

Exposure and sensitization to indoor allergens: Association with lung function, bronchial reactivity, and exhaled nitric oxide measures in asthma

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Background: Exposure to high levels of allergens in sensitized asthmatic patients causes worsening of pulmonary function in experimental studies. Chronic exposure to lower, naturally occurring levels of allergens might increase the severity of asthma.

Objective: We sought to study the associations between sensitization and exposure to common indoor allergens (dust mite, cat, and dog) in the home on pulmonary function, exhaled nitric oxide (eNO), and airway reactivity in asthmatic patients. **Methods:** Dust samples were collected from the living room carpet and mattress of 311 subject's homes, and Der p 1, Fel d 1, and Can f 1 concentrations were measured by using ELISAs. Spirometry, nonspecific bronchial reactivity, and eNO were measured.

Results: Subjects both sensitized and exposed to high levels of sensitizing allergen had significantly lower FEV₁ percent predicted values (mean, 83.7% vs 89.3%; mean difference, 5.6%; 95% CI, 0.6%-10.6%; $P = .03$), higher eNO values (geometric mean [GM], 12.8 vs 8.7 ppb; GM ratio, 0.7; 95% CI, 0.5-0.8; $P = .001$), and more severe airways reactivity (PD₂₀ GM, 0.25 vs 0.73 mg; GM ratio, 2.9; 95% CI, 1.6-5.0; $P < .001$) compared with subjects not sensitized and exposed. No significant effect of the interaction between sensitization and exposure was found for FEV₁ percent predicted and eNO values. However, there was a significant effect of the interaction between sensitization and exposure to any allergen ($P = .05$) and between sensitization and exposure to cat allergen ($P = .04$) for nonspecific bronchial reactivity.

Conclusion: Asthmatic subjects who are exposed in their homes to allergens to which they are sensitized have a more severe form of the disease. (J Allergy Clin Immunol 2003;112:362-8.)

Key words: Asthma severity and control, allergen exposure-sensitization, bronchial reactivity, lung function

Abbreviations used

BR:	Bronchial reactivity
eNO:	Exhaled nitric oxide
GM:	Geometric mean
NS/E:	Not sensitized but exposed to any study allergens
NS/NE:	Not sensitized and not exposed to any study allergens
S/E:	Sensitized and exposed to 1 or more specific allergens
S/NE:	Sensitized but not exposed to any study allergens

Considerable data have accumulated regarding allergen exposure in relation to specific sensitization in children.¹ The deleterious effect of sensitization to inhaled allergens on lung function and bronchial reactivity (BR) has been suggested,^{2,3} but data on the importance of individual allergens have often been conflicting. In one survey, sensitization to dust mite, cat, and mold was a predictor of increased BR, whereas sensitization to outdoor allergens (timothy grass and birch) was not.² Analysis of the Spanish data collected through the European Community Respiratory Health Study,⁴ however, showed that sensitization to timothy grass, *Alternaria* species, and cat allergen, but not mite allergen, was associated with a lower FEV₁ after adjusting for bronchial hyperreactivity, respiratory symptoms, and smoking.

The underlying mechanism of asthma is airway inflammation,⁵ and chronic inflammation might lead to airway remodeling. Recent studies in animals have demonstrated structural remodeling changes occurring in the airways of rats that were sensitized and chronically exposed to allergens.^{6,7} In human experimental studies allergen challenge causes early and late decreases in lung function.⁸ However, the concentration of allergen used in challenges is much greater than the exposure level in the domestic environment, albeit for a shorter period. The chronic exposure to allergens over long periods might contribute to the irreversible airway obstruction and severe BR seen in patients with chronic asthma. Very few studies have investigated the association of real-life allergen exposure and its interaction with sensitization on asthma control and severity in adults.

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Received for publication July 30, 2002; revised May 2, 2003; accepted for publication May 15, 2003.

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1654

We aimed to examine the relationship between sensitization and exposure to indoor allergens in homes and their interaction and objective measures of asthma severity in a large group of asthmatic patients with a range of severity of disease.

METHODS

Subjects provided written informed consent and were recruited into the study if they had a physician diagnosis of asthma with symptoms requiring treatment with at least a short-acting bronchodilator in the previous 12 months. Study participants were selected randomly from a clinical trial database of asthmatic patients. The subjects were from the Greater Manchester area, United Kingdom, and were a mixture of hospital and primary care patients.

The study protocol was approved by the local research ethics committee. Subjects attended the research unit having avoided short-acting bronchodilators (6 hours); long-acting bronchodilators, theophyllines, and leukotriene antagonists (12 hours); and antihistamines (48 hours). The following investigations were performed.

Pulmonary function testing. FEV₁ and forced vital capacity were measured with a Microlab 3000 spirometer (MicroMedical, Rochester, United Kingdom). FEV₁ percent predicted was calculated by using European Coal and Steel reference values.⁹

Assessment of airway reactivity. For subjects with an FEV₁ of greater than 60% of the predicted value, a modified Yan technique bronchial challenge¹⁰ was used to obtain a cumulative PD₂₀ FEV₁ value. For technical and supply reasons, either histamine or methacholine was used for the provocation. The concentrations differed slightly (ie, histamine, 3.13 to 50 mg/mL; methacholine, 1.56 to 50 mg/mL). Previous studies^{11,12} have shown that the 2 compounds produce similar results, and dose equivalence was presumed for our study.

A Koko DigiDoser dosimeter (PDS Research, Louisville, Colo) and "646" nebulizer pots (Devilbiss, Somerset, Pa) calibrated to deliver 10 µL per breath actuation during a tidal inspiration were used for the challenge. The PD₂₀ FEV₁ was calculated (by using cumulative doses) with the following formula:

$$PD_{20} = \text{Antilog}[\log D1 + (\log D2 - \log D1)(20 - R1)/(R2 - R1)].$$

The equation is derived from linear interpolation. In subjects who did not demonstrate a 20% decrease in FEV₁ after the challenge, a PD₂₀ of 12 mg was entered as the result (one doubling dose above the final cumulative dose).

Exhaled nitric oxide. Exhaled nitric oxide (eNO) was measured before spirometry and bronchial challenge with a chemiluminescence analyzer (LR 2000; Logan Research, Rochester, United Kingdom) with a resolution of 1 ppb, according to published guidelines.¹³ The response time (10%-90%) was less than 0.6 seconds, and the sampling rate was 250 mL/min. To measure eNO, subjects inhaled fully to total lung capacity and then immediately exhaled slowly (at a controlled rate of 6 L/min) to residual volume into a wide-bore Teflon tube with minimal resistance. The NO reading was taken when the level had reached a plateau and the CO₂ concentration exceeded 5%. The mean of 2 readings was taken. The pressure during expiration was kept constant at 3 ± 0.4 mm Hg (by using a visual light display of expiratory flow), which was measured by using the pressure and volume sensors in the analyzer.

Skin prick testing. Sensitization was assessed by means of skin prick testing to common indoor allergens (*Dermatophagoides pteronyssinus*, cat, and dog; Bayer, Elkhart, Ind). The result was considered positive if the mean of 2 perpendicular diameters across the wheal was at least 3 mm greater than that produced by the negative control.

Home visits and assessment of allergen exposure

Dust samples were collected by vacuuming a 1-m² area of the living room carpet and mattress. All samples were assayed for Der p 1, Fel d 1, and Can f 1 content by using ELISAs, as previously described.¹⁴⁻¹⁶ The results were expressed as micrograms of allergen per gram of fine dust, and the limit of detection was 0.05 µg/g for Der p 1, 0.08 µg/g for Fel d 1, and 0.2 µg/g for Can f 1.

Statistical analysis

Statistical analysis was performed with SPSS 10.0 for Windows (SPSS, Chicago, Ill). The conventional 5% level of statistical significance was used. A log_e transformation of PD₂₀ and eNO was required to produce an adequate approximation to a normal distribution, and geometric means (GMs) are presented as summary statistics. To test the hypothesis that the sensitized and exposed subgroup differs from the other 3 groups in the study, we performed an overall ANOVA together with a planned contrast of both sensitized and exposed patients versus patients from all other groups combined. This latter comparison does not necessarily prove that the sensitized and exposed group is significantly different from the 3 other subgroups, only that it is different from the average of the 3 other subgroups.

If the overall ANOVA result was found to be statistically significant, then individual comparisons between pairs of groups by using the Scheffe test were also reported.

The independent effects of sensitization and exposure were assessed by using an ANOVA model with main factors of sensitized versus not sensitized and exposed versus not exposed and the interaction of sensitized versus not sensitized × exposed versus not exposed together with the potential confounding factors (age, sex, family history of asthma, smoking history, and use of inhaled steroids). ANOVA was used because this is the conventional statistical approach to assess the effects of factors after adjusting for interactions and to evaluate the strength of the interaction between factors.

The following tests were performed to assess for the potential bias between the methacholine and histamine challenge:

- an independent-samples *t* test to compare PD₂₀ between the 2 groups,
- a χ^2 test to analyze the numbers of subjects classified as having normal, mild, moderate, or severe BR between the 2 groups,
- univariate ANOVA adjusted for the method of challenge, and
- a multivariate analysis to assess any interaction between the method and any associations observed.

RESULTS

Three hundred eleven subjects were recruited into the study. Demographics and baseline results are listed in Table I.

Indoor allergen levels

Allergen levels for all subjects, as well as for pet owners, are listed in Table II. Subjects were classified as being exposed to high levels of domestic allergens on the basis of previously proposed criteria (>2 µg/g Der p 1, >10 µg/g Fel d 1, and >10 µg/g Can f 1 in either carpet or mattress).^{17,18} By using this classification, 143 (48%) patients were exposed to high levels of Der p 1, 83 (28%) to high levels of Fel d 1, and 71 (24%) to high levels of Can f 1.

TABLE I. Demographic details and baseline results

Measure	Value
Age	10-68 y (mean, 42 y; 95% CI, 41-44 y)
Sex	
Male	128 (41.2%)
Female	183 (58.8%)
Smoking status	
Smokers	42 (13.5%)
Nonsmokers	269 (86.5%)
Asthma treatment	
Receiving inhaled steroids	197 (63%) (mean dose, 728 µg; median dose, 500 µg; 95% CI, 659-797 µg)
Not receiving inhaled steroids	114 (37%)
FEV ₁ percent predicted	n = 311; range, 25%-131% (mean, 88%; 95% CI, 85%-90%)
PD ₂₀	n = 179; range, 0.02-12.0 mg (GM, 0.44 mg; 95% CI, 0.34-0.57)
Exhaled nitric oxide	n = 265; range, 0.7-250 ppb (GM, 10.5 ppb; 95% CI, 9.4-11.7 ppb)
Atopic status (n = 311)	n = 306; not known, n = 5
Atopic	248 (80%)
Nonatopic	58 (19%)
Allergen sensitization (positive test results)	
Dust mite	186 (60%)
Cat	151 (49%)
Dog	125 (40%)

TABLE II. Allergen level in homes visited

	Der p 1	GM (µg/g), range (95% CI)	
		Can f 1	Fel d 1
All subjects (n = 311)			
Mattress	1.60, 0.04-305 (1.19-2.10)	0.74, 0.05-780 (0.56-0.97)	1.24, 0.05-940 (0.90-1.71)
Living room	1.72, 0.04-150.0 (1.33-2.23)	2.78, 0.05-2150 (2.06-3.75)	3.27, 0.05-1870 (0.38-4.46)
Pet owners (n = 135)		Dog owners (n = 58)	Cat owners (n = 81)
Mattress	2.03, 0.04-270 (1.28-3.23)	2.61, 0.05-780 (1.54-4.43)	8.80, 0.05-940 (5.21-14.87)
Living room	1.30, 0.04-120.0 (0.85-1.98)	11.63, 0.05-2150 (6.59-20.54)	17.08, 0.05-1870 (9.49-30.74)

Allergen sensitization, lung function, eNO, and airway reactivity

There was no difference in percent predicted FEV₁ between subjects sensitized to at least one allergen and nonsensitized subjects (mean [95% CI], 87.4% [84.7%-90.0%] vs 88.3% [82.8%-93.8%], $P = .77$). However, sensitized subjects had significantly lower PD₂₀ FEV₁ values than nonsensitized subjects (GM [95% CI], 0.37 mg [0.28-0.49 mg] vs 1.28 mg [0.63-2.59 mg]; GM ratio, 3.5-fold; 95% CI, 1.62 to 7.35; $P = .002$). eNO was significantly greater in sensitized compared with nonsensitized subjects (GM [95% CI], 11.6 ppb [10.3-13.0 ppb] vs 7.2 ppb [5.7-9.2 ppb]; GM ratio, 1.6-fold; 95% CI, 1.2-2.1; $P = .001$).

Subjects were divided into one of the following 4 groups on the basis of their sensitization and exposure status to any of the allergens: (1) not sensitized and not exposed to any study allergens (NS/NE); (2) sensitized but not exposed to any study allergens (S/NE); (3) not sensitized but exposed to any study allergens (NS/E); and (4) sensitized and exposed to 1 or more specific allergens (S/E).

The mean dose of inhaled steroid was 1043 µg (95% CI, 645-1440 µg) for the NS/NE group, 660 µg (95% CI,

583-738 µg) for the S/NE group, 830 µg (95% CI, 690-971 µg) for the NS/E group, and 632 µg (95% CI, 539-724 µg) for the S/E group (S/E vs NS/NE, $P < .01$).

Effect of sensitization and exposure on pulmonary function test results and eNO

When sensitization and exposure to all allergens was considered in the ANOVA, there was a significant difference between the groups for PD₂₀, eNO, and FEV₁ percent predicted. Subjects in the S/E group had significantly lower FEV₁ percent predicted values (mean difference, 5.6; 95% CI, 0.6-10.6; $P = .029$), greater BR (GM ratio, 2.9; $P < .001$), and higher eNO values (GM ratio, 0.7; $P = .001$) than the rest of the patients in the other groups combined (Table III). Individual analyses for mite, cat, and dog allergens were also performed (Table IV). For mite allergen, there was a difference between the S/E group and the rest of the groups, with the former group having a lower PD₂₀ and higher eNO. There was no difference between the groups in terms of FEV₁ percent predicted. For the cat allergen, there was a significant difference for all 3 measures between the S/E group and the rest, with all the measures demonstrating greater asthma severity in the S/E group. However, the important differ-

TABLE III. Analysis of pulmonary function in relation to allergen sensitization and exposure status

	Mean GM (95% CI)				S/E vs all other subgroups		
	NS/NE	S/NE	NS/E	S/E	Overall ANOVA	Linear contrast	2-Sample t test
FEV ₁ % predicted	(n = 21) 92.9 (85.2-100.6)	(n = 73) 86.9 (81.9-92.0)	(n = 61) 90.8 (85.9-95.7)	(n = 118) 83.7 (79.6-87.7)	F(3,269) = 2.2; P = .086	t(269) = 2.4; P = .018	t(271) = 2.2; P = .029; mean = 83.7 vs 89.3; mean difference = 5.6; 95% CI = 0.6-10.6
PD ₂₀	(n = 15) 0.70 (0.29-1.72)	(n = 47) 0.54 (0.32-0.90)	(n = 31) 1.03 (0.53-1.97)	(n = 65) 0.25 (0.16-0.37)	F(3,154) = 5.7; P = .001 S/E significantly lower than NS/E	t(154) = 3.8; P < .001	t(156) = 3.8; P < .001; GM = 0.25 vs 0.70; mean ratio difference = 2.9; 95% CI = 1.6-4.8
eNO	(n = 20) 6.8 (4.9-9.4)	(n = 59) 11.2 (9.2-13.6)	(n = 57) 7.8 (6.1-9.9)	(n = 104) 12.9 (10.9-15.3)	F(3,236) = 6.3; P = .001, S/E significantly higher than NS/E and NS/NE	t(236) = 3.7; P < .001	t(238) = 3.3; P = .001; GM = 12.9 vs 8.9; mean ratio difference = 0.7; 95% CI = (0.6-0.9)

ence appeared to be between those subjects who were sensitized and those who were not (rather than their exposure status), with the sensitized subjects having more severe asthma. In the dog allergen analysis the converse to cat allergen was seen, with a significant difference in FEV₁ percent predicted between S/E and the rest but not for PD₂₀ or eNO.

Results of the ANOVA model with the main factors of sensitized versus not sensitized and exposed versus not exposed and the interaction of sensitized versus not sensitized × exposed versus not exposed together with the potential confounding factors, assessing the independent effects of sensitization and exposure, are presented in Table V. No significant effect of the interaction between sensitization and exposure was found for FEV₁ percent predicted and eNO values. However, there was a significant effect of the interaction between sensitization and exposure to any allergen and between sensitization and exposure to cat allergen and nonspecific BR. There was no significant difference in the frequency of sensitized and exposed subjects between those with and without bronchial challenge (*P* = .2).

There was no significant difference in the numbers of subjects classified as having normal, mild, moderate, or severe BR between the subjects who underwent histamine challenge compared with methacholine challenge or in the PD₂₀ (GM [95% CI], 0.40 mg [0.28-0.56 mg] vs 0.52 mg [0.34-0.77 mg]; histamine vs methacholine challenge, *P* = .33). The combined analyses adjusted for the method of challenge showed no effect of the method on the outcomes, and no interaction was seen between the method and any of the sensitized-exposed groups or with the S/E group versus the rest of the groups. These data suggest dose equivalence between the 2 challenge methods.

DISCUSSION

This is one of the first large studies assessing the influence of both sensitization and exposure to indoor allergens on the severity of asthma, as measured on the basis of pul-

monary function tests, BR, and eNO. In a cohort of more than 300 asthmatic subjects with disease of varied severity, we have demonstrated significantly lower FEV₁ values, higher levels of eNO, and increased BR in subjects who were both sensitized and exposed to sensitizing allergen compared with that seen in other subjects. Furthermore, there was a significant interaction between sensitization and exposure to any allergen and between sensitization and exposure to cat allergen for nonspecific BR but not eNO or baseline pulmonary function. Of the potential confounding factors, age was the most common significant associate for all measures. Smoking history was associated with a lower eNO value, and male sex and inhaled steroid use were associated with lower FEV₁ values.

Several studies¹⁹⁻²¹ have reported an association between the risk of admission to the hospital with acute asthma and allergic sensitization, but the relationship between atopy and progressively severe asthma is less well proved. Studies in the general population have shown a relationship between FEV₁ decline and the presence of atopy,^{22,23} whereas a study in asthmatic patients²⁴ did not demonstrate this effect. One study²⁵ actually showed a greater decline in lung function in nonatopic asthmatic subjects.

The relationship between eNO and atopy has been less studied. Two studies^{26,27} have demonstrated increased eNO values in atopic subjects aged 5 to 15 years compared with nonatopic asthmatic patients but no difference between atopic and nonatopic (ie, nonasthmatic) control subjects. Epithelial cells of asthmatic subjects show greater expression of inducible NO synthase,²⁸ and this suggests that the airway epithelial lining might differ between atopic and nonatopic subjects and that this difference is greater in asthmatic subjects.

Several studies have suggested that exposure to allergens worsens asthma control. Allergen avoidance studies have demonstrated an improvement in BR and symptoms.²⁹ We have previously reported a relationship between mite allergen exposure and clinical activity of asthma (measured by level of BR, peak flow variability,

TABLE IV. Lung function outcomes, sensitization, and exposure to individual allergens

	Mean GM (95% CI)				S/E vs all other subgroups		
	NS/NE	S/NE	NS/E	S/E	Overall ANOVA	Linear contrast	2-Sample t test
Dust mite							
FEV ₁ % predicted	(n = 59) 91.4 (86.8-96.3)	(n = 93) 86.1 (81.5-90.8)	(n = 57) 89.1 (83.7-94.6)	(n = 81) 84.0 (79.3-88.7)	F(3,286) = 1.6; P = .18	t(286) = 1.8; P = .08	t(288) = 1.6; P = .11; mean = 84.0 vs 88.4; mean difference = 4.4; 95% CI = -1.0 to 9.8
PD ₂₀	(n = 41) 0.92 (0.52-1.60)	(n = 58) 0.36 (0.23-0.57)	(n = 27) 0.45 (0.22-0.93)	(n = 45) 0.26 (0.16-0.42)	F(3,167) = 4.1; P = .008	t(167) = 2.4; P = .02	t(169) = 2.2; P = .027; GM = 0.26 vs 0.51; mean ratio difference = 1.99; 95% CI = 1.1 to 3.6
eNO	(n = 54) 7.7 (6.0-10.0)	(n = 80) 11.2 (9.4-13.3)	(n = 54) 10.4 (8.2-13.0)	(n = 68) 13.0 (10.2-16.4)	F(3,252) = 3.6; P = .015	t(252) = 2.4; P = .02	t(254) = 2.2; P = .03; GM = 13.0 vs 9.8; mean ratio difference = 0.76; 95% CI = 0.59 to 0.97
Cat							
FEV ₁ % predicted	(n = 114) 88.3 (84.5-92.1)	(n = 100) 89.3 (85.4-93.1)	(n = 37) 87.8 (79.9-95.8)	(n = 42) 80.6 (73.2-88.0)	F(3,289) = 1.8; P = .15	t(289) = 2.2; P = .03	t(291) = 2.3; P = .023; mean = 80.6 vs 88.6; mean difference = 8.0; 95% CI = 1.1 to 14.9
PD ₂₀	(n = 65) 0.54 (0.35-0.83)	(n = 65) 0.33 (0.22-0.52)	(n = 22) 1.26 (0.58-2.75)	(n = 22) 0.21 (0.10-0.42)	F(3,170) = 4.9; P = .003	t(170) = 2.6; P = .009	t(172) = 2.1; P = .034; GM = 0.21 vs 0.50; mean ratio difference = 2.37; 95% CI = 1.1 to 5.2
eNO	(n = 102) 9.0 (7.6-10.8)	(n = 35) 13.0 (10.7-15.8)	(n = 35) 6.9 (5.2-9.1)	(n = 39) 14.8 (11.6-19.0)	F(3,253) = 7.5; P < .001	t(253) = 3.0; P = .003	t(255) = 2.6; P = .010; GM = 14.8 vs 9.9; mean ratio difference = 0.67; 95% CI = 0.49 to 0.91
Dog							
FEV ₁ % predicted	(n = 132) 89.8 (86.1-93.5)	(n = 90) 86.0 (81.6-90.4)	(n = 41) 89.3 (83.6-95.0)	(n = 42) 78.2 (69.7-86.6)	F(3,288) = 2.7; P = .048	t(288) = 2.4; P = .015	t(290) = 2.5; P = .013; mean = 78.2 vs 88.4; mean difference = 10.2; 95% CI = 2.2 to 18.3
PD ₂₀	(n = 83) 0.63 (0.42-0.94)	(n = 56) 0.34 (0.22-0.52)	(n = 24) 0.32 (0.15-0.71)	(n = 10) 0.24 (0.08-0.70)	F(3,169) = 2.1; P = .097	t(169) = 0.9; P = .37	t(171) = 1.1; P = .28; GM = 0.24 vs 0.46; mean ratio difference = 1.89; 95% CI = 0.60 to 5.97
eNO	(n = 120) 9.1 (7.8-10.7)	(n = 73) 13.5 (11.1-16.3)	(n = 36) 9.5 (6.6-13.6)	(n = 28) 11.6 (8.3-16.3)	F(3,253) = 3.2; P = .024	t(253) = 0.5; P = .59	t(255) = 0.6; P = .54; GM = 11.6 vs 10.4; mean ratio difference = 0.90; 95% CI = 0.63 to 1.28

and FEV₁ percent predicted).³⁰ A case-control study³¹ demonstrated that the incidence of sensitization and exposure to indoor allergens was significantly greater in subjects with brittle asthma compared with that in subjects with stable asthma. A study in patients with mild asthma (not taking inhaled steroids)³² showed higher eNO values in subjects sensitized and exposed to allergens compared with that in subjects sensitized but not exposed to allergens. Also, allergen avoidance has been associated with decreases in eNO values,³³ and, in an experimental study, an increase in eNO after allergen challenge.³⁴ In the current study subjects who were both sensitized and exposed to high levels of the sensitizing allergen had more severe

asthma, as measured on the basis of eNO, BR, and FEV₁ than subjects who were not sensitized and exposed. However, in the case of eNO, the lack of interaction between sensitization and exposure could suggest that the effect was predominantly caused by sensitization, whereas for BR there was a significant interaction between sensitization and chronic exposure in the home.

When exposure and sensitization to individual allergens was examined in the present study, a significant difference was seen for FEV₁ percent predicted between the subjects sensitized and exposed to either cat and dog allergen and the rest of the group. In a previous study in children,³⁵ there was a trend toward lower lung function

TABLE V. Independent effects of sensitization and exposure by using an ANOVA model together with the potential confounding factors of age, sex, family history of asthma, smoking history, and use of inhaled steroids

	Sensitization	Exposure	Interaction
FEV ₁ % predicted			
Overall	$P = .057$ Significant confounders: age, $P < .001$; sex, $P < .001$; inhaled steroids, $P = .029$	$P = .62$	$P = .12$
Mite	$P = .003$ Significant confounders: age, $P < .001$; sex, $P = .001$; inhaled steroids, $P = .019$	$P = .70$	$P = .75$
Cat	$P = .014$ Significant confounders: age, $P < .001$; sex, $P = .001$; inhaled steroids, $P = .036$	$P = .43$	$P = .12$
Dog	$P = .014$ Significant confounders: age, $P < .001$; sex, $P = .001$	$P = .52$	$P = .18$
PD ₂₀			
Overall	$P = .005$ Significant confounder: family history, $P = .04$	$P = .35$	$P = .048$
Mite	$P = .009$ Significant confounder: age, $P = .05$	$P = .72$	$P = .47$
Cat	$P = .002$ Significant confounder: age, $P = .05$	$P = .25$	$P = .038$
Dog	$P = .44$ Significant confounder: age, $P = .016$	$P = .56$	$P = .88$
eNO			
Overall	$P = .025$ Significant confounders: age, $P = .02$; smoker, $P = .003$	$P = .87$	$P = .18$
Mite	$P = .079$ Significant confounders: age, $P = .018$; smoker, $P < .001$	$P = .06$	$P = .81$
Cat	$P < .001$ Significant confounder: smoker, $P = .002$	$P = .41$	$P = .37$
Dog	$P = .021$ Significant confounders: age, $P = .028$; smoker, $P = .001$	$P = .53$	$P = .93$

in subjects sensitized to these allergens, and sensitivity to dog had the strongest effect on BR. This latter finding was not seen in our study. Analysis of exposure and sensitization to individual allergens was also performed in a study in children in the United States.³⁶ This study demonstrated greater asthma morbidity in subjects exposed and sensitized to cockroach allergen. This finding did not occur with exposure to dust mite or cat allergen. However, the percentage of subjects considered to be exposed to high levels of dust mite (9.7%) and cat allergen (12.6%) was considerably lower than that seen in our study.

Some subjects in our study did not have a bronchial challenge. Most of these subjects' lung functions precluded the test, and some refused the investigation. It is recognized that this could theoretically influence the results. However, because there was no difference in the frequency of sensitized and exposed subjects in the 2 groups, it is presumed that the comparison of BR between the S/E group and the rest of the subgroups is valid.

In conclusion, our study has shown that asthmatic patients who are sensitized to at least one allergen have evidence of more severe asthma, as measured on the basis of eNO and BR. Furthermore, markers of asthma activity within the sensitized group are significantly worse in those subjects who are also exposed to the allergens causing sensitization.

REFERENCES

1. Custovic A, Woodcock A. Exposure and sensitization in infants and children. *Curr Opin Allergy Immunol* 2001;1:133-8.
2. Omenaas E, Bakke P, Eide GE, Elsayad S, Gulsvik A. Serum house dust mite antibodies: predictor of increased bronchial responsiveness in adults of a community. *Eur Respir J* 1996;9:919-25.
3. Omenaas E, Bakke P, Eide GE, Elsayad S, Gulsvik A. Serum house-dust mite antibodies and reduced FEV₁ in adults of a Norwegian community. *Am J Respir Crit Care Med* 1995;152:1158-63.
4. Sunyer J, Soriano J, Anto JM, et al. Sensitization to individual allergens as risk factors for lower FEV₁ in young adults. *Int J Epidemiol* 2000;29:125-30.
5. Djukanovic R, Roche WR, Wilson JW, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;142:434-57.
6. Palmans ELS, Kips JC, Pauwels RA. Prolonged allergen exposure induces structural airway changes in sensitized rats. *Am J Respir Crit Care Med* 2000;161:627-35.
7. Salmon M, Walsh DA, Koto H, Barnes PJ, Chung KF. Repeated allergen exposure of sensitized Brown-Norway rats induces airway cell DNA synthesis and remodelling. *Eur Respir J* 1999;14:633-41.
8. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977;7:503-13.
9. Quanjer PhH, ed. Standardization of lung function tests—1993 Update. Report of the Working Party for the European Community for Steel and Coal. *Eur Respir J* 1993;6(suppl):5-40.
10. Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;38:760-5.
11. Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 1978;33:705-10.
12. Salome CM, Schoeffel RE, Woolcock AJ. Comparison of bronchial reac-

tivity to histamine and methacholine in asthmatics. *Clin Allergy* 1980;10:541-6.

13. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. ERS Task Force Report. *Eur Respir J* 1997;10:1683-93.
14. Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman M. A two-site monoclonal antibody ELISA for the quantitation of the major *Dermatophagoides* spp. allergens Der p 1 and Der f 1. *J Immunol Methods* 1989;118:227-35.
15. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE. Monoclonal antibodies to the major feline allergen *Fel d* 1: II. Single step affinity purification of *Fel d* 1, N-terminal sequence analysis and development of a two site immunoassay to assess *Fel d* 1 exposure. *J Immunol* 1988;140:812-8.
16. Schou C, Hansen GN, Lintner T, Lowenstein H. Assay for the major dog allergen Can f 1: investigation of house dust samples and commercial dog extracts. *J Allergy Clin Immunol* 1991;88:847-53.
17. Chapman MD, Woodfolk JA, Heyman PW. Cat Allergy. *Ann Allergy* 1992;69:273-5.
18. Ingram JM, Sporik R, Rose G, Honsinger R, Chapman MD, Platts-Mills TAE. Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relation to sensitization and asthma among school children living in Los Alamos, New Mexico. *J Allergy Clin Immunol* 1995;96:449-56.
19. Gelber LE, Seltzer LH, Bouzoukis K, Pollart SM, Chapman MD, Platts-Mills TAE. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 1993;147:573-8.
20. Nelson RP Jr, DiNicolo R, Fernandez-Caldas E, Seleznick MJ, Lockey RF, Good RA. Allergen-specific IgE levels and mite allergen exposure in children with acute asthma first seen in an emergency department and in nonasthmatic controls. *J Allergy Clin Immunol* 1996;98:258-63.
21. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TAE. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J Allergy Clin Immunol* 1989;83:875-82.
22. Gottlieb DJ, Sparrow D, O'Connor GT, Weiss ST. Skin test reactivity to common aeroallergens and decline of lung function. The Normative Aging Study. *Am J Respir Crit Care Med* 1996;153:561-6.
23. Tracey M, Villar A, Dow L, Coggon D, Lampe FC, Holgate ST. The influence of increased bronchial responsiveness, atopy and serum IgE on decline in FEV₁. *Am J Respir Crit Care Med* 1995;151:656-62.
24. Peat JK, Woolcock AJ, Cullen K. Rate of decline of lung function in subjects with asthma. *Eur J Respir Dis* 1987;70:171-9.
25. Ulrik CS, Backer V, Dirksen A. A 10 year follow up of 180 adults with bronchial asthma: factors important for the decline in lung function. *Thorax* 1992;47:14-8.
26. Frank TL, Adisesh A, Pickering AC, et al. Relationship between exhaled nitric oxide and childhood asthma. *Am J Respir Crit Care Med* 1998;158:1032-6.
27. Henriksen AH, Lingsaas-Holmen T, Sue-Chu M, Bjerner L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. *Eur Respir J* 2000;15:849-55.
28. Hamid Q, Springall DR, Riveros-Moreno V, et al. Induction of inducible nitric oxide synthase in human bronchial epithelial cells. *Lancet* 1993;342:1510-3.
29. Custovic A, Simpson A, Chapman MD, Woodcock A. Allergen avoidance in the treatment of asthma and atopic disorders. *Thorax* 1998;53:63-72.
30. Custovic A, Taggart SCO, Francis HC, Chapman MD, Woodcock A. Exposure to house dust mite allergens and the clinical activity of asthma. *J Allergy Clin Immunol* 1996;98:64-72.
31. Tunnicliffe WS, Fletcher TJ, Hammond K, et al. Sensitivity and exposure to indoor allergens in adults with differing asthma severity. *Eur Respir J* 1999;13:654-9.
32. Simpson A, Custovic A, Pipis S, Adisesh A, Faragher B, Woodcock A. Exhaled nitric oxide, sensitisation and exposure to allergens in patients with asthma who are not taking inhaled steroids. *Am J Respir Crit Care Med* 1999;160:45-9.
33. Piacentini GL, Bodini A, Costella S, et al. Allergen avoidance is associated with a fall in exhaled nitric oxide in asthmatic children. *J Allergy Clin Immunol* 1999;104:1323-4.
34. Kharitonov SA, O'Connor BJ, Evans DJ, Barnes PJ. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;151:1894-9.
35. Nelson HS, Szefler SJ, Jacobs J, et al. The relationship among environmental allergen sensitization, allergen exposure, pulmonary function, and bronchial hyperresponsiveness in the Childhood Asthma Management Program. *J Allergy Clin Immunol* 1999;104:775-85.
36. Rosenstreich DL, Eggleston P, Kattan M, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med* 1997;336:1356-63.

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