

Association between Der p 1 concentration and peak expiratory flow rate in children with wheeze: A longitudinal analysis

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Background: House dust mite (HDM) allergen exposure has been well documented as an environmental cause of airway hyperresponsiveness (AHR) and asthma symptoms. The relationship between asthma morbidity and exposure to low concentrations of HDM allergen suggests that there may be no safe exposure threshold to HDM allergen.

Objective: We aimed to investigate the associations between Der p 1 in bedding and lung function in 30 children with a history of wheezing in a longitudinal study.

Methods: After a cross-sectional study of school children, which included histamine challenge for AHR and skin testing for dust mite atopy, we made repeated measurements of HDM allergens in children with a history of wheeze over a 12-month period. These children also kept a daily asthma diary in which they recorded their peak expiratory flow rates (PEFRs). We used a repeated measures model to determine the association between PEFR and HDM allergen concentration.

Results: There was a significant association between PEFRs and HDM allergen concentration (β -coefficient = -14.17 , $P = .0024$) in children with HDM atopy. An association was not found in children without HDM atopy.

Conclusions: These findings support the hypothesis that HDM allergens have an adverse effect on the lung function of children with wheeze and highlight the importance of maintaining low dust mite allergen levels throughout the year in the home environment of children sensitized to HDMs. (*J Allergy Clin Immunol* 1998;102:382-6.)

Key words: Der p 1, asthma, children, dust mite atopy, peak expiratory flow rate, random effects model, longitudinal analysis

Abbreviations used

AHR: Airway hyperresponsiveness

HDM: House dust mite

95% CI: 95% confidence interval

PEFR: Peak expiratory flow rate

Asthma is the most common chronic disease in childhood, with a prevalence in Australia of about 12%. A further 12% of children have episodic wheeze and/or have used a bronchodilator in the previous 12 months.¹

House dust mite (HDM) allergen exposure has been well documented as an environmental factor associated with airway hyperresponsiveness (AHR) and asthma symptoms.²⁻⁴ The relationship between HDM sensitization and exposure to low concentrations of HDM allergen⁵ suggests that there may be no safe exposure threshold to house dust allergen.

There are few longitudinal epidemiologic studies reported in the literature that have investigated the relationship between HDM allergen (Der p 1) and lung function (as measured by peak flowmeter) in children with asthma. In this study we aimed to investigate the effect of Der p 1 in bedding on lung function in 30 children with a history of wheezing in a longitudinal study spanning 12 months.

METHODS

Subject selection

In August 1993, we performed a cross-sectional study of children in school years 3 to 5 (aged 8 to 12 years) in 6 primary schools in western and southwestern Sydney.⁶ The cross-sectional study consisted of a parent-completed questionnaire seeking information about demography, history of asthma and wheeze, family history of atopy and asthma morbidity, measurement of children's AHR by histamine bronchial challenge, and assessment of children's atopy status by skin prick tests to 9 commercially available allergens.

We then invited children and parents to participate in a 12-month longitudinal study (commencing in January 1994) of lung function and air pollution if a child had a history of wheezing in the previous 12 months. This cohort of children had baseline HDM allergen levels measured in their bedding at the commencement of the longitudinal study. We then selected 15 children with the highest Der p 1

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levels in their bedding and another 15 children with the lowest Der p 1 levels. Children were selected by one of the investigators who was blinded to baseline data from the questionnaire and the results of the bronchial histamine challenge and of skin testing to the commercially available allergens. Repeated dust mite measurements of the bed were made in June, August, October, and December 1994.

Ethics approval was obtained from the Western Sydney Area Health Service before the cross-sectional and longitudinal studies.

Peak expiratory flow rate measurements

We asked each child in the longitudinal study to complete an asthma diary twice a day, when they woke up in the morning and in the evening before going to bed. The asthma diary included information about peak expiratory flow rate (PEFR), asthma symptoms, and medications used. Each child performed 3 PEFR measurements in the standing position with a mini-Wright peak flow meter (Clement Clarke International Ltd). All 3 PEFR measurements were to be recorded in the diary. We instructed children to leave the diary blank if they forgot to complete their diary and not to complete the diary retrospectively.

Trained research assistants visited all children in the study at least once every 6 to 8 weeks. Between each home visit, we made 1 telephone contact with each household/child. At the first visit, the children and parents were taught to use the peak flowmeter and to complete the asthma diary. At each subsequent home visit, we observed the child's PEFR measurement technique and corrected the PEFR measurement technique if necessary. We asked parents to supervise their child's diary keeping and PEFR measurements.

House dust collection

This cohort of children had baseline HDM allergen measurements of the bed performed in March/April 1994. House dust was collected from the bed with a portable vacuum cleaner (Makita, Model 4071D) modified to collect fine dust in a 25- μ m pore size nylon bag. We vacuumed the items of the bedding starting at the bottom and working up through the layers. We vacuumed for a total of 1 minute and sampled an area of about 1 to 1.5 m². Items vacuumed included mattress, upper bedding, pillows, pillow casings, and sheets.

After collection, we stored the dust samples at -20° C to prevent mite allergen degradation and fungal growth. Dust was sieved through a 400- μ m wire mesh, and 50 mg of fine dust was extracted in 1 mL 0.2% BSA, PBS, and 0.05% Tween 20 at room temperature. The samples were vortexed, rotated for at least 20 minutes, and centrifuged at 3000 g for 10 minutes, and the supernatant was stored at -20° C until analysis.

HDM allergen Der p 1 concentration was measured in the supernatant by using a double monoclonal ELISA assay (purchased from University of Virginia) according to the method described by Luczynska et al,⁷ with the exception that the monoclonal antibody was used at half the recommended concentration, and by using the Der p 1 allergen standard provided with the kit. The HDM allergen concentrations are reported as micrograms of Der p 1 per gram of fine dust.

Statistical methods

We used the highest morning PEFR measure from the 3 attempts as the values to be used in all analyses. The main outcome measure was the weekday minimum morning PEFR over the 1-month period when Der p 1 concentration was measured. Der p 1 concentrations were log transformed (natural log) before analysis.

We used the *t* test and chi square test to compare continuous and categorical variables, respectively. We used analytical methods for

TABLE I. Demographic and risk factor characteristics of children by HDM atopy status (*n* = 30)

	HDM atopy (<i>n</i> = 17)	No HDM atopy (<i>n</i> = 13)
Mean age $\pm$ SD* (y)	9.6 $\pm$ 0.70	9.5 $\pm$ 1.05
Boys (%)	11 (65)	5 (38)
Height $\pm$ SD (cm)	139.6 $\pm$ 7.53	133.5 $\pm$ 4.70*
Any atopy (%)	17 (100)	4 (31)
AHR (%)	10 (59)	5 (38)
Asthma diagnosed by a doctor (%)	13 (76)	12 (92)
Age of first wheeze, mean age $\pm$ SD (y)	3.1 $\pm$ 1.45	3.1 $\pm$ 1.32
$\geq$ 4 episodes of acute asthma in past 12 months (%)	8 (47)	3 (23)
Visited local doctor for asthma in past 12 months (%)	4 (24)	1 (8)
Used β -agonist in past 12 months†	11 (69)	11 (85)
Used inhaled steroids in past 12 months‡	8 (62)	7 (78)
Mother smoked during pregnancy (%)	1 (6)	6 (46)§
One parent smoked in home in first 2 years of child's life (%)	7 (41)	7 (54)
Carpet in either bedroom or living room (%)	16 (94)	13 (100)
Gas cooker (%)	4 (24)	4 (31)
Combustion heating (%)	10 (59)	5 (38)

*Standard deviation.

†Missing = 1.

‡Missing = 8.

§*P* < .05.

||*P* = .001.

repeated measures over time (Proc Mixed in SAS statistical package)⁸ to model the association between PEFR and Der p 1 concentrations. In a random effects model, PEFR was the response variable, Der p 1 concentration and linear time trend were treated as fixed effects, and subjects were treated as random effects. An indicator variable was used to adjust for linear time trend. A *P* value of less than .05 indicated statistical significance.

RESULTS

For this substudy, 30 children were selected for repeated HDM measurements at approximately 2 monthly intervals. Fifteen children had initial high Der p 1 concentrations in their bedding (high Der p 1 homes: mean Der p 1 = 148.8 μ g/g, 95% CI = 106.7-206.4), whereas the other 15 had initial low Der p 1 concentrations in their bedding (low Der p 1 homes: mean Der p 1 = 5.5 μ g/g, 95% CI = 4.0-7.5).

Demographic data and asthma characteristics for the 30 children by HDM atopy status are presented in Table I. Overall, there were nearly equal numbers of boys and girls (boys: *n* = 16; 53%), and the mean age was 9.6 years. Seventeen children (57%) had HDM atopy as measured by skin prick testing, 15 children (50%) had AHR on histamine challenge, and 25 children (83%) had received a

TABLE II. Mean values for Der p 1 concentration and minimum morning PEFR measured on 5 separate occasions

	March 1994	June 1994*	August 1994	October 1994	December 1994
Children with HDM atopy (n = 17)					
Log _e Der p 1 (μg/g)					
mean ± SD	3.4 ± 1.84	3.5 ± 1.57	3.6 ± 1.38	3.1 ± 1.27	2.8 ± 1.35
Minimum morning PEFR (L/min)					
mean ± SD	251 ± 78.8	265 ± 56.1	292 ± 40.9	308 ± 28.4	307 ± 33.4†
Children without HDM atopy (n = 13)					
Log _e Der p 1 (μg/g)					
mean ± SD	3.4 ± 1.77	3.6 ± 1.29	3.64 ± 1.24	3.4 ± 1.16	2.9 ± 1.32
Minimum morning PEFR (L/min)					
mean ± SD	263 ± 72.3	278 ± 77.5	275 ± 64.8	290 ± 74.9	301 ± 72.9

*Two subjects missing (1 from each group).

†One subject missing.

diagnosis of asthma from a doctor. More children with HDM atopy had atopy to at least 1 of the 9 commercial allergens used ($P < .001$), and fewer of their mothers smoked cigarettes during pregnancy compared with children without HDM atopy ($P < .05$).

Table II shows the repeated mean Der p 1 concentration and minimum morning PEFR at baseline and for the 4 other occasions when Der p 1 measurements were obtained. For both children with HDM atopy and without HDM atopy, there were no significant differences in the Der p 1 concentrations on each of the occasions it was measured. Der p 1 concentrations were lowest in December, but not significantly different from the other times. There was no difference in the minimum PEFR between the 2 groups at baseline. Minimum PEFR improved over the study period in both groups.

At baseline, compared with children without HDM atopy, children with HDM atopy had a greater proportion of person-days with morning wheeze (4.3% vs 3.5%; $P = .55$), use of salbutamol in the morning (8.9% vs 1.9%; $P < .01$), and use of inhaled steroids in the morning (13.8% vs 0.5%; $P < .01$) (data not shown). Children with HDM atopy had a greater proportion of person-days of morning wheeze in June (9.4% vs 1.2%; $P < .01$) and lesser proportion of person-days of morning wheeze in December (1.1% vs 6.0%; $P < .01$) compared with children without HDM atopy. For salbutamol and inhaled steroids used, differences between the 2 groups of children were maintained over the remaining 4 occasions, except for December when there was no difference in the proportion of person-days in the use of salbutamol in the morning (4.3% vs 3.6%; $P = .64$).

Correlation coefficients between Der p 1 and minimum morning PEFR for the 5 occasions when they were measured are presented in Table III. For children with HDM atopy, there was a moderate negative correlation between log Der p 1 and minimum morning PEFR on each of the 5 occasions (correlation coefficient range: -0.35 to -0.61). The correlation coefficients were statistically significant except for March 1994 and December 1994. For children without HDM atopy, correlation coef-

ficients between log_e Der p 1 and minimum morning PEFR were generally negative (correlation coefficient range: -0.39 to 0.22) but not statistically significant. Fig 1 presents the PEFR and log_e Der p 1 data for all children for August 1994.

In a random effects model regression analysis for repeated measures, when all 30 children were included in the analysis and after adjusting for time trend, there was a significant association between PEFR and log_e Der p 1 concentration (β -coefficient = -7.83 , $P = .048$) (Table IV).

However, when only children with HDM atopy were considered, the association between PEFR and log_e Der p 1 concentration was much larger and highly significant (β -coefficient = -14.17 , $P = .0024$). The association between PEFR and log_e Der p 1 concentration was not statistically significant for children without HDM atopy.

DISCUSSION

In our study we found that in children with HDM atopy, there was a consistent moderate correlation between log_e Der p 1 concentration and PEFR on 4 of the 5 occasions when Der p 1 was measured. In the linear regression model, which takes into account clustered data with repeated measures, there was a highly significant association between Der p 1 concentration and PEFR after adjusting for linear time trend. An increase in a one natural log concentration of Der p 1 would lead to an approximately 15 L/min decrease in PEFR, reflecting a fall in PEFR of about 6% from baseline. In this group of children, Der p 1 concentration ranged from about 3.7 to 665 μg/g. We did not find any significant associations between Der p 1 concentration and PEFR in children without HDM atopy.

The associations between HDM atopy and airway responsiveness⁹⁻¹¹ and HDM atopy and asthma symptoms¹²⁻¹³ are well documented. There are also cross-sectional studies that demonstrate an association between HDM atopy and indices of PEFR.¹⁴ However, in the few reported studies that have investigated the relationship between HDM allergen concentration, mite count, or both and PEFR over time, the results are inconsistent.

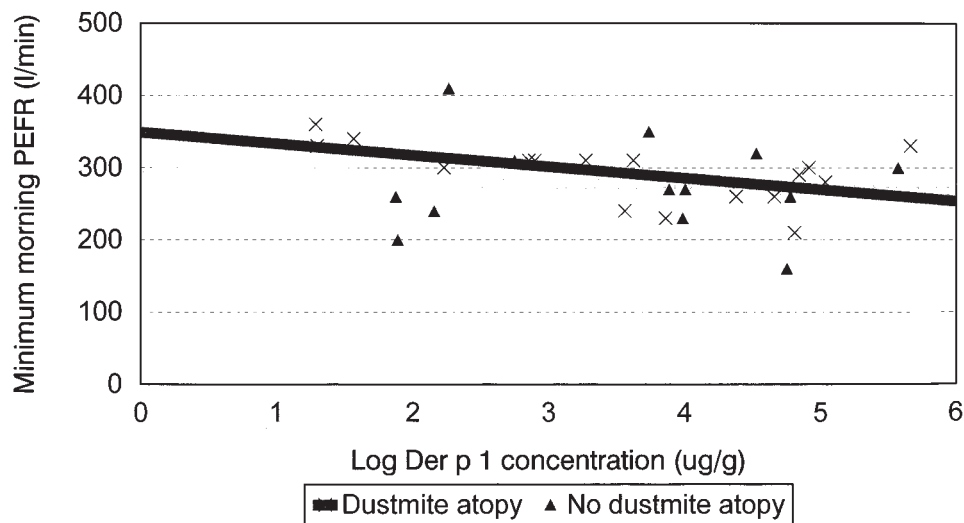


FIG 1. Scatter plot of minimum morning PEFR and log Der p 1 concentrations for August 1994.

TABLE III. Correlation coefficients between minimum morning PEFR and log_e Der p 1 concentration

	March 1994	June 1994*	August 1994	October 1994	December 1994
Children with HDM atopy (n = 17)	-0.35	-0.61†	-0.54†	-0.51†	-0.44
Children without HDM atopy (n = 13)	0.22	0.14	-0.08	-0.39	-0.06
All children (n = 30)	-0.12	-0.26	-0.28	-0.39†	-0.19

*Two subjects missing.

†P < .05.

In a study of 17 children followed up over 6 months with monthly measurements of HDM count from mattresses and daily measurement of PEFR, Kivity et al¹⁵ did not find any correlation between HDM counts and peak flow variability. In 34 HDM-atopic adults with asthma followed for between 3 and 12 months, Marks et al¹⁶ demonstrated significant negative associations between changes in allergen concentration in bedding with changes in AHR and symptom score, but not with peak flow variability or morning PEFR.

Chan-Yeung et al,¹⁷ in a 12-month study of 52 children and 68 adults with asthma after controlling for seasonality, demonstrated a significant negative relationship between mean PEFR (percent predicted) and HDM allergen concentration (sum of Der p 1 and Der f 1 for mattress and floor) for children allergic to mites only. In this group of children, there was about a 5% decrease in mean PEFR (percent predicted) for every 1 log (microgram per gram of dust) increase in HDM allergen concentration.

Although we found a significant association between Der p 1 and PEFR in HDM-atopic children, we cannot assume causality between the 2 factors. We have not

TABLE IV. Associations between log_e Der p 1 concentration and PEFR from a random effects regression model

	β-Coefficient	Standard error	P value
Children with HDM atopy (n = 17)	-14.17	4.47	0.0024
Children without HDM atopy (n = 13)	-2.27	4.40	0.61
All children (n = 30)	-7.83	3.92	0.048

*Adjusted for time trend.

taken into account or adjusted for a number of potential confounders, such as pollens, molds, ambient and indoor air pollution, and viral infections, all of which have a role in asthma.^{18,19}

The subjects for this substudy presented in this report were drawn from a larger prospective study examining the associations between ambient air pollution and asthma in a group of children with a history of recent wheeze. By the nature of our subject selection, there were great differences in Der p 1 concentration between the high and low Der p 1 homes. At baseline, Der p 1 concentration in the low Der p 1 homes did not exceed 15 µg/g (and was generally less than 10 µg/g), whereas in the high Der p 1 homes, Der p 1 concentrations ranged from about 80 to 650 µg/g. However, the concentrations were no different between HDM-atopic and HDM nonatopic homes. This is consistent with other studies, which have not demonstrated an association between prevalence of HDM atopy and concentrations of Der p 1 in a similar population.²⁰

Although a seasonal variation for Der p 1 was not demonstrated in our data, mean Der p 1 concentrations were lowest in December than at other times of the year. This is unusual because in Australia December is a summer month with higher ambient temperatures and relative humidity than other times of the year. The average daily

temperature and relative humidity in December 1994 were 21.4° C and 68.8%, respectively, whereas in August 1994 (a winter month), the mean daily outdoor temperature and humidity were 11.3° C and 63.3%, respectively (data provided by NSW Environment Production Authority, 1995). Although similar nonseasonality for Der p 1 concentration in Australia has also been demonstrated in the past,²⁰ others have demonstrated increased HDM allergen concentrations in bedroom floors from late summer to early autumn and lower levels in winter.²¹

The minimum morning PEFR (lowest of daily morning PEFR) expressed as a percentage of the highest PEFR is strongly correlated with AHR and moderately correlated with asthma symptoms.²² However, the minimum morning PEFR expressed as a percentage of the highest PEFR was not normally distributed, and we used the minimum morning PEFR over each 1-month period as the outcome measure for lung function. In our data the minimum morning PEFR and the minimum morning PEFR expressed as a percentage of the highest PEFR were highly correlated ($r = 0.85$, $P = .0001$).

This study has demonstrated that natural changes in HDM levels can lead to significant reductions in PEFR in children who are allergic to HDMs. These findings highlight the importance of maintaining low HDM levels throughout the year in the home environment of children sensitized to HDMs.

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