blocked nose during the past 12 months – “Has your child been sneezing or did he/she have a runny/blocked nose accompanied by itchy, watering eyes when he/she did not have a cold during the past 12 months?”; hay fever ever – “Has your child ever had hay fever?”. Atopic eczema during the past 12 months was defined as an itchy rash which was coming and going in the last 12 months at one of the following locations: in the folds of the elbows, behind the knees, in front of the ankles, around the ears or eyes.

Bronchial hyperresponsiveness

BHR was determined according to the protocol of the European Community Respiratory Health Survey (2). A KoKo or Rosenthal-French dosimeter and DeVilbiss 646 nebulizers were used to nebulize methacholinebromide with an air pressure of 30 psi (2 bars) up to a cumulative dose of 2.46 mg.

Specific IgE

Specific immunoglobulin (Ig) E levels for HDM (*Dermatophagoides pteronyssinus*), cat, dog, *Dactylis glomerata* and birch pollen, *Alternaria alternata*, egg and milk were measured in blood samples by a radioallergosorbent test-like method used at the Sanquin Laboratories (Amsterdam, The Netherlands).

Collection and analysis of house dust samples

In brief, mattress dust has been collected after the blanket was removed by vacuuming the entire upper surface of the sheets, on which the subjects actually slept, for 2 minutes. After weighing, the whole dust sample including the filter was extracted with phosphate-buffered saline. Aliquots of the extracts were stored at -20°C , until analysis. Dust extracts were analyzed for house dust mites *Der p 1* and *Der f 1* using reagents for a sandwich enzyme immunoassay, purchased from Indoor Biotechnologies (Cardiff, UK). The lower limit of detection was 8 ng/mL for *Der p 1* and 6 ng/mL for *Der f 1* for 5-fold diluted samples. Samples with undetectable amounts of allergen were assigned a value of two-thirds of the detection limit. House dust mite allergen levels were expressed as nanogram per per gram of dust (ng/g). For samples with amounts of dust below the limit of detection (< 11 mg), the amount of allergen per gram dust was not calculated.

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Table S1. House dust mite allergen levels in the child's mattress dust at age 8 years for the three study groups expressed as geometric means (GM) with geometric standard deviations (GSD), and geometric means ratios (GMR) with 95% confidence intervals (CI) for all participants.

	N	< LOD ¹	GM (GSD)	GMR (95% CI) ²
<i>Der f1 (ng/g)</i>				
Placebo cover	30	3	1,930 (6.0)	1
Active cover	28	1	1,372 (3.9)	0.71 (0.31-1.65)
NHS-HR	30	1	3,001 (5.6)	1.55 (0.68-3.56)
<i>Der p1 (ng/g)</i>				
Placebo cover	30	5	459 (4.3)	1
Active cover	28	8	341 (5.9)	0.74 (0.32-1.71)
NHS-HR	30	7	392 (5.1)	0.85 (0.38-1.94)

¹ number of samples below the limit of detection

² placebo cover is reference group

Table S2. Adjusted ¹ associations ² between the intervention and asthma-related health outcomes at age 8 years by allergic sensitization at ages 1 and 8 years.

	Active cover vs placebo cover			
	Sensitized ³		Non-sensitized	
	RR	(95% CI)	RR	(95% CI)
Sensitization at age 1 year				
Asthma past 12 months	1.27	(0.30-5.39)	1.49	(0.55-4.07)
Wheeze past 12 months	0.51	(0.19-1.33)	0.94	(0.47-1.92)
Bronchial hyperresponsiveness	0.94	(0.63-1.41)	1.00	(0.74-1.36)
Sensitization at age 8 years				
Asthma past 12 months	n.e.	n.e.	n.e.	
Wheeze past 12 months	0.88	(0.48-1.61)	0.85	(0.28-2.57)
Bronchial hyperresponsiveness	1.06	(0.78-1.43)	1.05	(0.74-1.48)

¹ adjusted for all variables from Table 1 that were significantly ($p < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, pets at home.

² Associations are presented as relative risks (RR) with 95% confidence intervals (CI)

³ Approximately one-third of the participants of the active cover group (75/237, 31.6%) and one-fourth of the participants of the placebo group (54/203, 26.6%) with health data at the age of 8 years were sensitized at age 1 year

n.e. = not estimable

Table S3. Distribution of house dust mite allergen levels in house dust samples collected on the child's mattress at age 3 months.

	n	<LOD *	> LOD				
			Min	P33	Median	P66	Max
<i>Der f 1</i> (ng/g)	820	56%	111	571	870	1,575	401,291
<i>Der p 1</i> (ng/g)	818	68%	186	805	1,222	2,115	131,271
<i>Der p1 + Der f1</i> (ng/g)	818	47%	260	1,115	1,777	2,761	532,562

* percentage of samples below the limit of detection (LOD)

Table S4. Adjusted ¹ associations ² between house dust mite allergen levels (*Der p1* + *Der f1*) per gram of mattress dust collected at age 3 months and health outcomes at age 8 years in participants of the intervention study and high-risk participants of the natural history study. Children with non-detectable allergen levels served as reference category.

	1 st tertile (LOD–1,115 ng/g)		2 nd tertile (1,115–2,761 ng/g)		3 rd tertile (≥ 2,761 ng/g)	
	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
BHR	0.91	(0.63-1.32)	1.18	(0.79-1.78)	1.05	(0.77-1.43)
Allergic sensitization	0.98	(0.69-1.38)	0.82	(0.53-1.26)	0.61	(0.38-1.98)
Allergic sensitization to house dust mite	1.38	(0.90-2.11)	0.74	(0.44-1.23)	0.88	(0.51-1.52)

¹ adjusted for all variables from Table 1 that were statistically significantly ($p < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, pets in the home

² Associations are presented as relative risks (RR) with 95% confidence intervals (CI)

LOD Limit of detection

Table S5. Adjusted ¹ associations ² between levels of house dust mite allergens Der p1 and Der f1 per gram of mattress dust collected at age 3 months and health outcomes at age 8 years in participants of the intervention study and high-risk participants of the natural history study. Children with non-detectable allergen levels served as reference category.

	<i>Der f 1</i>				<i>Der p 1</i>			
	< median ³ vs < LOD		≥ median ³ vs < LOD		< median ³ vs < LOD		≥ median ³ vs < LOD	
	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)
BHR	1.00	(0.71-1.41)	1.26	(0.97-1.61)	0.87	(0.62-1.23)	0.82	(0.58-1.17)
Allergic sensitization	1.01	(0.71-1.44)	0.84	(0.58-1.23)	0.90	(0.61-1.32)	0.55	(0.34-0.90)
Allergic sensitization to house dust mite	1.36	(0.90-2.06)	1.00	(0.64-1.59)	1.09	(0.70-1.70)	0.69	(0.40-1.19)

¹ adjusted for all variables from Table 1 that were statistically significantly ($p < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, pets in the home

² Associations are presented as relative risks (RR) with 95% confidence intervals (CI)

LOD Limit of detection

Figure S1. Flow chart of data availability in the PIAMA intervention study.

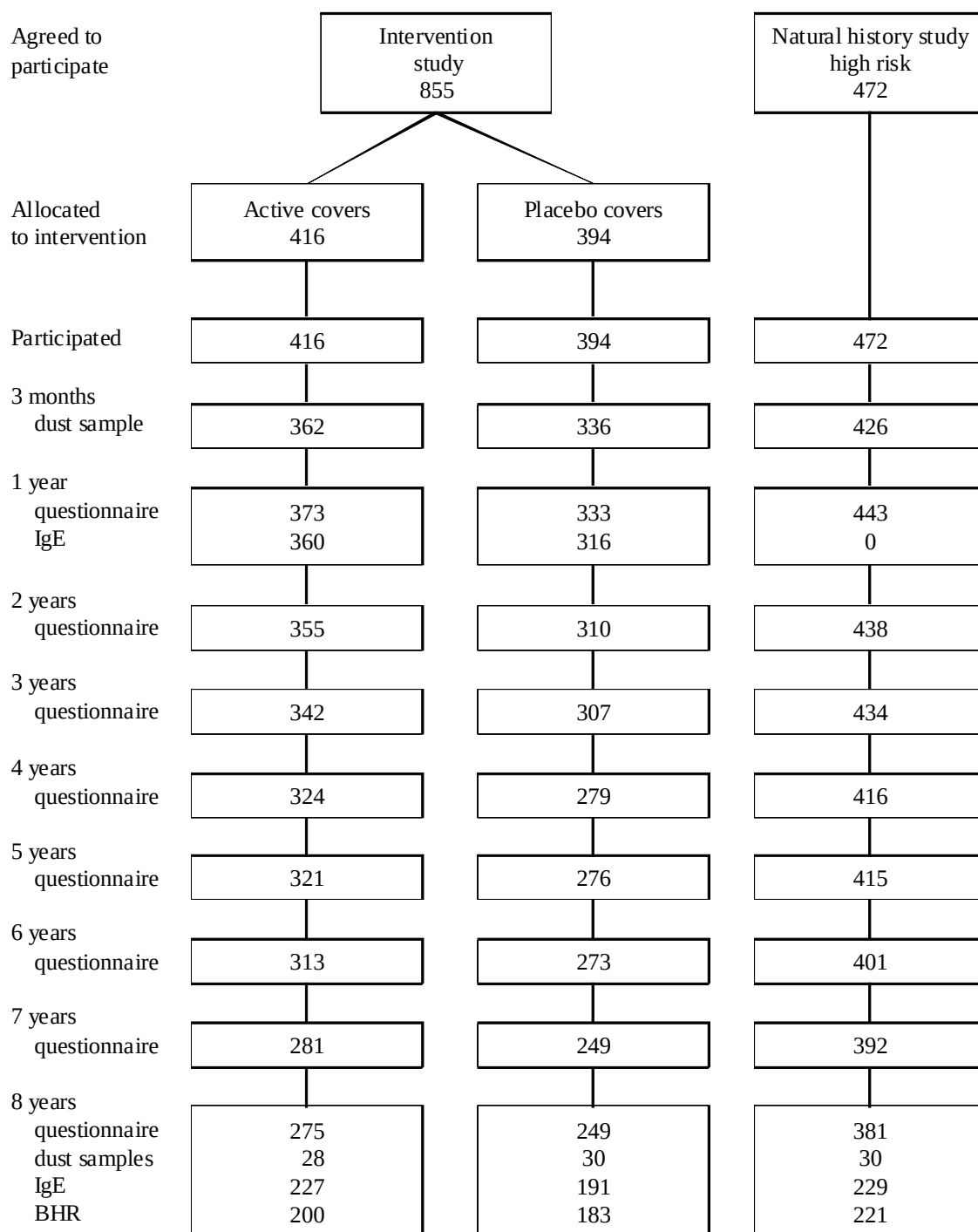
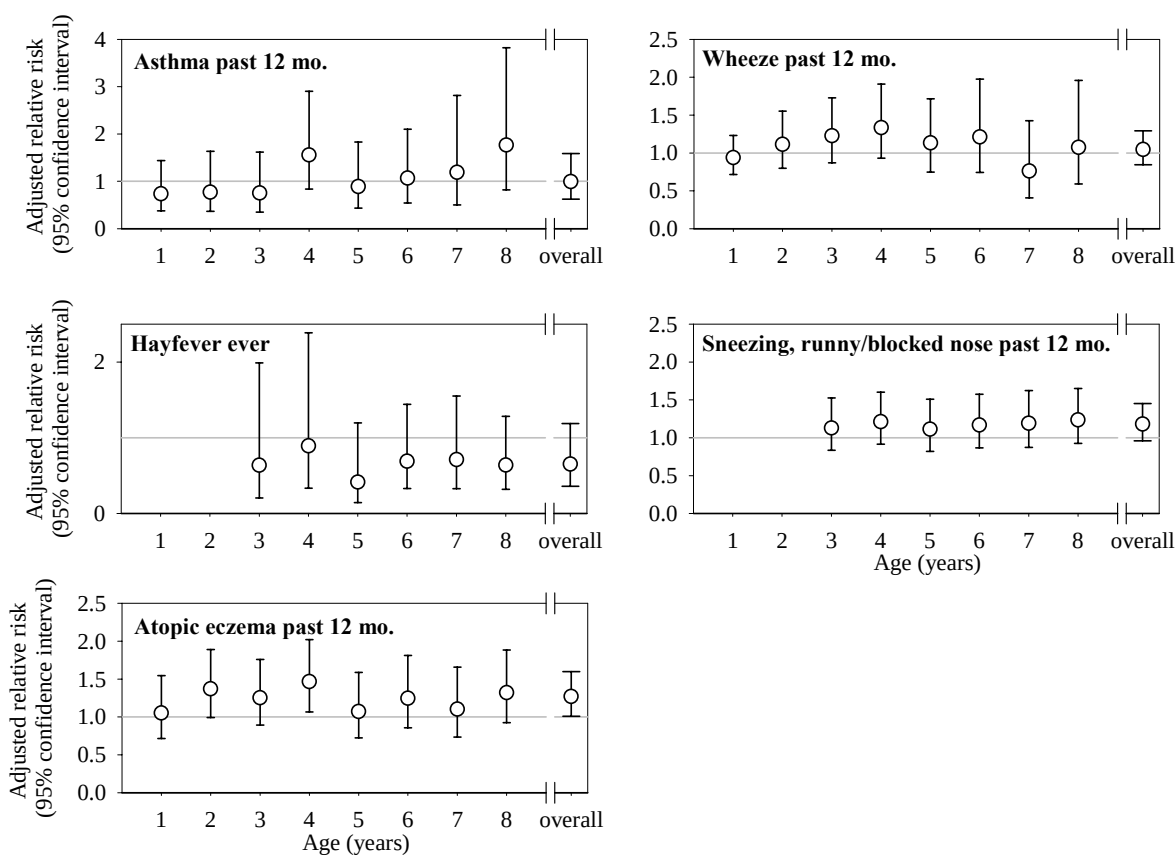


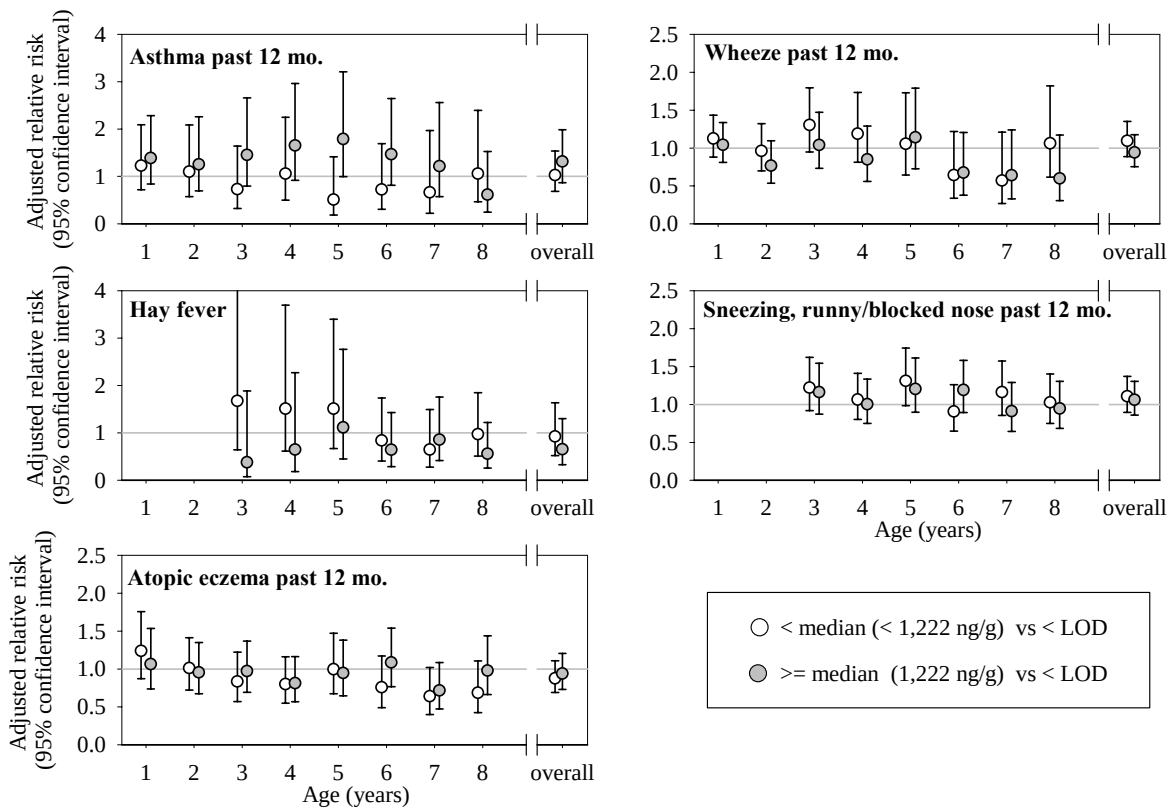
Figure S2. Adjusted ¹ age-specific and overall associations between the intervention (active covers vs. natural history high risk group) and asthma, hay fever, eczema and related symptoms during the first 8 years of life.



¹ adjusted for all variables from Table 1 that were statistically significantly ($p < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, and presence of pets.

² Associations are presented as relative risks with 95% confidence intervals

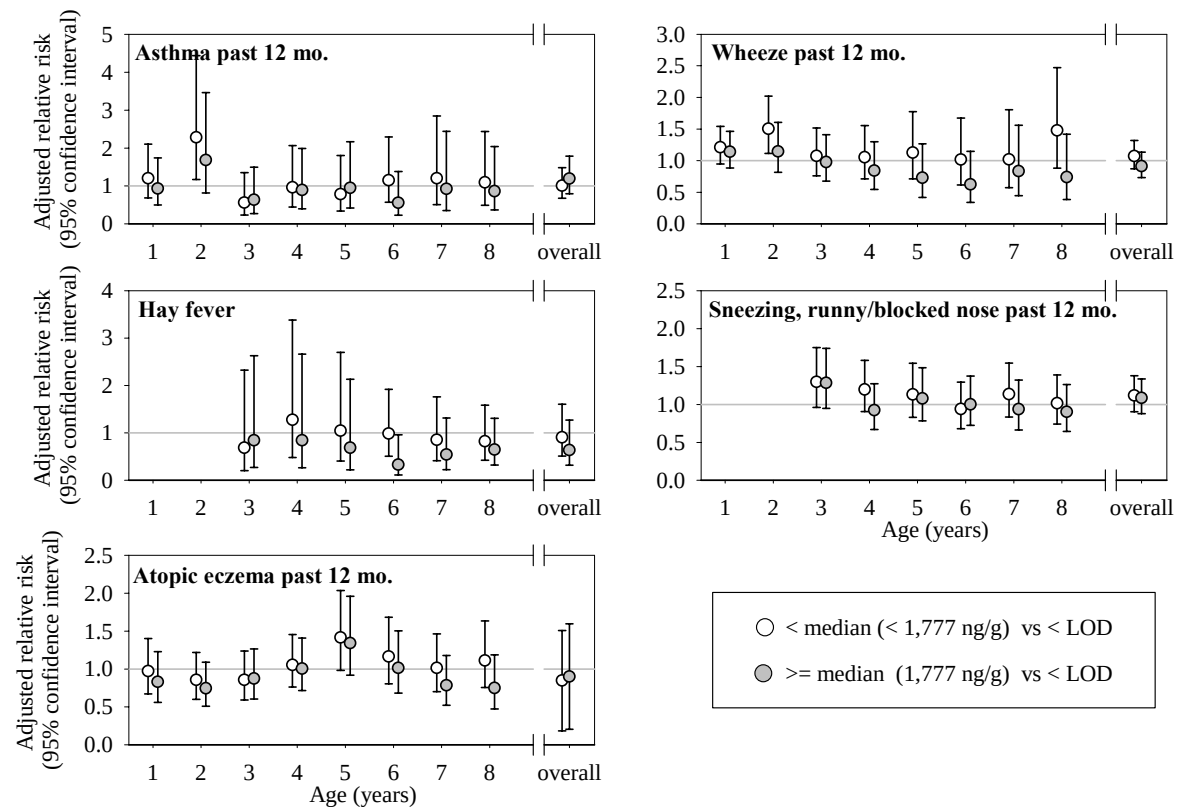
Figure S3. Adjusted ¹ age-specific and overall associations between Der p1 levels at the age of 3 month and asthma, hay fever, eczema and related symptoms during the first 8 years of life.



¹ adjusted for all variables from Table 1 that were statistically significantly (p < 0.05) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, and pets at home

² Associations are presented as relative risks with 95% confidence intervals

Figure S4. Adjusted ¹ age-specific and overall associations between Der f1 levels at the age of 3 month and asthma, hay fever, eczema and related symptoms during the first 8 years of life.



¹ adjusted for all variables from Table 1 that were statistically significantly ($p < 0.05$) different between active cover group and placebo group or natural history high risk group and placebo group, respectively, i.e. mother asthma, mother allergic to house dust mite, gender, and pets at home

² Associations are presented as relative risks with 95% confidence intervals