Clin Exp Allergy 2003; **33**:490–495

Mattress encasings and mite allergen levels in the Prevention and Incidence of Asthma and Mite Allergy study

R. T. van Strien*, L. P. Koopman†, M. Kerkhof‡, M. Oldenwening*, J. C. de Jongste†, J. Gerritsen§, H. J. Neijens†, R. C. Aalberse¶, H. A. Smit** and B. Brunekreef*

**Department of Environmental and Occupational Health, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, †Department of Paediatrics, Sophia Children's Hospital, Rotterdam, Departments of ‡Epidemiology and Statistics and §Paediatric Respiratory Medicine, University Hospital Groningen, Groningen, ¶Central Laboratory for the Blood Transfusion Service, Department of Allergy, Amsterdam and **National Institute of Public Health and the Environment, Bilthoven, the Netherlands*

Summary

Background Reduction of allergen exposure from birth may reduce sensitization and subsequent allergic disease.

Objective To measure the influence of mite allergen-impermeable mattress encasings and cotton placebo encasings on the amount of dust and mite allergen in beds.

Methods A total of 810 children with allergic mothers took part in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study. Allergen-impermeable and placebo mattress encasings were applied to the childrens' and the parents' beds before birth. Dust samples were taken from the beds of children and their parents before birth and 3 and 12 months after birth. Extracts of dust samples were analysed for mite allergens (Der p 1 and Der f 1).

Results Active mattress encasings were significantly more effective in reducing dust and mite allergen levels than placebo encasings. Mite allergen levels were low in general and the treatment effect was modest. Twelve months after birth, mattresses with active mattress encasings had about half the amount of Der 1 (Der p 1 + Der f 1)/m^2, compared to mattresses with placebo encasings, for the child's and the parental mattress.

Conclusion This study shows that mite-impermeable mattress encasings have a significant but modest effect on dust and mite allergen levels of mattresses with low initial mite allergen levels, compared to placebo.

Keywords allergen avoidance, infants, intervention, mite allergens

Submitted 7 June 2002; revised 11 September 2002; accepted 9 December 2002

Introduction

Allergies to house dust mites are common in asthmatics in the Netherlands and many other countries where climatic conditions favour the growth of mites [1–3]. Mite-allergic asthmatics benefit from the use of mite-impermeable mattress encasings, as these lower mite allergen exposure [4–8]. A number of studies have suggested that allergen exposure during early childhood is associated with a higher risk of sensitization and possibly with asthma later in life [9–12]. Some of these studies addressed the issue of primary prevention of mite allergy by influencing the home environment of the newborn child. Avoidance measures should preferably be as simple and feasible as possible in such studies, because the measures are intended for symptom-free children who are not allergic yet, which may influence compliance with more demanding interventions.

Correspondence: B. Brunekreef, IRAS-EOH, PO Box 80176, 3508 TD Utrecht, the Netherlands. Tel. + 31 30 2535400, Fax: + 31 30 2535077.
E-mail: b.brunekreef@iras.uu.nl

In the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, 'high risk' subjects were included in the third trimester of pregnancy. The subjects were randomly allocated to an active or a placebo group. The active group was supplied with mite-impermeable mattress and pillow encasings for both the parents' bed and the child's bed (Allergy Control®). The placebo group was supplied with placebo cotton encasings. In the present study, we examined the influence of this relatively simple avoidance measure on mite allergen levels in dust collected from beds. Effects on health status of the infants are reported elsewhere.

Methods

Study population

The PIAMA study is a prospective multicentre birth cohort study, which is partly natural history and partly intervention study. A total of 10 232 pregnant women in three regions of the Netherlands completed a validated screening questionnaire on allergies and asthma in 1996 and 1997 [13]. For the intervention study, 2062 allergic mothers were invited to participate and 855

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(41.5%) agreed. In 810 of these 855, the intervention measures were actually applied (416 in the active group and 394 in the placebo group). At 3 months after birth, dust samples were taken in 352 (active) and 335 (placebo) homes. The Medical Ethical Committees of all participating institutes approved the study protocol. Children were born between May 1996 and December 1997. The subjects were randomly allocated to two groups. The active group was supplied with mite-impermeable encasings (Acb, Allergy Control Products, Saratoga Springs, NY, USA) and the placebo group was supplied with placebo cotton encasings. Before the intervention was applied, a home visit was performed in the third trimester of pregnancy. Dust samples were taken from the parental mattress and, if already present, the child's mattress. Mattress and pillow encasings for the parental bed and mattress encasings for the child's bed were supplied, including instructions on how to use the encasings. No encasings were supplied for blankets or duvets. The parents were instructed to take all bedding from the mattress and to put the encasing around the mattress. After this, all bedding should be re-installed as it was previously. All parents were advised to wash the sheets regularly at temperatures high enough ($> 60\,^{\circ}C$) to kill mites. Furthermore, 'allergen avoidance' brochures from the Netherlands Asthma Fund were supplied to all participants. About 3 and 12 months after the birth of the child, a second and a third home visit were conducted and new dust samples were taken.

Dust sampling and analysis

Dust was sampled, by trained fieldworkers, from the parental mattress and the child's mattress after the blankets (but not the undersheets) were removed. In the Netherlands it is common to have a fleecy cotton underblanket underneath the sheets, to protect the mattress. Furthermore, the child's mattress was often equipped with a plastic sheet against bedwetting. All these items, if present, were left in place during sampling. Dust was sampled from the sheets, on which the subjects actually slept. Before birth, the child's bed was usually not equipped with any bedding.

Dust was sampled, using a Rowenta Dymbo vacuum cleaner at 1200 Watt, with an ALK-filter holder. Paper filters (Schleicher & Schuell 589, Dassel, Germany) were put in plastic Petri dishes and weighed before sampling after at least 24 h equilibration at 20 °C and 50% relative humidity. At the sampling site, the filter was placed in the filter holder using a forceps. Sampling was done from each bed for a period of 2 min, regardless of the surface area of the mattress. After sampling, the dust and the filter were put back into the Petri dish and frozen at the end of the day ($-20\,^{\circ}C$). After thawing and adjustment to the controlled temperature and relative humidity for 24 h, the Petri dish was weighed again and the total amount of dust was recorded. The limit of detection for this procedure was 11 mg of dust, based on the average of three times the standard deviation of the repeated registration of the weight of blank samples. All dust samples containing less than 11 mg of dust were set at two-thirds of that weight, and for these samples only the amount of allergen per m^2 was calculated and not the amount per gram dust. The dust and the filter were extracted for 2 h by shaking in phosphate-buffered saline (PBS) with 0.05% Na-azide (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4/L; pH 7.2). For < 300 mg dust 3 mL was used, for 300–500 mg 5 mL was used, for 500–1000 mg 10 mL was used and for more than 1000 mg 20 mL was used. After this, aliquots of the extracts were stored at $-20\,^{\circ}C$ until analysis.

Dust extracts were analysed using reagents for a sandwich EIA, supplied by Indoor Biotechnologies (Cardiff, UK). As catching antibody, the Der p 1 and Der f 1 assays use mouse IgG anti-mite monoclonal antibodies 5H8 and 6A8, respectively. Both assays use biotinylated mouse IgG1 anti-mite monoclonal 4C1 for marker antibody. Avidine peroxidase was used as conjugate (Dako, Glostrup, Denmark). OPD was used as substrate and the reaction stopped with 2 M HCl. After this, absorbance was read at 492 nm. All extracts were diluted five-, 10- and 20-fold, and more if required. The median coefficient of variance (CV) was 7.9% and 8.1% for Der p 1 and Der f 1. The lower limit of detection was 8 ng/mL for Der p 1 and 6 ng/mL for Der f 1 for fivefold diluted samples. About 20% of the samples were analysed in duplicate from a second aliquot on a different day. In about half of these samples, allergen levels were undetectable both times. For samples detectable on one or both occasions, the CV was calculated. For Der p 1 and Der f 1 the median CV was 15.2% and 19.0%, respectively.

A limited number of samples was not analysed for allergens, due to laboratory problems. Of the samples taken during the third visit (at 12 months), a randomly selected subset of 200 was analysed for both allergens, 100 from homes with active mattress encasings and 100 from homes with placebo encasings.

Statistical analysis

Statistical analysis was done using SAS statistical software for PC v6.12 (SAS, Cary, NC, USA). As distributions were found to be extremely right-skewed, due to a large number of undetectable samples, medians and interquartile ranges were used to describe the distributions of dust, Der p 1, Der f 1 and total Der 1 (sum of Der p 1 and Der f 1)/m^2 and the amount of Der p 1, Der f 1 and total Der 1 per gram of dust. Spearman correlation coefficients were calculated to investigate the correlation between Der p 1 and Der f 1 in samples taken from the parental and the child's beds. Differences in median dust and allergen levels between active and placebo encasings were tested for statistical significance using Wilcoxon rank sum tests. Differences over time were tested using Wilcoxon's signed rank test (for paired analysis). *P*-values smaller than 0.05 were considered statistically significant.

Results

Tables 1 and 2 show dust and allergen levels in the child's and the parental mattress before birth and 3 and 12 months after birth. Table 1 shows results for the child's mattress and Table 2 shows results for the parental mattress. The intervention was implemented 1–2 months before birth so that samples taken after birth were taken 4–5 months after the intervention had been implemented. A total of 77% of the samples taken from childrens' mattresses before birth, and 94% of the samples taken 3 months after birth contained a detectable quantity of dust. A detectable amount of dust was almost always obtained from the parental mattress. Of all samples taken from the child's mattress, 41–46% did not contain detectable amounts of Der p 1 or Der f 1 (Table 1). Of all samples taken from the

Table 1. Levels of dust (mg/m^2) and Der 1 (Der p 1 + Der f 1; ng/m^2 and ng/g dust) before the intervention and after the intervention in the child's mattress

	2 months before birth			3 months after birth			12 months after birth		
	n/nd	Median	IQR	*n*/nd	Median	IQR	*n*/nd	Median	IQR
Placebo									
mg dust/m^2	322/81	94	26–221	333/15	159	86–268	94/1	271	142–572
ng Der 1/m^2	282/132	114	65–575	294/122	154	65–455	95/26	398	130–1676
ng Der 1/g	207/79	1346	726–4782	282/117	974	629–2310	93/24	1468	634–7313
Active									
mg dust/m^2	340/74	87	30–223	352/29	129*	68–240	97/1	201	123–572
ng Der 1/m^2	314/140	136	65–668	327/146	130*	65–298	97/32	190†	83–691
ng Der 1/g	247/92	1525	753–6030	300/126	1028	657–1928	96/31	913‡	569–2443

n/nd = number of samples/number of non-detectable samples; IQR, interquartile range. Differences between active and placebo assessed using Wilcoxon rank sum test: *$P < 0.01$, †$P < 0.05$, ‡$P < 0.10$.

Table 2. Levels of dust (mg/m^2) and Der 1 (Der p 1 + Der f 1) (ng/m^2 and ng/g dust) before the intervention and after the intervention in the parental mattress

	2 months before birth			3 months after birth			12 months after birth		
	n/nd	Median	IQR	*n*/nd	Median	IQR	*n*/nd	Median	IQR
Placebo									
mg dust/m^2	350/0	202	133–341	335/0	216	146–323	95/0	176	104–265
ng Der 1/m^2	295/38	414	85–1361	299/46	281	88–809	95/13	309	87–1268
ng Der 1/g	293/38	1851	607–5957	297/45	1507	470–3202	95/13	2097	568–6002
Active									
mg dust/m^2	382/3	202	128–313	351/0	167*	96–247	98/0	154	89–234
ng Der 1/m^2	338/40	298	73–1140	321/79	113*	47–430	98/21	158†	59–496
ng Der 1/g	336/40	1676	419–5602	321/79	807*	357–2424	98/21	1018	465–3179

n/nd = number of samples/number of non-detectable samples; IQR, interquartile range. Differences between active and placebo assessed using Wilcoxon rank sum test: *$P < 0.01$, †$P < 0.05$.

parental mattress, 12–24% did not contain detectable quantities of Der p 1 or Der f 1. For the child's bed it is important to realize that many of the beds were new and therefore never used when the baseline samples were taken.

Figures 1 and 2 show the association between the amounts of Der p 1 and Der f 1 expressed per gram dust and per m^2 for the child's bed and the parental bed. Only when the levels of Der p 1 and Der f 1 were both undetectable, samples were not entered into the figures. The figures show that Der p 1 and Der f 1 are hardly related to each other. Correlations are low, and for different mattresses the amount of Der p 1can be high while the amount of Der f 1 is low and *vice versa*.

Figures 3 and 4 show the concentrations of Der 1/m^2, Der 1/g, Der p 1/g and Der f 1/g for the active and the placebo group at the three different moments dust samples were taken (2 months before birth, 3 months after birth and 12 months after birth), for the child's and the parental bed, respectively. To make sure concentrations are comparable over time, in this figure, data are shown only for the subset of mattresses for which samples were analysed at 12 months after birth. The figure shows that, 3 and 12 months after birth, the total amount of mite allergen/m^2 is reduced in the parental beds with active encasings, whereas in the placebo group, allergen levels remained about the same. For the child's bed, the total amount of mite allergen/m^2 increased significantly in the placebo group (Wilcoxon signed rank test), while the increase in the active group was not statistically significant. In the child's as well as in the parental bed, the differences between active and placebo encasings were larger at 12 months than at 3 months. At 12 months after birth, total mite allergen levels, expressed per m^2 as well as per gram dust were about twofold lower in the active group compared to placebo.

Discussion

This study showed that at relatively low mite allergen levels, the application of mite-impermeable mattress encasings resulted in modest, but statistically significant, changes in dust and mite allergen exposure, compared to placebo. The effect of the application of mite-impermeable mattress encasings, compared to placebo, was bigger after 1 year than it was after 3 months.

The dust and mite allergen content of the mattresses studied here was low, not only in the children's mattresses, which was expected after other studies [12, 14], but also in the mattresses of the parents. These low allergen levels are in contrast with our previous findings in population-based studies, where allergen levels in mattress dust were up to 10 times higher [15, 16]. Possible explanations include a strong downward shift of allergen exposure in recent years and self-selection of subjects joining this study who might be actively practicing mite allergen avoidance measures already. In a previous study, allergic

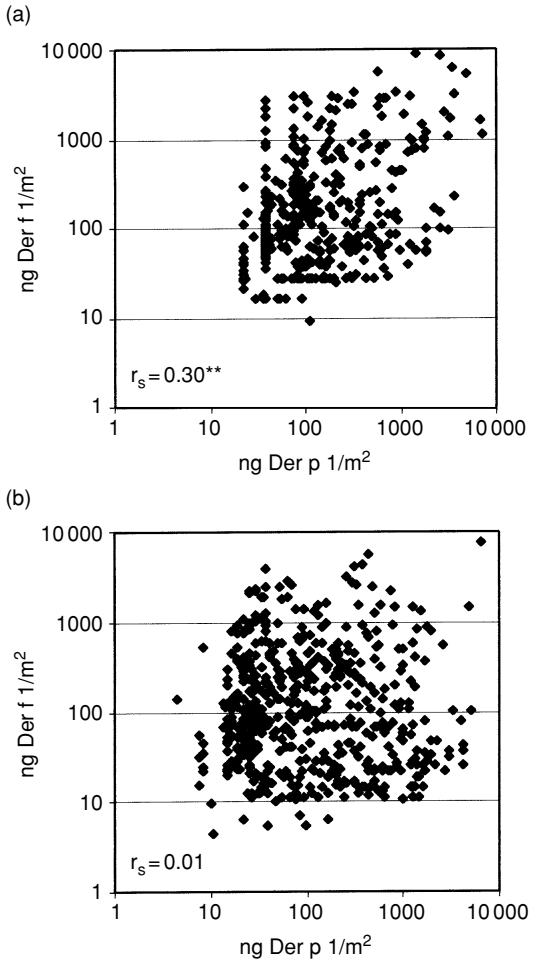


Fig. 1. The amount of Der p 1 versus Der f 1/m^2 for the child's (a) and the parental (b) mattress (samples for which Der p 1 and Der f 1 were both undetectable are not shown). r_s = Spearman correlation coefficient: $^{**}P < 0.01$.

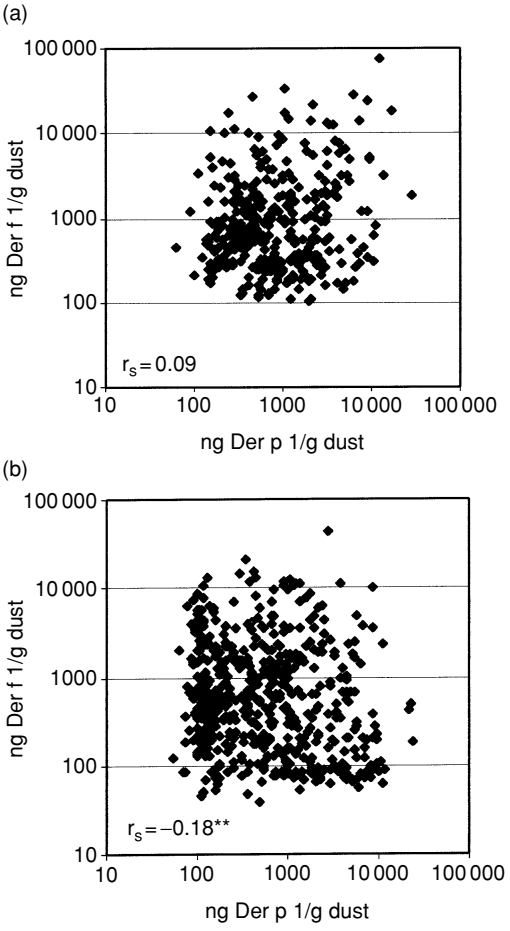


Fig. 2. The amount of Der p 1 versus Der f 1/g dust for the child's (a) and the parental (b) mattress (samples for which Der p 1 and Der f 1 were both undetectable are not shown). r_s = Spearman correlation coefficient: $^{**}P < 0.01$.

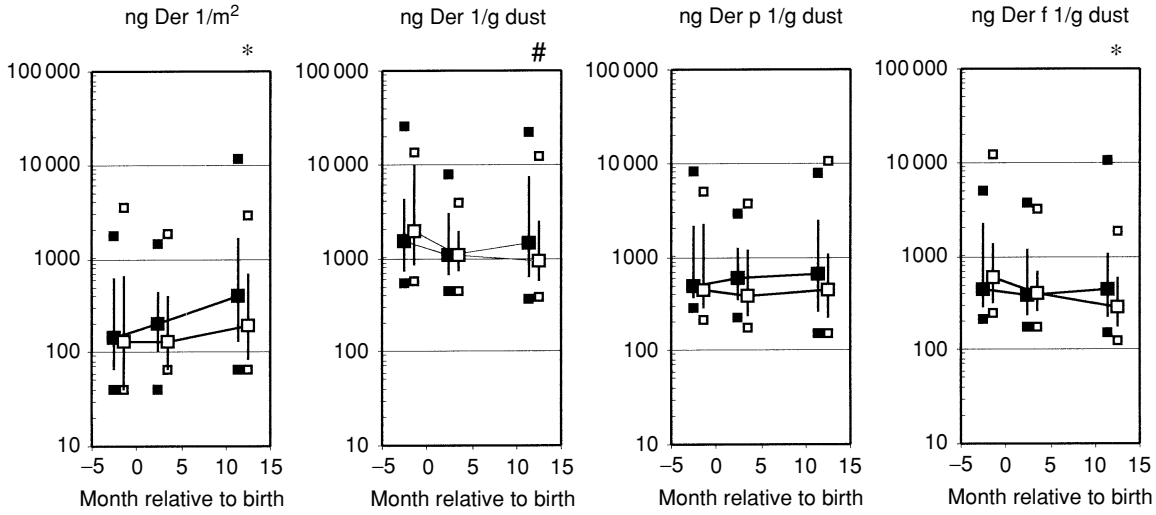


Fig. 3. Total mite allergen content and Der p 1 and Der f 1 concentrations in dust from the child's mattress, shown for the placebo (■) and the active covers (□) separately. The figures show median, interquartile range (error bars) and 10 and 90 percentiles (small squares), for the samples taken before birth, at 3 months after birth and at 12 months after birth. Differences between active and placebo assessed using Wilcoxon rank sum test: $\#P < 0.10$; $^{*}P < 0.05$.

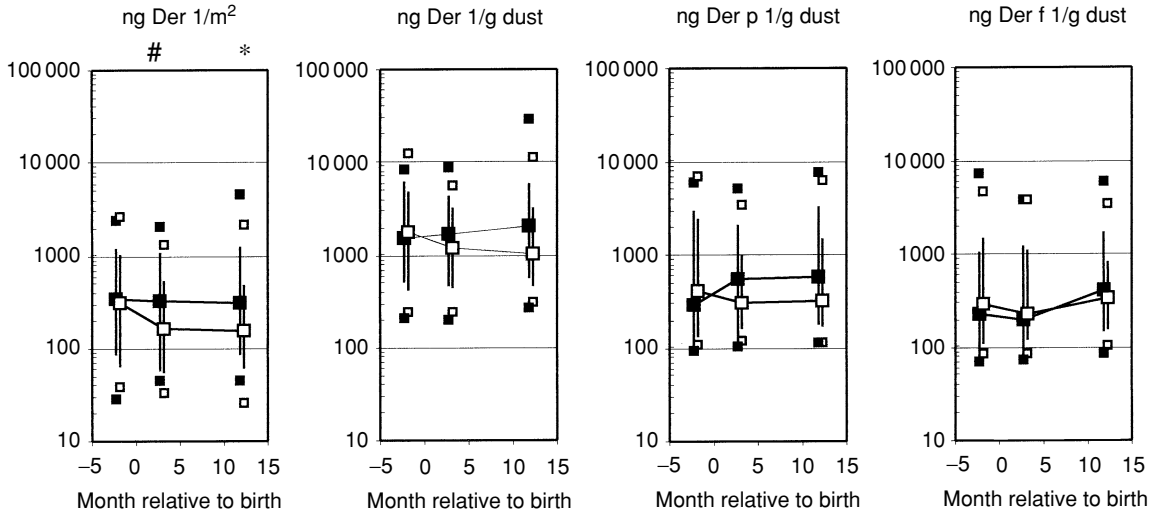


Fig. 4. Total mite allergen content and Der p 1 and Der f 1 concentrations in dust from the parental mattress, shown for the placebo (■) and the active covers (□) separately. The figures show median, interquartile range (error bars) and 10 and 90 percentiles (small squares), for the samples taken before birth, at 3 months after birth and at 12 months after birth. Differences between active and placebo assessed using Wilcoxon rank sum test: $\#P < 0.10$; $^*P < 0.05$.

parents were shown to have lower mite allergen levels in their homes than non-allergic parents [16]. This makes the low mite allergen levels found in the PIAMA intervention study plausible, because participants were chosen on the basis of self-reported allergies and asthma. Another possible explanation is that in previous studies all dust samples were taken in October and November, which is the season in which mites are most abundant. Due to the nature of the PIAMA study, dust samples were taken year round, and this could have been another reason for the low allergen levels found in this study. When more samples, including samples from homes of non-allergic parents will be available in the PIAMA study, these possible explanations will be examined further.

This finding of low mite allergen levels in the study population participating in the intervention part of the study has some possibly interesting consequences. If families with an allergic mother are already reducing mite allergen exposure fairly effectively, the relatively small gains related to the use of mite-impermeable mattress encasings cannot be expected to have the same clinical effect as when baseline levels of allergen exposure would have been higher, and thus more amenable to change.

Low correlations were found between Der p 1 and Der f 1 levels. This was in accordance with results found by Gross and coworkers [17]. Der p 1 is important in some mattresses with no Der f 1 and vice versa. This finding makes it important to not only determine Der p 1, but also Der f 1. Furthermore, for the child's bed, the difference between active and placebo was especially apparent for Der f 1, and not so much for Der p 1.

The main goal of the PIAMA study is to find out whether mite allergen-impermeable mattress encasings were able to reduce the allergen exposure of children and thereby, possibly, the development of allergic diseases. For the child's mattress, the total amount of mite allergen (Der 1/m^2) increased during use of the mattress in the first year of life, significantly so in the placebo group. However, the amount of mite allergen/g dust (Der 1/g) does not change considerably over time, and only the amount of Der f 1/g dust was significantly lower in the active group compared to the placebo group at 12 months after birth. This means that mite-impermeable mattress encasings delayed the increase in the amount of mite allergen found on the child's bed, thereby possibly decreasing exposure of the child. For the parental mattress, the total amount of mite allergen (Der 1/m^2) did not change over time considerably in the placebo group, and decreased, after installation of the mattress and pillow encasings, in the active group. At 12 months after birth of the child, the difference was statistically significant. Differences between the active and placebo groups were, on average, never larger than a factor two.

The Der p 1 concentrations (ng/g) found by Custovic et al. at 12 months after birth were similar to the concentrations (ng/g) found in this study (Fig. 3) at 12 months after birth [14]. The main difference lies in the fact that we had a placebo-controlled design whereas no encasings were used at all in the study reported from Manchester. Mite allergen concentrations were reduced in the active group as well as in the placebo group, but in the active group the effect seemed to persist for a longer period of time compared to the placebo group. Relative to a control group using no mattress encasings at all, the effect of the active intervention may have been underestimated. Nevertheless, the active intervention reduced the mite allergen concentration in dust from the parental and the child's bed more than placebo, especially after 12 months.

At this moment a number of other studies is going on in which the development of sensitization and allergic disease is being studied in relation to different types of intervention [11, 12, 14]. In a few years time, we will be able to reach more definitive conclusions about the effect of allergen avoidance measures for preventing the development of sensitization and allergic disease in early childhood.

Acknowledgements

This study was supported by a grant of the Dutch Asthma Foundation and the Dutch Ministry of Housing and the Environment.

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