

Manchester Asthma and Allergy Study: Low-allergen environment can be achieved and maintained during pregnancy and in early life

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Background: Early exposure to dust mite allergens may be critical for primary sensitization. Reducing exposure may offer a realistic chance for primary prevention of sensitization and asthma, but it is essential to implement measures that can achieve and maintain the low-allergen environment.

Objective: Our purpose was to assess the effectiveness of mite allergen avoidance measures in achieving and maintaining a low-allergen environment during pregnancy and in the first year of life.

Methods: The Manchester Asthma and Allergy Study is a prospective, prenatally randomized study that follows the development of asthma and atopy in a cohort of infants at high risk (both parents atopic) who are randomly allocated to full mite allergen avoidance or to a normal regimen. Avoidance measures comprise (1) mite-proof covers (mattress, pillow, and quilt) for parental bed, (2) high-filtration vacuum cleaner, (3) vinyl flooring in infant's bedroom, (4) new crib and portable crib mattresses encased in mite-proof material, (5) benzyl benzoate (Acarosan) applied on carpets and soft furniture, (6) bed linens washed in hot water weekly, and (7) washable soft toys. Dust samples from the parental bed, bedroom floor, living room floor, infant's mattress, and nursery floor were collected between the 10th and 14th weeks of pregnancy, immediately after birth, and then at age 6 months and 1 year, and Der p 1 levels were determined by mAb-based ELISA.

Results: Recovered Der p 1 from maternal mattress was reduced by 97.25% (95% confidence interval [CI] 95.25%-98.41%) during the second and third trimesters of pregnancy, with the effect persisting for 6 months (98% reduction, 95% CI 97.25%-99.1%) and 12 months (97.6% reduction, 95% CI 95.7%-98.6%) after the birth (active vs control, $P < .000001$). Total Der p 1 from bedroom floor in the active group was reduced by 53.7% (95% CI 25.7%-71.2%) in samples collected within 4 weeks of the child's birth, with the percentage reduction being 62.8% (95% CI 39.3%-77.2%) at 6 months and 26.5% (95% CI -24% to 57.1%) at 1 year (active compared vs control, $P < .007$). Der p 1 levels in crib mattress and nursery

floor in the active group were extremely low (crib mattresses geometric mean [95% CI] 2.3 ng [1.6-3.4] at birth, 6.8 ng [4.5-10] at age 6 months, and 15.6 ng [9.8-24.8] at age 1 year [active vs control, $P = .001$]; nursery 1 ng [0.9-1.1] at birth, 1.7 ng [1.2-2.5] at age 6 months, and 2 ng [1.3-3.5] at age 1 year [active vs control, $P < .00001$]). The total amount of allergen recovered at age 1 year was 29-fold (95% CI 15.1- to 56.7-fold) higher in the control group than in the active group.

Conclusions: The avoidance measures used in this study achieved and maintained a low mite allergen environment during pregnancy and in the first year of life in homes of infants at risk of atopy. (J Allergy Clin Immunol 2000;105:252-8.)

Key words: Primary prevention, dust mite, avoidance, atopy, asthma

Sensitization to dust mites is a major independent risk factor for asthma in areas where mite allergen levels in most of the homes are high.¹ Furthermore, even in areas with a low mite allergen level there is a close association between mite sensitization and asthma.² Evidence about the importance of dust mites in the causation of asthma is emerging from developing countries, suggesting that this is a worldwide problem.³

Exposure to allergens in early infancy has been suggested as a critical period for primary sensitization. Children born just before the birch pollen season in Scandinavia have a higher risk of sensitization to birch than do those born after the season.⁴ Similar observations were made in relation to house dust mite exposure in children born in the autumn, when mite numbers tend to be higher than at other times of the year.⁵ The importance of early exposure to mite allergens has been highlighted in a prospective study of children at high risk of atopy in Poole, Dorset.^{6,7} Exposure to Der p 1 $>2 \mu\text{g/g}$ of dust during infancy was associated with an increased prevalence of sensitization to mites at age 5 years and levels above $10 \mu\text{g/g}$ with a 4.8-fold relative risk for development of asthma at age 11 years.^{6,7} In the German Multi-centre Atopy Study a large number of infants were prospectively followed up to investigate the influence of exposure to indoor allergens on atopic sensitization.⁸ Children who had sensitization to dust mite by age 3 years had significantly higher levels of mite allergen in their homes than did those in the unsensitized group. In the areas with low mite allergen levels in homes fewer children seemed to have allergy to mites. In a group of 86 children at high risk prospectively followed up from birth

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Abbreviations used

- BBS-T: Borate-buffered saline solution with 0.1% Tween 20
CI: Confidence intervals
GM: Geometric mean
MAAS: Manchester Asthma and Allergy Study

until age 5 years in Linköping, Sweden, only one child had sensitization to mites.⁹

There is an increasing body of evidence that the initial priming of the T-cell system to environmental allergens may even occur before birth, probably during late gestation, and that this process in infants at high risk of atopy may be skewed from a T_H1-like to a T_H2-like phenotype.¹⁰ This has been the subject of 2 recent review articles.^{11,12} It has recently been suggested that maternal exposure to allergens beyond the 22nd week of pregnancy plays an important role in fetal T-cell priming.¹³

Reducing exposure to mite allergens may offer a realistic opportunity for primary prevention of atopy and asthma, provided the measures can achieve effective reduction in allergen exposure. The Manchester Asthma and Allergy Study (MAAS) investigates whether dust mite allergen avoidance can prevent sensitization and allergic disease in high-risk children of atopic parents. We report on the effectiveness of avoidance measures used in the MAAS in achieving a low allergen environment over an 18-month period during the second and third trimesters of pregnancy and through the first year of life.

METHODS

The MAAS is a prospective, prenatally randomized, parallel group study that follows the development of asthma and other atopic disorders in a cohort of infants at high risk of atopy who are randomly allocated to house dust mite allergen avoidance or a normal regimen. Cat and dog ownership is an exclusion criterion for the study. The setting is the maternity catchment area of Wythenshawe and Stepping Hill Hospitals, comprising 50 square miles of South Manchester and Cheshire, United Kingdom. The hospitals have a total of 8000 deliveries per annum from a stable mixed urban-rural population.

Screening and recruitment

All mothers were screened for eligibility at "booking" antenatal visits (8th-10th week of pregnancy). The study was explained to the parents, and informed consent for initial questionnaires and skin testing was obtained.

Skin prick testing. Skin tests were performed by prick method on the volar surface of the forearm. The extracts of the 4 most common inhalant allergens (*Dermaphagoides pteronyssinus*, cat, dog, mixed grasses) and negative and positive (histamine dihydrochloride 10 mg/mL) controls were used (Bayer, Elkhart, Ind). Test sites were inspected after 15 minutes and the size of reaction was recorded as the mean of the longest and the midpoint orthogonal diameters. A wheal diameter at least 3 mm in diameter greater than the negative control was regarded as a positive response.

Once an atopic mother was identified, both parents were interviewed for a more detailed allergic history, and the father was skin tested.

Inclusion criteria. Couples were identified as "high risk" and eligible for the study if the following criteria were met: (1) both parents were atopic (ie, prick test positive), (2) the mother was sensitized to indoor allergen (house dust mite, cat, or dog), and (3) there were no pets in the home.

Those parents meeting the entry criteria gave further informed consent before being randomized either to house dust mite allergen avoidance or the control group.

Allergen avoidance measures

Prenatal (completed by the 16th week of pregnancy)

1. Parental bed (pillow, mattress and quilt) was covered with mite-proof bedding (Intervent, WL Gore & Associates, Woking, UK) through pregnancy and after delivery. Two study nurses visited the home at the start of the trial and fitted the bedding covers.
2. Parents were provided with a high-filtration vacuum cleaner (Nilfisk GM 210, Nilfisk, Brøndby, Denmark).

Infant's bedroom

1. At 36 weeks of pregnancy carpet in the infant's bedroom was removed and replaced with a vinyl floor (Forbo-Nairn, Fife, UK).
2. Families were provided with brand-new custom-made crib mattresses encased in mite allergen-proof material (Slumberland, Wilmslow, UK).
3. New portable crib mattresses encased in mite allergen-proof material were provided (Slumberland).
4. Any bed linen was subjected to hot washing (55°C cycle) fortnightly.
5. A washable soft toy was supplied.

Rest of the house. Benzyl benzoate (Acarosan) was applied to carpets and sofas.

Home visits/assessment of allergen exposure

After recruitment a home visit was made; dust sampling was performed and the environmental questionnaire was completed. Homes were visited again immediately after birth and then at 6 months and 1 year. The active group had an additional 3-month "compliance" visit. Dust samples from the parental bed, neonate's bed, bedroom floor, nursery floor, living room floor, and upholstered furniture were collected on each occasion. Dust samples were collected with a Medivac dust sampler (Medivac Plc, Wilmslow, UK) with an air-flow rate of 45 L/s through a 355- μ m diameter mesh screen onto a 5- μ m vinyl filter, thus enabling collection of fine dust samples. The filters were supported in a plastic dust trap located behind the cleaner attachment (nozzle). A 1 m² area of carpet and mattress (upper part) were sampled for 2 minutes. Each sample was transferred into a preweighed petri dish, weighed, coded, and stored at -35°C until extraction. One hundred milligrams of fine dust was extracted with 2 mL of borate-buffered saline solution with 0.1% Tween 20 (BBS-T), pH 8.0. For samples less than 50 mg, 1 mL of BBS-T was added. Samples between 50 and 100 mg were extracted in the proportional amount of BBS-T (1:20). The dust was resuspended with a vortex mixer. Samples were then mixed end over end on an orbital rotator for 2 hours at room temperature before being centrifuged for 20 minutes at 2500 revolutions/min at 4°C. Supernatants were removed and stored at -35°C for future analysis of allergen content.

ELISA measurement of indoor allergens. Der p 1 was assayed with use of a 2-site immunometric ELISA as previously described.¹⁴ The standard used to establish the control curve for Der p 1 assay (UVA 93/02) was considered to contain 2500 ng of Der p 1 milliliter (relative to the World Health Organization/International

Union of Immunological Societies *D pteronyssinus* standard National Institute for Biological Standards and Controls 82/518, which has been estimated to contain 12.5 µg Der p 1 per ampul). Dust extracts were initially assayed neat and at 5-, 25-, and 125-fold dilutions.

The allergen data were found to follow a log-normal distribution; the results are thus reported as geometric means (GM). Changes over the study period both within and between groups were examined with a 2-factor ANOVA with repeated measures. Significant ANOVA findings were investigated in depth with use of the multiple comparison test. The computations were carried out with the SPSS (SPSS, Chicago, Ill) 8.0 computer program. Statistical significance was set at a conventional <5% level. The results are expressed both as allergen concentration (ie, micrograms per gram of fine dust) and total allergen recovered (ie, nanograms per area sampled).

RESULTS

Recruitment for the MAAS commenced on October 1, 1995, and was completed July 1, 1997. We attended 654 antenatal clinics and skin tested a total of 3618 mothers and 2172 fathers. Three hundred twenty-nine "at-risk" couples fulfilled the inclusion criteria, of whom 291 were willing to participate in the study and were randomized into the active (n = 145) and control groups (n = 146). Four couples decided not to participate because of the required environmental control measures. Twelve participating families in the active and 23 in the control group dropped out between the recruitment and the birth of the baby (6 of those in the active group discontinued because of the avoidance measures that had to be implemented). There were no further dropouts from birth to age 1 year, leaving a total of 133 participants in the active and 125 in the control group with a full set of data for the analysis. A total of 1032 home visits have been made at which dust sampling was performed, and more than 6000 dust samples were collected.

Exposure during pregnancy: parental bedroom

Der p 1 concentrations in parental mattresses and bedroom carpets (allergen concentration and total allergen recovered) are shown in Fig 1. Although there were some numeric differences in the baseline allergen levels, this did not reach statistical significance (mattress: recovered allergen, $P = .26$, allergen concentration, $P = .62$; floor: recovered allergen, $P = .25$, allergen concentration, $P = .37$).

Recovered Der p 1 from maternal mattress was reduced by 97.25% (95% CI 95.25%-98.41%) after the introduction of covers, and the effect persisted 6 months (98% reduction, 95% CI 97.25%-99.1%) and 12 months (97.6% reduction, 95% CI 95.7%-98.6%) after the birth of the child. The observed between-group difference in the reduction in Der p 1 levels over the 18-month period was highly significant (ANOVA with repeated measures: recovered allergen, $F = 256.7$, $P < .000001$; allergen concentration, $F = 91.5$, $P < .000001$). In addition, there was a significant reduction in Der p 1 in carpets in the active compared with the control group when the results were expressed as total recovered allergen ($F = 7.5$, $P < .007$).

There was a clear trend that just failed to reach statistical significance when the results were expressed as allergen concentration ($F = 3$, $P = .08$). Recovered Der p 1 from bedroom floor was reduced by 53.7% (95% CI 25.7%-71.2%) in samples collected within 4 weeks of the child's birth. The percentage reduction compared with the baseline allergen levels was 62.8% (95% CI 39.3%-77.2%) at 6 months and 26.5% (95% CI -24% to 57.1%) at 1 year.

Nursery

Der p 1 levels in the crib mattress and on the nursery floor (allergen concentration and total allergen recovered) are shown in Fig 2. Mite allergen levels in all sampling sites in infants' rooms in the active group were extremely low. The GM of the total recovered Der p 1 from infants' mattresses was 2.3 ng (95% CI 1.6-3.4) at birth, 6.8 ng (95% CI 4.5-10) at 6 months, and 15.6 ng (95% CI 9.8-24.8) at 1 year. Allergen levels increased at significantly higher rates in the control group compared with the active group (crib mattress: recovered allergen, $F = 10.66$, $P = .001$; allergen concentration, $F = 6.2$, $P = .014$). Minute quantities of allergen were recovered from the nursery floor in the active group (1 ng [95% CI 0.9-1.1] at birth, 1.7 ng [95% CI 1.2-2.5] at 6 months, and 2 ng [95% CI 1.3-3.5] at 1 year). Highly significant differences in mite allergen levels between the active and control groups over the 18-month period were observed (recovered allergen, $F = 103.3$, $P < .00001$; allergen concentration, $F = 32.3$, $P < .00001$). The total amount of allergen recovered at age 1 year was 29-fold (95% CI 15.1- to 56.7-fold) higher in the control than in the active group.

Some infants may spend most of the time during the first year of life in the parental bedroom rather than in the nursery. During the home visits we asked and recorded this information to measure allergen levels from the floor of the room in which the child spends most of the time. The data are presented in Table I. Der p 1 was significantly lower in the active compared with the control group when allergen from the floor of the room in which the child spends most of the time was measured (recovered allergen, $F = 68.3$, $P < .000001$; allergen concentration, $F = 22.5$, $P < .0001$).

Living room

Mite allergen levels in the living room floor at baseline were significantly higher compared with bedroom floor levels (GM 1.00 µg/g, 95% CI 0.77-1.3 vs GM 0.71 µg/g, 95% CI 0.54-0.93; 29.4% difference, 95% CI 5.0-47.5, $P = .02$). Dust mite allergen levels in the carpet and upholstered furniture from the living room are presented in Table II. Der p 1 level in the active group fell significantly within the active group at birth and 6 months compared with baseline both in the carpet and upholstered furniture (carpet: recovered allergen, birth 44.7% reduction, 95% CI 17.4-62.9, $P = .004$, 6 months 55.3% reduction, 95% CI 29.5-71.7, $P = .001$; allergen concentration, birth 37.6% reduction, 95% CI 11.9-55.8, $P = .008$, 6 months 49.4% reduction, 95% CI 26.6-65.2, $P < .001$; upholstered furniture: recovered allergen, birth 38.7%

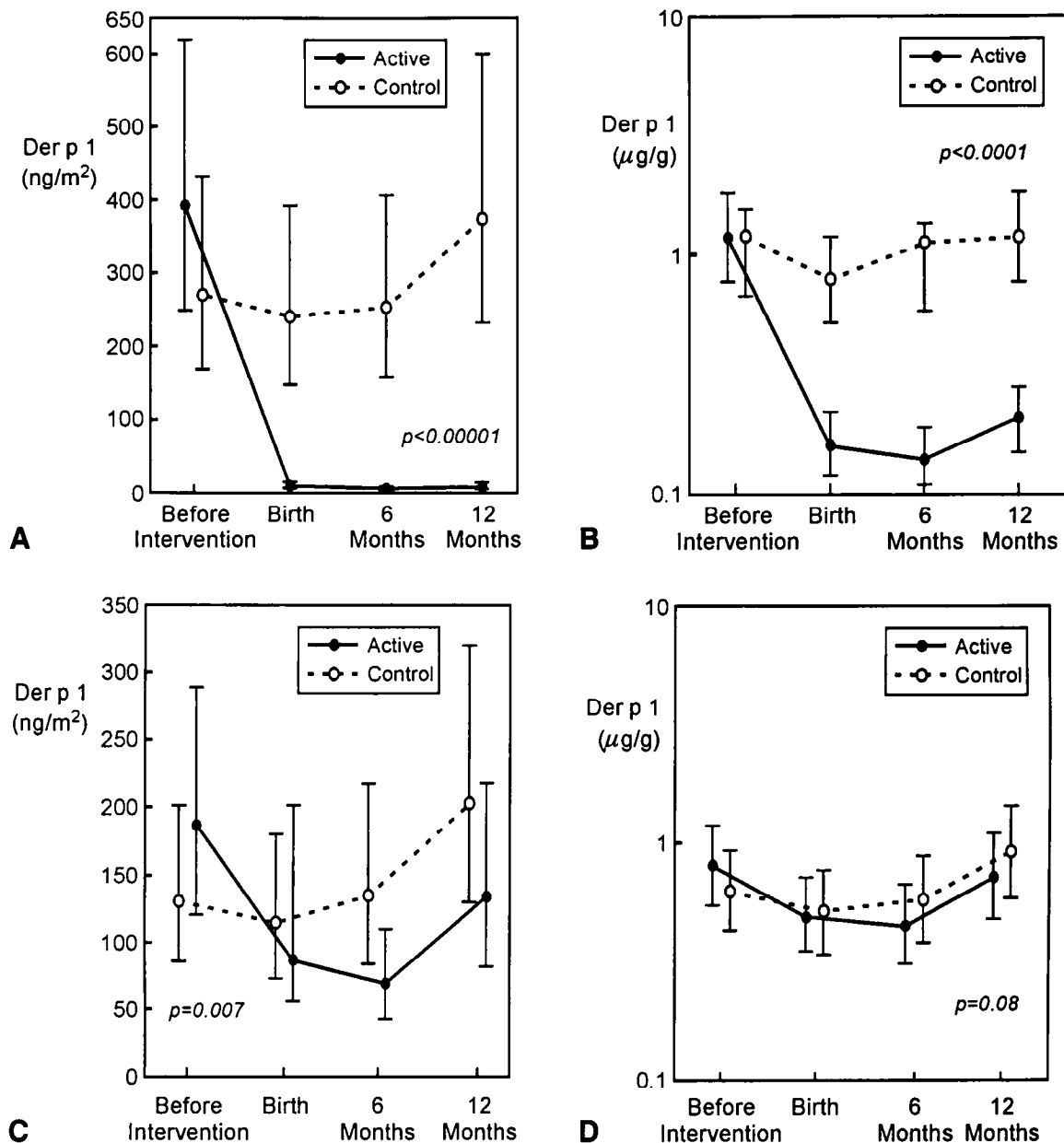


FIG 1. House dust mite allergen levels (GM and 95% confidence intervals [CI]) in maternal bedroom at base-line (10th week of pregnancy) and then at birth, 6 months, and 12 months. **A**, Mattress, total allergen recovered. **B**, Mattress, allergen concentration. **C**, Bedroom floor, total allergen recovered. **D**, Bedroom floor, allergen concentration.

reduction, 95% CI 11.3-57.6, $P = .01$, 6 months 54% reduction, 95% CI 28.8-70.3, $P = .001$; allergen concentration, birth 31.8% reduction, 95% CI 8.7-49, $P = .01$, 6 months 37.6% reduction, 95% CI 13.8-54.8, $P < .004$). However, there was no significant difference between the groups.

DISCUSSION

Prevalence rates of asthma in the United Kingdom are among the highest in the world, and the disease places

enormous demands on health care services.^{15,16} Thus it is essential to devise and implement methods of primary prevention. Peat and Li¹⁷ have recently reviewed the roles of risk factors for which there is evidence of a primary involvement in asthma etiology, focusing on those with the greatest potential for use in primary prevention (ie, mite allergens, breast-feeding, passive smoking, diet). The evidence for risk factors with a direct role in the causation of asthma is most complete for indoor allergens, particularly for dust mites.¹⁷⁻²¹ The relevance of allergens other than mites is inconsistent between differ-

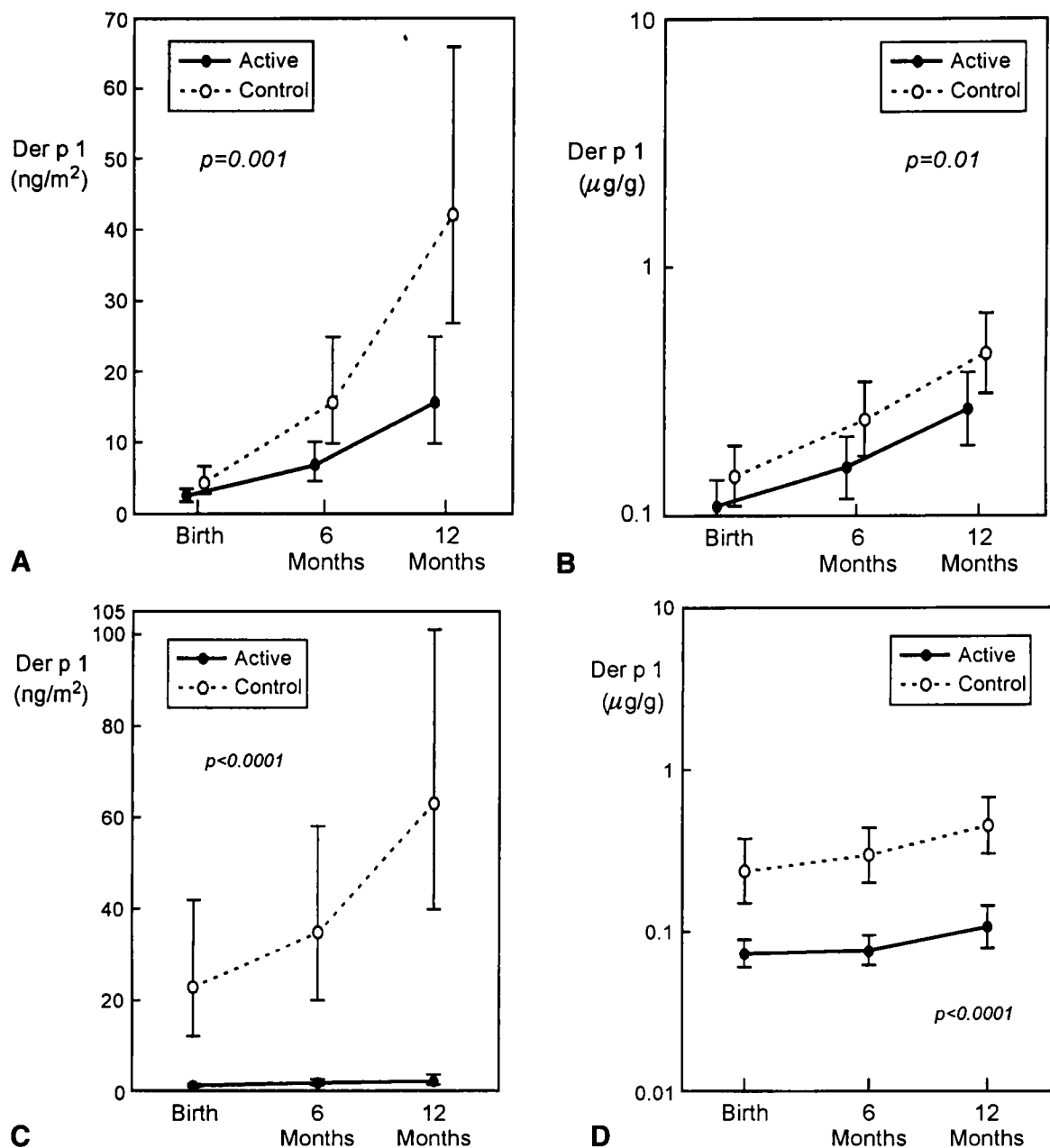


FIG 2. House dust mite allergen levels (GM and 95% CI) in nursery room at birth, 6 months, and 12 months. **A**, Crib mattress, total allergen recovered. **B**, Crib mattress, allergen concentration. **C**, Floor, total allergen recovered. **D**, Floor, allergen concentration.

ent areas and depends on the climate, habits, and socioeconomic features of the local community.²¹ Both increasing exposure and increasing sensitivity to dust mites represent progressively higher risks for the development of asthma. In a recent study of children from 6 regions of New South Wales the proportion of children sensitized to mites was higher in areas with higher mite allergen levels.¹⁹ Children who were skin test positive to mites in each of the areas were at significant risk for having asthma, and the risk of mite-sensitized children hav-

ing asthma doubled for every doubling of the level of exposure to mite allergens.

Successful environmental control could be anticipated to have a greater impact on prevalence of asthma than interventions directed at other risk factors such as exposure to environmental tobacco smoke, dietary interventions, and breast-feeding.²² Any environmental control strategy should be effective in reducing allergen exposure, flexible to suit individual needs, clinically effective, and not prohibitively expensive.²³ In a population at risk

TABLE I. House dust mite allergen levels in carpet and upholstered furniture from living room (GM and 95% CI)

	Baseline	Birth	6 months	12 months
Living room floor	246	133	111	175
Active (ng/m ²)	165-365	87-204	70-175	111-274
Living room floor	152	123	122	166
Control (ng/m ²)	87-239	79-191	78-189	102-271
Living room floor	1.21	0.74	0.63	0.90
Active (μg/g)	0.84-1.74	0.51-1.07	0.42-0.93	0.60-1.37
Living room floor	0.79	0.68	0.73	0.88
Control (μg/g)	0.54-1.17	0.46-1.03	0.49-1.07	0.56-1.39
Upholstered furniture	196	123	92	134
Active (ng/m ²)	125-308	78-195	57-151	83-214
Upholstered furniture	136	137	121	86
Control (ng/m ²)	85-217	84-222	73-201	52-142
Upholstered furniture	0.96	0.67	0.61	0.82
Active (μg/g)	0.69-1.34	0.47-0.95	0.44-0.86	0.57-1.19
Upholstered furniture	0.78	0.72	0.68	0.62
Control (μg/g)	0.55-1.10	0.51-1.01	0.47-0.99	0.43-0.90

TABLE II. House dust mite allergen levels from floor of room in which child spends most of time (GM and 95% CI)

	Birth	6 months	12 months
Child's bedroom floor	47.4	3.4	3.6
Active (ng/m ²)	29-76.7	2.2-5.4	2.1-6.1
Child's bedroom floor	98.6	62.5	82.6
Control (ng/m ²)	62.5-155.8	38.8-100.9	52.3-130.6
Child's bedroom floor	0.347	0.106	0.143
Active (μg/g)	0.243-0.495	0.080-0.139	0.103-0.200
Child's bedroom floor	0.469	0.421	0.551
Control (μg/g)	0.313-0.704	0.286-0.619	0.368-0.825

the most vigorous mite avoidance measures should be implemented to minimize exposure as far as possible.¹⁸

In the current study we have demonstrated that an integrated dust mite allergen avoidance regimen is highly effective in achieving and maintaining a very low allergen environment in homes of children at high risk of allergic disease. In designing the study we considered carefully where and how an infant spends his or her early life. Although not easy, it is possible to substantially reduce allergen exposure. We have confirmed that covering mattresses, pillows, and quilts with covers that are impermeable to mite allergens is an exceptionally effective long-term avoidance measure.²⁴ Allergen levels were dramatically reduced after the introduction of covers. The covers proved to be robust, easily fitted, and easily cleaned as the effectiveness in maintaining low allergen levels was confirmed over an 18-month period.

Carpets are an important microhabitat for mite colonization and a possible source of allergen from which beds can be reinfested. In the current study carpeted living rooms had higher concentrations of mite allergens than carpeted bedrooms, which is consistent with the finding of the study from the Netherlands²⁵ but is in contrast with the recent Australian study.²⁶ We have confirmed that replacement of fitted carpets with vinyl flooring leads to a very substantial reduction in mite allergen levels. Furthermore, practically no allergen buildup on these hard floors was observed over a 1-year period. This

is in line with the data from the Melbourne House Dust Mite Study showing that low bedroom allergen levels were found in bedrooms with no carpet and impermeable mattress encasement even when adjacent rooms were carpeted.²⁶ In the current study the presence of fitted carpets in other parts of the house did not affect mite allergen levels on the nursery floor. However, the attempt to control the dust mite population in carpets in the rest of the house by the use of benzyl benzoate was considerably less effective. It is worth noting that the research team applied all other avoidance measures (ie, we fitted impermeable covers on mattresses, pillows, and quilts; delivered high-filtration vacuum cleaners and serviced them regularly; delivered and put in place crib and portable crib mattresses; removed carpets and replaced them with vinyl floors). Benzyl benzoate was delivered to the participants with advice on how to use it (allowing the powder to remain on carpet for 12 to 18 hours with repeated brushing followed by vigorous vacuum cleaning), but participants were asked to apply it themselves.

The avoidance measures used in the MAAS achieved and maintained very low mite allergen levels during pregnancy and in the first year life. The advantages of this integrated approach to environmental control is that once all the measures are in place very little additional effort is required from the families in maintaining a low allergen environment. The practical acceptance of the aggressive environmental control was excellent and only

10 families found the avoidance measures too stringent. We intend to encourage the families to continue to use these measures as long as possible (eg, children's beds will be covered in mite-impermeable covers once they move from cribs to beds).

In the Isle of Wight Study even modest reductions in house dust mite allergen levels in homes of infants at risk of allergy (by relatively ineffective acaricides) reduced the prevalence of sensitization to mites and recurrent wheezing during the first years of life and had some benefits on allergic disease at age 4 years.^{27,28} We have established that it is possible to substantially reduce allergen levels and achieve and maintain a very low allergen environment in homes. The long-term follow-up of the children in this study is under way to obtain the critical data to address whether living in a low-allergen environment prevents sensitization and the risk of developing asthma and other atopic disorders.

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