

Low domestic exposure to house dust mite allergens (*Der p 1*) is associated with a reduced non-specific bronchial hyper-responsiveness in mite-sensitized asthmatic subjects under optimal drug treatment

P. MAESTRELLI, L. ZANOLLA*, P. PUCCINELLI†, M. POZZAN‡ and L. M. FABBRI§

*Institute of Occupational Medicine, University of Padova, Padova, *General Hospital, Verona, †Alk-Abello, Milano, ‡Study Group of Ricerca Sanitaria Finalizzata n.395/03/93 Regione Veneto, Venezia, and §Research Centre on Asthma and COPD, University of Ferrara, Ferrara, Italy*

Summary

Background Airway inflammation in asthma causes symptoms, airflow limitation and bronchial hyper-responsiveness. The strategy of asthma management is to reduce airway inflammation by drug treatment and avoidance of triggers, including allergens.

Objective We determined the effect of exposure to house dust mite (HDM) allergens on bronchial responsiveness in asthmatics sensitive to mites while under optimal drug treatment.

Methods We studied 71 mild to moderate HDM-sensitive asthmatics. Drug treatment sufficient to keep asthma under control was administered to each patient for 1 year. Subjects were divided into two groups, according to the amount of *Der p 1* in their bedrooms measured after standard HDM reduction measures: low *Der p 1* exposure ($0.64 \pm 0.5 \mu\text{g/g}$ dust) (Group 1, $n = 34$) and high *Der p 1* exposure ($12.5 \pm 11.4 \mu\text{g/g}$) (Group 2, $n = 37$). Bronchial responsiveness to methacholine (PD20FEV₁) was determined at the beginning and end of the study.

Results In Group 1, PD20FEV₁ increased 2.15-fold at the end of the study from 57 to 123 μg ($P < 0.05$), whereas in Group 2 no significant changes were observed. The subjects in Group 2 tended to increase the use of inhaled steroids and bronchodilators in the autumn months compared with subjects in Group 1, but the difference was not significant.

Conclusion This long-term study shows that exposure to lower levels of mite allergens in the bedroom is associated with a decrease of bronchial hyper-responsiveness in sensitized asthmatic subjects under optimal drug treatment.

Keywords: bronchodilators, corticosteroids, indoor antigens, lung function, prevention

Clinical and Experimental Allergy, Vol. 31, pp. 715–721. Submitted 13 January 2000; revised 11 August 2000; accepted 18 August 2000.

Introduction

Current management of asthma is based on avoidance or control of asthma triggers and on medication, including anti-inflammatory agents and bronchodilators. Both interventions are intended to suppress or reverse airway inflammation which causes symptoms, variable airflow limitation and bronchial hyperresponsiveness [1].

Domestic mites are the most common indoor allergen and a major cause of asthma world-wide [2,3]. Occurrence of asthma symptoms in subjects sensitized to house dust

On behalf of the Regione Veneto Study Group on 'Effect of Immunotherapy in allergic asthma': L. Andri‡, L. Berardino‡, P. Bernardis‡, V. Calvo‡, O. Cappellato‡, S. Crescioli‡, M. Crivellaro‡, A. De Rossi‡, B. Di Campli‡, C. Gemignani‡, S. Macaluso‡, G. Marcer‡, G. Moro‡, L. Murer‡, G. Piacentini‡, I. Piazza‡, G. Senna‡, P. Serena‡, A. Sirena‡, C. Sivini‡ and E. Volpato‡.

Correspondence: Piero Maestrelli MD, Istituto di Medicina del Lavoro, Università di Padova, Ospedale Giustiniano, via Giustiniani 2, 35128 Padova, Italy. E-mail: pmaestr@ux1.unipd.it

mites has been related to the amount of environmental mite allergens [4]. Moving sensitized asthmatics to allergen-free mountain Institutions or Hospitals results in improvement in symptoms and bronchial hyper-responsiveness [5–7]. Reducing mite allergens in patients' homes is a difficult task and complete success is difficult to achieve [8]. One of the most effective interventions is the application of allergen-proof encasing of mattresses and pillows in the bedroom [9,10].

Controlled trials of mite avoidance in the domestic environment have been conducted, but the evidence that lower house dust mite (HDM) exposure contributes to improvement of clinical and functional parameters in asthma remains controversial [11]. In contrast, several long-term clinical studies in asthma have shown that anti-inflammatory agents are effective for control of symptoms, improvement of lung function and decrease of bronchial hyper-responsiveness [12,13]. Most of the studies on allergen avoidance either kept drug regimen constant irrespective of the changes in patients' conditions during trials or did not standardize the drug treatment [14–18]. The aim of this study was to determine whether exposure to different levels of mite allergen in the bedroom may influence the outcome of asthma in sensitized asthmatic subjects given the drug treatment necessary to achieve control of the disease [1]. To do this we studied a group of asthmatic subjects sensitized to HDM. Standard allergen-avoidance measures in bedrooms and drug treatment, adequate to the severity of the disease, were provided for the patients. After 1 year, we assessed whether changes in bronchial responsiveness to methacholine and in the use of antiasthma drugs were related to measured levels of house dust mite allergens in the patients' bedrooms.

Methods

Subjects

We recruited 71 asthmatic subjects from the out-patient clinic of 11 participating centres: 31 female and 41 male patients, with a mean age of 23 years (range 8–43 years). Thirteen were children < 15 years old.

The patients included in the study had the following characteristics: (i) a typical history of bronchial asthma for at least 1 year [1]; (ii) confirmation of bronchial asthma by reversibility of bronchial obstruction (increase in forced expiratory volume in 1 s (FEV₁) of at least 15% after inhaled salbutamol) or bronchial hyperresponsiveness to methacholine challenge; (iii) sensitization to HDM confirmed by positive skin prick test to HDM extract (ALK-Abellò, Milano, Italy) and/or presence of serum-specific IgE to *Dermatophagoides pteronyssinus* (Phadebas RAST; Pharmacia, Uppsala, Sweden; $\geq$ class 3); (iv) absence of

concomitant sensitization to perennial allergens other than HDM, such as cat, dog, *Alternaria*, *Aspergillus* and cockroach; (v) residence in north-east Italy < 1000 m altitude above sea level. Patients presenting at least one of the following characteristics were excluded: respiratory diseases other than asthma, cardiovascular or immunological diseases and other severe illnesses; active smokers; FEV₁ persistently < 70% of predicted; previous immunotherapy with HDM within 3 years from the start of the study; recurrent asthma exacerbations or more than two emergency Hospitalizations for asthmatic attack in the previous year, work-related symptoms of asthma or occupational asthma. Informed consent to participate in the study was given by all patients. The protocol was the observational year preceding an ongoing study on immunotherapy with dust mite in allergic asthma and was approved by Ethical Committees of University Hospital of Padova and of participating centres.

Design of the study

Patients were recruited over a period of 5 months (November to March) and were studied for a period of at least 12 months.

At entry (visit 0), general medical and allergy evaluation, lung function tests, including methacholine challenge, were performed. Severity of asthma was assessed by standard criteria and drug treatment was assigned according to the recommendations of the 'International Consensus Report on Diagnosis and Management of Asthma' [1]. Salbutamol (Meter dose inhaler (MDI), 100 µg per actuation) was used as bronchodilator. Beclometasone dipropionate (MDI, 250 µg per actuation) was used as regular anti-inflammatory agent. No other antiasthma drugs were allowed, except oral antihistamine tablets (cetirizine) for treatment of rhinitis. Then, patients were instructed to fill in a diary card for assessment of symptoms and medication use, and to perform peak expiratory flow (PEF) monitoring. In addition, they were given written recommendations of measures for reducing mite exposure and were supplied with allergen-impermeable covers (CFB, Nibionno, Italy) to encase mattresses and pillows in their bedroom.

Clinical assessment was repeated in spring (visit 1), in September–October (visit 2) and in December–January (visit 3). At each visit, diary card and PEF records were reviewed, spirometry was performed, drug treatment was modified, if required, according to severity and clinical activity of asthma. Indicators for step-down or step-up in medication were frequency of symptoms, FEV₁ values, PEF variability [1]. In addition, bronchial provocation testing with methacholine was repeated before visit 3. In October–November, between visits 2 and 3, dust samples were collected from patients' bedrooms.

Symptom scores and drug use

The patients kept a diary for assessment of asthma and rhinitis symptoms, and anti-asthmatic drug use during five 4-week periods: March, May, September, October, November. The following were registered daily:

1. Night asthma symptoms noted on a scale from 0 to 3 (maximum score of 6).
2. Daytime asthma symptoms on a scale from 0 to 3 (maximum score of 6).
3. Nasal symptoms on a scale from 0 to 3 (maximum score of 12).

Intake of antiasthmatic drugs, inhaled β_2 -agonists (short acting) and inhaled steroids was recorded as number of MDI actuations per day.

Mean symptom scores per month and drug use in spring (March, May) and autumn (September, October, November) months were calculated for each subject and the values were used for the comparison.

PEF monitoring

Patients were supplied with peak flow meters (fdE; Ferraris Medical Ltd, London, UK) and were instructed to measure PEF three times a day (morning, noon, evening) during five 4-week periods: March, May, September, October, November. The best of three measurements performed each time was recorded. They were advised not to record PEF for 6 h after use of short-acting bronchodilator medication. Daily PEF variability was calculated as amplitude percent mean.

Pulmonary function tests

Lung function was measured at each visit with a dry spirometer (PFT 922; SensorMedics, Anaheim, CA). Subjects were tested at least 12 h after the last inhaled short-acting bronchodilator. Forced expiratory manoeuvres were repeated until two values of FEV₁ within 100 mL of each other were obtained. The highest of these values was recorded. Values were expressed as a percentage of predicted values [19].

Airway responsiveness to methacholine was assessed at entry into the study and at the end, before the last visit, in subjects whose FEV₁ was > 80% predicted, as previously described [20]. Briefly, aerosols were generated by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA) connected to a dosimeter driven by compressed air. PBS was inhaled first, followed by doubling concentrations of methacholine from 0.05 to 32 mg/mL. Ventilatory function was monitored by measurement of FEV₁ with a spirometer. Inhalations continued until FEV₁ had fallen by at least 20% below the post-control value.

The cumulative dose of methacholine in μ g producing a 20% fall in FEV₁ (PD₂₀FEV₁) was calculated by interpolation of the dose-response curve and used to measure airway responsiveness. Ten and four subjects, respectively, at entry and at the end of the study, did not perform methacholine challenge due to bronchoconstriction (FEV₁ < 80% predicted).

Domestic dust sampling and analysis

Bedroom dust was collected by standardized vacuum cleaning. Bedding (pillows and quilt), mattress and bedroom floor were vacuumed by the same technician with the same vacuum cleaner (Folletto, Vorwerk, Germany). In each bedroom, a sampling time of 1 min/m² was applied to both floor and mattress. Dust samples were sealed in plastic bags, coded and stored at 4 °C until extracted. Each sample was sieved, weighed and 1 g of dust was extracted under magnetic stirring for 90 min with 10 mL (10% p/v) PBS, 0.1 M at pH 7.4. After incubation, the samples were centrifuged for 20 min at 1350 g at 4 °C. Supernatants were filtered and aliquots stored at -20 °C until analysed.

Der p 1 and Der f 1 were measured with a solid-phase ELISA according with the method described by Ventas *et al.* [21] using monoclonal antibodies (Pt1513 and Fa1511; ALK-Abellø, Madrid, Spain). Der 2 (Der p 2 + Der f 2) was determined according to the method described by Heymann [22] (ALK-Abellø Labs). The results were expressed as μ g of antigen/g of dust.

Statistical analysis

Quantitative data are presented as mean $\pm$ SD. Comparison between means was performed using Student's *t*-test for unpaired data. Multiple logistic regression was used to assess the influence of multiple independent variables on binary variables. Subjects who did not perform methacholine challenge due to bronchoconstriction were given a PD₂₀FEV₁ arbitrary value of 0 [23]. Logarithmic transformation of PD₂₀FEV₁ was used for the analysis. Before log transformation, + 1 was added to each original PD₂₀FEV₁ value [24]. The analysis of time trend of FEV₁ and PEF data was done by a mixed model analysis of variance. The distribution of dust concentration, drug scores and symptoms scores is reported as median and interquartile range. Comparison between groups was performed using two-sample Wilcoxon rank sum test. Probability values of < 0.05 were considered significant. Statistical adjustment for multiple comparison was made by Bonferroni correction.

	Group 1 (<i>Der p</i> 1 ≤ 2 µg/g)	Group 2 (<i>Der p</i> 1 > 2 µg/g)
Sex (M/F)	16/18	24/13
Age (years)	23.2 ± 7.6	22.8 ± 8.5
Severity of asthma (mild/moderate)	21/13	17/20
Allergy to grass pollen, no. (%)	7 (20)	10 (27)
Rhinitis, no. (%)	20 (59)	23 (62)
Exposure to passive smoking, no. (%)	12 (35)	7 (19)
Social class (no. inhabitants/house m ²)	0.04 ± 0.02	0.04 ± 0.01
Location of the house (town/country)	25/9	20/17
Bedroom mite allergens†:		
<i>Der p</i> 1 (µg/g)	0.47 (0.23–0.88)	8.4 (4.3–14.4)*
<i>Der f</i> 1 (µg/g)	1.5 (0.5–5.7)	0.8 (0.4–1.6)
<i>Der</i> 2 (µg/g)	0.79 (0.4–2.6)	2.88 (2.1–6.4)*
FEV ₁ at entry (% predicted)	99.3 ± 19.5	93.7 ± 14.9
FEV ₁ < 80% predicted, no. (%)	16 (13.1)	21 (14.4)
PD20FEV ₁ (µg methacholine)‡	57 (26–126)	156 (84–291)

Quantitative data are expressed as mean ± SD, †median (interquartile range) or ‡geometric mean (95% confidence interval); **P* < 0.001.

Results

The study population comprised 71 patients with mild or moderate asthma. *Der p* 1 concentrations within the exposure value of 2 µg/g of dust [2] were obtained in the homes of 34 subjects (Group 1), while in the remaining 37 subjects the concentrations were > 2 µg/g (Group 2) (Table 1). The characteristics of the low and high exposure subjects are reported in Table 1. The groups did not differ for any of the variables except the concentrations of dust mite antigens, *Der p* 1 and *Der* 2, in the bedrooms. *Der f* 1 was not significantly different between the two groups and its levels were not correlated with those of *Der p* 1. Multiple logistic regression did not show any influence of individual or environmental confounders listed in Table 1, as independent variables, on the HDM exposure groups of patients.

The symptom scores of the spring months and autumn months are compared in Table 2. Daytime and nocturnal asthma and rhinitis were not significantly different between the two groups in any period of the study. Similar results were obtained when grass pollen-sensitive subjects were excluded.

The subjects with high exposure to *Der p* 1 (Group 2) tended to increase the use of steroids and bronchodilators in the autumn months compared with the subjects with lower *Der p* 1 exposure, but the difference was not statistically significant (Table 3).

Morning and evening PEF values did not change throughout the study in either group (Table 4). No significant differences in FEV₁ were detected between the two groups at any visit (Table 5). The mixed model

Table 1. Characteristics at the time of enrolment of subjects with low (*Der p* 1 ≤ 2 µg/g dust) (Group 1) and high (*Der p* 1 > 2 µg/g dust) (Group 2) exposure levels to house dust mite

analysis of variance did not evidence a significant difference in time trend for PEF or FEV₁ in the two groups.

The degree of bronchial responsiveness (as indexed by the PD20FEV₁) in the two study groups varied widely. However, the mean values for PD20FEV₁ at the start of the study were not statistically different between the two groups (Table 1). In Group 1 exposed to low levels of *Der p* 1, PD20FEV₁ increased 2.15-fold at the end of the study (from 57 µg, 95% confidence interval (CI) 26–126, to 123 µg, 95% CI 61–245; *P* < 0.05), whereas in the group exposed to high levels of *Der p* 1 the 1.26-fold difference increment of PD20FEV₁ was not statistically significant

Table 2. Comparison of asthma and rhinitis symptoms in patients with low (*Der p* 1 ≤ 2 µg/g dust) (Group 1) and high (*Der p* 1 > 2 µg/g dust) (Group 2) exposure levels to house dust mite

Symptom score	Group 1	Group 2
Daytime asthma:		
spring	3.5 (1–17)	5.2 (0–16)
autumn	3.5 (0–11)	5.7 (0–15)
Nocturnal asthma:		
spring	1 (0–11)	3.3 (0–10)
autumn	2 (0–10)	4.2 (0–9)
Nasal symptoms:		
spring	28 (8–82)	35 (14–79)
autumn	28 (13–79)	36 (23–76)

Results are expressed as median (interquartile range) of mean monthly scores of the spring (March, May) and autumn (October, November, December) months.

Table 3. Comparison of anti-asthma drug use during spring and autumn in patients with low (*Der p* 1 ≤ 2 $\mu\text{g/g}$ dust) (Group 1) and high (*Der p* 1 > 2 $\mu\text{g/g}$ dust) (Group 2) exposure levels to house dust mite

Drug score	Group 1	Group 2
Inhaled beclomethasone ($\mu\text{g/day}$):		
spring	17 (0–500)	38 (0–425)
autumn	11 (0–333)	171 (17–533)
Inhaled bronchodilators (actuations per month):		
spring	2.5 (0–19)	4.0 (0–18)
autumn	1.0 (0–15)	7.2 (0–27)

Results are expressed as medians (interquartile range) of average daily dose of beclomethasone and of number of actuations of meter dose inhaler of bronchodilator during spring (March, May) and autumn (October, November, December) months.

(from 156 μg , 95% CI 84–291, to 165 μg , 95% CI 96–284; $P = \text{NS}$) (Fig. 1).

Discussion

In this study we showed that exposure to levels < 2 $\mu\text{g/g}$ of *Der p* 1 in the bedrooms of asthmatic subjects allergic to HDM and adequately treated with anti-asthma drugs for 1 year is associated with a significant reduction of bronchial responsiveness to methacholine. In contrast, bronchial responsiveness was unchanged after 1 year in a

Table 4. Comparison of morning and evening peak expiratory flow (PEF) measurements in patients with low (*Der p* 1 ≤ 2 $\mu\text{g/g}$ dust) (Group 1) and high (*Der p* 1 > 2 $\mu\text{g/g}$ dust) (Group 2) exposure levels to house dust mite

Month	Group 1	Group 2
Morning PEF (L/min):		
March	476 $\pm$ 20	494 $\pm$ 19
May	483 $\pm$ 21	502 $\pm$ 20
September	484 $\pm$ 17	486 $\pm$ 18
October	491 $\pm$ 19	497 $\pm$ 20
November	494 $\pm$ 18	494 $\pm$ 21
Evening PEF (L/min):		
March	479 $\pm$ 21	504 $\pm$ 18
May	489 $\pm$ 21	511 $\pm$ 18
September	486 $\pm$ 17	501 $\pm$ 18
October	485 $\pm$ 25	511 $\pm$ 19
November	501 $\pm$ 18	504 $\pm$ 20

Results are the average values of each month and are expressed as mean $\pm$ SD.

Table 5. Comparison of average FEV₁ values in patients with low (*Der p* 1 ≤ 2 $\mu\text{g/g}$ dust) (Group 1) and high (*Der p* 1 > 2 $\mu\text{g/g}$ dust) (Group 2) exposure levels to house dust mite over the study year

FEV ₁ (% predicted)	Group 1	Group 2
Visit 0	99.3 $\pm$ 19.5	93.7 $\pm$ 14.9
Visit 1	100.2 $\pm$ 19.7	95.7 $\pm$ 15.7
Visit 2	101.9 $\pm$ 20.9	96.9 $\pm$ 18.1
Visit 3	101.4 $\pm$ 21.8	93.9 $\pm$ 15.1

Results are expressed as mean $\pm$ SD.

comparable asthmatic group exposed to higher levels of mite allergens.

The changes in bronchial responsiveness to methacholine we observed in the lower dust mite exposure group were small (one doubling dose on average), but significant when compared with the absence of changes in the group exposed to > 2 $\mu\text{g/g}$ of mite antigen. The improvement in PD20 was not influenced by alterations of baseline airway caliber since no changes in FEV₁ were detected. Our findings are in agreement with those of Marks *et al.* [25], who found that bronchial hyper-responsiveness had the strongest association with changes in allergen level.

Long-term administration of inhaled steroid in asthma is associated with a reduction of bronchial hyper-responsiveness. Improvement in airway responsiveness ranging from 1.4- to four-fold after 1 year of treatment with inhaled steroids was demonstrated [13,26]. In these studies, no patient was on steroids at the beginning of the trial and all were under treatment at the end. Since the measurements of bronchial responsiveness in our study were obtained in patients receiving drug treatment adequate to the severity of their disease, we did not expect changes higher than those actually observed in relationship with mite antigen exposure.

Conflicting results on relationship between changes in bronchial hyper-responsiveness and HDM exposure in homes of asthmatics were obtained by previous studies. Reduction in bronchial hyper-responsiveness was detected by some investigators after intervention with mattress encasing. However, the combined effect of drug treatment was not determined. Van der Heide *et al.* [18] kept constant drug use during trial. Drug regimen was not controlled in other studies [27,28]. In a German study of similar allergen-avoidance measures, a 4.5-fold improvement of bronchial responsiveness was observed [14]. However, information about medication and sensitization patterns in the eight asthmatic children sensitized to HDM, who initially had very high levels of *Der p* 1 allergen in their bedrooms, was not reported. No significant changes in

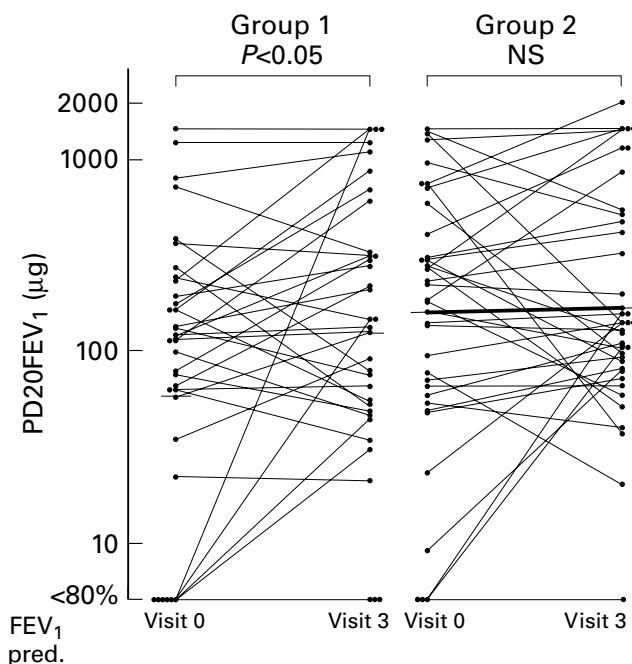


Fig. 1. Individual changes of bronchial responsiveness to methacholine (PD20FEV₁) in patients with low (*Der p* 1 ≤ 2 µg/g dust) (Group 1) and high (*Der p* 1 > 2 µg/g dust) (Group 2) exposure levels to house dust mite. Horizontal bars represent geometric means.

bronchial responsiveness were detected in other studies on mite avoidance [16,17,29].

The amount of inhaled steroids necessary to achieve optimal control of the disease during autumn months was on average 15-fold higher in asthmatic subjects exposed to > 2 µg/g of *Der p* 1 than in those exposed to < 2 µg/g of *Der p* 1. A possible explanation for the lack of statistical significance of this difference may be the wide intersubject variability in the use of inhaled steroids. In fact, several subjects with mild asthma in both study groups did not require topical steroids. According to the study design, the achievement of an optimal control of asthma was assessed considering symptoms, baseline lung function, and PEF measurements [1]. The finding of no differences in such variables between the two groups of patients confirms that adequate treatment was indeed given to the patients.

Allergen levels were not measured at the beginning of the study, which can be viewed as a limitation. The primary purpose of the present study was to look at the association between outcome of asthma and allergen in the home, not to examine the success of avoidance measurements. Previous studies clearly showed that antigen levels after mattress encasing remain steady over periods of several months (3–12 months) [18]. In addition, *Dermatophagoides* antigens in homes in north Italy remained relatively

constant during the year [30]. Therefore, we are confident that the measured levels of *Der p* 1 made between visits 2 and 3 are representative of the effective exposure to dust mite of our patients, at least during the autumn months.

In conclusion, the results of this long-term study show that exposure to lower levels of mite allergens in the bedroom is associated with a decrease of airway hyper-responsiveness in sensitized asthmatic subjects under optimal drug treatment. It remains to be shown if this provides additional benefit to the long-term outcome of asthma.

Acknowledgements

The writers thank Giovanna Fulgeri for secretarial assistance, Fiorenza Greggio, Adriano Battan, Paola Bortolami, Ida Adinolfi and Fabrizio Bortolami for technical assistance. Supported by a grant of the Regione Veneto, Giunta Regionale, Ricerca Sanitaria Finalizzata, Venezia, and by ALK-Abellò S.p.A., Lainate, Milano, Italy.

References

- 1 Sheffer AL, ed. International Consensus Report on Diagnosis and Management of Asthma. National Institute of Health. NIH Publication No 92-3091. Eur Respir J 1992; 5:601–41.
- 2 Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol 1997; 100:S2–S24.
- 3 Sporik RB, Chapman MD, Platts-Mills TAE. House dust mite exposure as a cause of asthma. Clin Exp Allergy 1992; 22:897–906.
- 4 Sporik RB, Holgate ST, Platts-Mills TAE, Cogswell J. Exposure to house dust mite allergen (*Der p* 1) and the development of asthma in childhood: a prospective study. N Engl J Med 1990; 323:502–7.
- 5 Platts-Mills TAE, Tovey ER, Mitchell EB, Moszoro H, Nock P, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982; 2:675–7.
- 6 Vervloet D, Penaud A, Razzouk H et al. Altitude and house dust mites. J Allergy Clin Immunol 1982; 69:290–6.
- 7 Peroni DG, Boner AL, Vallone G, Antonelli I, Warner JO. Effective allergen avoidance at high altitude reduced allergen-induced bronchial hyperresponsiveness. Am J Resp Crit Care Med 1994; 149:1442–6.
- 8 Huss K, Squire EN Jr, Carpenter GB. Effective education of adult asthmatics who are allergic to dust mites. J Allergy Clin Immunol 1992; 82:836–43.
- 9 Kuehr J. Preventing exposure to mite allergens. Clin Asthma Rev 1998; 2:117–22.
- 10 Sporik R, Hill DJ, Thompson PJ et al. The Melbourne House Dust Mite Study: long-term efficacy of house dust mite reduction strategies. J Allergy Clin Immunol 1998; 101:451–6.

- 11 Gotzsche PC, Hammarquist C, Burr M. House dust mite control measures in the management of asthma: meta-analysis. *Br Med J* 1998; 317:1105–10.
- 12 Haahtela T, Jarvinen M, Kava T et al. Comparison of a beta 2-agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. *N Engl J Med* 1991; 325:388–92.
- 13 Van Essen-Zandvliet EE, Hughes MD, Waalkens HJ, Duiverman EJ, Pocock SJ, Kerrebijn KF. Effects of 22 months of treatment with inhaled corticosteroids and/or beta-2-agonists on lung function, airway responsiveness, and symptoms in children with asthma. The Dutch Chronic Non-Specific Lung Disease Study Group. *Am Rev Respir Dis* 1992; 146:547–54.
- 14 Ehbert B, Lau-Schadendorf S, Weber A, Buettner P, Schou C, Wahn U. Reducing domestic exposure to house dust mite allergen reduces bronchial hyperreactivity in sensitive children with asthma. *J Allergy Clin Immunol* 1992; 90:135–8.
- 15 Dietemann A, Bessot JC, Hoyet C, Ott M, Verot A, Pauli G. A double-blind, placebo controlled trial of solidified benzyl benzoate applied in dwellings of asthmatic patients sensitive to mites: clinical efficacy and effect on mite allergens. *J Allergy Clin Immunol* 1993; 91:738–46.
- 16 Marks GB, Tovey ER, Green W, Shearer M, Salome CM, Woolcock AJ. House dust mite allergen avoidance: a randomized controlled trial of surface treatment and encasement of bedding. *Clin Exp Allergy* 1994; 24:1078–83.
- 17 Chang JH, Becker A, Ferguson A et al. Effect of application of benzyl benzoate on house dust mite allergen levels. *Ann Allergy Asthma Immunol* 1996; 77:187–90.
- 18 Van der Heide S, Kauffman HF, Dubois AEJ, de Monchy JGR. Allergen-avoidance measures in house-dust-mite-allergic asthmatic patients. Effects of acaricides and mattress encasings. *Allergy* 1997; 52:921–7.
- 19 Commission des Communautés Européennes. Commentaires relatifs au questionnaire de la CECA pour l'étude de la bronchite chronique et de l'emphysème pulmonaire 1967. Collection d'hygiène et de médecine du travail, no. 14, 2nd edn. Luxembourg: Office des Publications Officielles des Communautés Européennes, 1972.
- 20 Mapp CE, Polato R, Maestrelli P, Hendrick DJ, Fabbri LM. Time course of the increase in airway responsiveness associated with late asthmatic reactions to toluene diisocyanate in sensitized subjects. *J Allergy Clin Immunol* 1985; 75:568–72.
- 21 Ventas Alguacil P, Lombardero Vega M, Duffort Guatire O, Carreira Garea J. Cuantificación de los alérgenos *Der p 1* y *Der f 1* de los ácaros del polvo doméstico y *Fel d 1* de gato mediante un ELISA en fase sólida. *Rev Esp Alergol Inmunol Clin* 1990; 5:71–5.
- 22 Heymann PW, Chapman MD, Alberse RC, Fox JW, Platts-Mills TA. Antigenic and structural analysis of group II allergens (*Der f II* and *Der p II*) from house dust mite (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989; 83:1055–67.
- 23 Peat JK, Unger WR, Combe D. Measuring changes in logarithmic data, with special reference to bronchial responsiveness. *J Clin Epidemiol* 1994; 47:1099–108.
- 24 Van Schayck CP, Graafsma SJ, Visch MB, Dompeling E, van Weel C, van Herwaarder CLA. Increased bronchial hyperresponsiveness after inhaling salbutamol during 1 year is not caused by subsensitization to salbutamol. *J Allergy Clin Immunol* 1990; 86:793–800.
- 25 Marks GB, Tovey ER, Green W, Shearer M, Salome CM, Woolcock AJ. The effect of changes in house dust mite allergen exposure on the severity of asthma. *Clin Exp Allergy* 1995; 25:114–8.
- 26 Juniper EF, Kline PA, Vanzielegheem MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid (Budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. *Am Rev Respir Dis* 1990; 142:832–6.
- 27 Carswell F, Birmingham K, Oliver J, Crews A, Weeks J. The respiratory effects of reduction of mite allergen in the bedrooms of asthmatic children—a double blind controlled trial. *Clin Exp Allergy* 1996; 26:386–96.
- 28 Walshaw MJ, Evans CC. Allergen avoidance in house dust mite sensitive adult in asthma. *Quart J Med* 1986; 58:199–215.
- 29 Hayden ML, Perzanowzki M, Matheson L, Scott P, Call RS, Platts-Mills TAE. Dust mite allergen avoidance in the treatment of hospitalized children with asthma. *Ann Allergy Asthma Immunol* 1997; 79:437–42.
- 30 Minelli M, Luca P, Musarra A et al. Annual biological rhythm of house mites allergenic. Preliminary results relative to the months May–December '96. *Ital J Allergy Clin Immunol* 1997; 7:218–25.