

Factors associated with increased bronchial responsiveness in adolescents and young adults: The importance of adjustment for prechallenge FEV₁

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Background: Asymptomatic increased bronchial responsiveness (BR) appears to be a major risk factor for later development of asthma, but risk factors for increased BR are not well established.

Objective: The relationship of age, gender, house dust mite allergy, number of blood eosinophils, and FEV₁ to the degree of nonspecific BR was studied in a population sample of 665 adolescents and young adults, aged 13 to 23 years.

Methods: Case history, especially concerning smoking habits and respiratory symptoms, was obtained by interview and a self-administered questionnaire. Pulmonary function, number of blood eosinophils, BR to inhaled histamine, and skin test reactivity to house dust mites were measured with standard techniques. BR was analyzed as both a continuous variable (dose-response slope) and a categorical variable (cutoff: PC₂₀ 16 mg/ml).

Results: Increasing number of blood eosinophils, sensitivity to house dust mite, and former or current symptoms of asthma were associated with increasing levels of BR, independently of the level of FEV₁. Adjustment for prechallenge FEV₁ revealed a strong association between lower prechallenge FEV₁ and increasing level of BR ($p < 0.0001$), whereas the previously observed association between female gender and higher level of BR vanished. Repeating the analyses after exclusion of subjects with former or current asthma ($n = 85$) showed an unchanged relationship between house dust mite allergy and level of BR whether or not prechallenge FEV₁ was included in the regression model, whereas gender only influenced the level of BR when no adjustment was made for prechallenge FEV₁. On the contrary, the number of blood eosinophils was only significantly associated with BR after adjustment for baseline FEV₁. Comparable findings were observed when patients with former or current symptoms of asthma and subjects with a prechallenge FEV₁ less than 80% of predicted value ($n = 46$) were excluded from the analyses.

Conclusions: This analysis suggests that adjustment for prechallenge level of FEV₁ should be considered in studies concerned with risk factors for symptomatic and asymptomatic bronchial hyperresponsiveness. (*J ALLERGY CLIN IMMUNOL* 1996;97:761-7.)

Key words: Bronchial responsiveness, risk factors, allergy, eosinophils, asthma, prechallenge FEV₁

A close relationship between current asthma and increased nonspecific bronchial responsiveness (BR) has been clearly established, not only in

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Abbreviations used

BR: Bronchial responsiveness

FVC: Forced vital capacity

selected groups of patients¹ but also in population studies.² Nonspecific challenge testing with either histamine or metacholine in combination with respiratory symptoms has been suggested as diagnostic for asthma in population studies.³ However, there is considerable overlap between the levels of

BR observed in asthmatic and nonasthmatic subjects, making selection of a cutoff between normal and abnormal arbitrary.

In previous studies,^{1,4,5} a close association was found between a lower level of prechallenge FEV₁ and an increased responsiveness to inhaled histamine in adults with asthma, which has also been demonstrated in healthy adults.⁶ The relationship between nonspecific BR and prechallenge level of pulmonary function has not been extensively studied in asthmatic and nonasthmatic adolescents and young adults. Some of the factors suggested to be associated with increased levels of BR are known to be associated with a lower level of FEV₁ (e.g., cigarette smoking and respiratory symptoms). Such factors therefore may be associated with higher levels of responsiveness primarily because of their association with a lower level of pulmonary function. Adjustment for prechallenge FEV₁ may be considered in order to ascertain that an observed association between any particular factor and the level of BR is not driven by its relation to the prechallenge level of pulmonary function.

The purpose of this analysis was to investigate the relationship of age, gender, number of blood eosinophils, respiratory symptoms, and sensitization to house dust mite to level of nonspecific BR in a population sample of adolescents and young adults, taking the level of prechallenge pulmonary function into account.

METHODS

Subjects

All subjects included in the analysis took part in a prospective population study of asthma, allergy, and bronchial hyperresponsiveness initiated in 1986. A random sample of 983 children and adolescents living in the area surrounding the National University Hospital in Copenhagen was drawn from the civil registration list; all subjects were born in the first week of each month in the years 1969 to 1979.

Subjects were invited to the examination by letter; 527 subjects responded, and the first part of the study was carried out in 1986. The subjects ($n = 983$) were invited to a second examination in 1992, and a total of 665 (68% of those invited) participated. All data used in this cross-sectional analysis were taken from the second examination.

The question of nonresponder bias has been addressed in a previous article.⁷ Briefly, apart from a significantly higher number of current smokers among the subjects who only participated in the 1992 examination, no significant differences were found with respect to anthropometric data, pulmonary function, or prevalence of positive skin prick test results, bronchial hyper-

responsiveness, and allergic diseases, indicating that the subjects included in this analysis are representative of the entire sample.

Exclusion criteria

The subjects were asked to abstain from smoking for at least 2 hours before the examination. In case they were taking medication for asthma or allergy, they were asked not to use theophylline or an antihistamine for at least 24 hours, astemizole for 6 weeks, an oral β_2 -agonist for 18 hours, or an inhaled bronchodilator for 6 hours before the tests. They were allowed to continue use of any inhaled or oral corticosteroids they had been taking.

Case history

The participants and, if present, their parents were interviewed by one person about birth weight, diseases (respiratory and nonrespiratory), active and passive smoking, use of medication, known or suspected allergic disease or allergy, pets in the household, use of eider-down-stuffed pillows and continental quilts, and carpets in bedrooms.

Furthermore, all participants filled in a questionnaire about asthmatic and allergic symptoms, that is, rhinitis (sneezing, runny or blocked nose not associated with a cold) and eczema (an itchy dry rash on face, arms, or legs), as related to themselves, their siblings, and their parents.

The questionnaire concerning respiratory symptoms and the definition of asthma were adopted from studies by the American Thoracic Society, Division of Lung Disease of the National Heart, Lung and Blood Institute^{8,9} and Hopp et al.^{10,11} Asthma was defined by questionnaire criteria on the basis of responses to the following questions.

1. Have you ever had asthma?
2. Does your breathing ever sound wheezy or whistling?
3. Do you have attacks of shortness of breath with wheezing?
4. Do you experience wheezing, chest tightness, cough, or breathlessness at rest, on exertion, during emotional stress, on exposure to cold air, or with chest infections or head cold?
5. Do you experience wheezing after exposure to dust, fumes, molds, pollen, food, pets, or drugs?
6. Have you ever been hospitalized or observed and treated by a doctor because of asthma?
7. Have you ever received medication for your asthma?
8. What was the medication used?
9. Did it help?
10. How many episodes of wheezing have you had during the last year?
12. Have you ever had attacks of wheezing, shortness of breath, or dry coughing at night?

Asthma was defined on the basis of positive responses to at least questions 2 and 3, and 4, and/or 5. Current

asthma was defined as symptoms of asthma within the preceding 12 months.

Furthermore, all participants reported whether they were current smokers, ex-smokers, or life-long non-smokers; for the first two categories they reported the duration of smoking. Current and ex-smokers reported their daily tobacco consumption, and an estimate of their lifetime tobacco exposure was calculated as pack-years (current tobacco consumption [gm/day] $\div$ 20) $\times$ duration of smoking (years).

Pulmonary function tests

FEV₁ and forced vital capacity (FVC) were measured with a 7 L dry wedge spirometer (Vitalograph, Inc., London, U.K.), which was calibrated weekly. Each measurement consisted of at least three maximal expiratory maneuvers from total lung capacity to residual volume with a variation of less than 5% in FEV₁. The highest FEV₁ and FVC values were used in the analyses.

House dust mite sensitivity

Skin prick test reactivity to house dust mite (*Dermaphagoides pteronyssinus* and *D. farinae*) was tested on the volar surface of the forearm with standard dilutions (100,000 BU/ml) of the allergens in 50% glycerol. Histamine HCl, 10 mg/ml, in 50% glycerol was used as a positive control; a negative control (50% glycerol) was also included. The reactions were read after 15 to 20 minutes. The reaction to each test was regarded as positive if the largest diameter of the wheal was at least 3 mm.¹² In case of reaction to the negative control, the result was read as the difference between the largest diameter of the reaction to the allergen and the largest diameter of the reaction to the negative control. Subjects were considered sensitized to house dust mite if they had a positive reaction to either of the two house dust mite allergens.

Blood eosinophils

For each subject, the standard differential leukocyte counts were estimated by a Technicolor Hemalog (Technicon, Tarrytown, N.Y.), and the number of eosinophilic leukocytes was counted in a counting chamber.¹³ Eosinophilia was considered to be present at a count of greater than $0.25 \times 10^9/L$.¹⁴

Histamine challenge test

BR to inhaled histamine was measured with the method described by Cockcroft et al.¹ Aerosols of the test solution were generated by a Wright nebulizer (Aerosol Products Ltd., London, England) operated to provide an output of 0.14 ml/min. Each aerosol was inhaled through the mouth by tidal breathing for 2 minutes. The first aerosol was saline solution (0.9%), and it was followed at 4-minute intervals by twofold increasing concentrations of histamine (0.075 to 16.0 mg/ml). The response was measured by the FEV₁ 1 minute after each inhalation. The test was terminated

when a decline of 20% or more in FEV₁ from the postsaline value occurred or at the end of the dose schedule if such a decline did not occur. For all subjects having a decline of at least 20% in FEV₁ by the end of the dose schedule, the PC₂₀ was calculated by linear interpolation from the individual log-dose response curve as follows¹⁵:

$$PC_{20} = \text{antilog}[(\log C_1 + (\log C_2 - \log C_1)(20 - R_1) \div (R_2 - R_1))]$$

where C_1 = second last concentration of histamine (<20% FEV₁ fall), C_2 = last concentration of histamine (>20% FEV₁ fall), R_1 = percent fall FEV₁ after C_1 , and R_2 = percent fall FEV₁ after C_2 . A positive test result was defined as a PC₂₀ of ≤ 16.0 mg/ml histamine.

Histamine responsiveness was also analyzed with use of an estimate of the overall slope of the dose-response relationship as described by O'Connor.¹⁶ The dose-response slope was calculated as the decline in FEV₁ from the postsaline value (expressed as a percentage of the postsaline value) after the final dose divided by the dose of histamine administered¹⁶; a constant of 3 was added to all dose-response slopes to eliminate negative and zero values.

Statistical methods

Data were analyzed with use of the statistical package program SPSS (SPSS Inc., Chicago, Ill.). The distribution of the data was checked for normality, or, if appropriate, the variables were log-transformed to obtain normality and expressed as geometric means and percentages of standard deviations. Other variables are reported as arithmetic means and standard deviations. Multiple linear and logistic regression analyses were used to assess factors of possible importance for BR.

RESULTS

A total of 665 subjects (68% of the original sample) had complete data required for this analysis; general characteristics of the subjects are given in Table I. Bronchial hyperresponsiveness to inhaled histamine was found in 103 subjects (16%, 54 male subjects), whereas a history of former or current symptoms of asthma was reported by 45 (14%) male subjects and 40 (12%) female subjects. Positive skin reactions to house dust mite and blood eosinophilia were found in 172 (26%) and 127 (19%) subjects, respectively.

Most of the subjects with former or current asthma had bronchial hyperresponsiveness, whereas only 5% of the studied subjects had asymptomatic bronchial hyperresponsiveness (Table II). Five of the subjects with current asthma (i.e., symptoms within the preceding 12 months) had a PC₂₀ greater than 16 mg/ml histamine; seven

TABLE I. Characteristics of the sample of adolescents and young adults ($n = 665$) by gender

Characteristic	Male subjects ($n = 322$)	Female subjects ($n = 343$)
Age (yr) [SD]	18.5 [2.8]	18.7 [2.9]
Range	14-23	13-23
Smoking status (no.)		
Life-long nonsmokers	212	191
Ex-smokers	3	13
Current smokers	106 (33%)	138 (40%)
Prechallenge FEV ₁		
Percent predicted	96.3 [15.0]	98.2 [12.4]
Range	53-143	45-138
L	4.52 [0.52]	3.49 [0.88]
Range	1.80-7.40	1.40-5.20
Asthmatic status (no.)		
Nonasthmatic	276	302
Ever asthma (%) [*]	45 (14%)	40 (12%)
Current asthma (%) [†]	39 (12%)	37 (11%)
HDM reactivity (no.)		
Positive	99 (31%)	73 (21%)
Negative	223	216
Eosinophils (10 ⁹ /L) [SD]	0.18 [0.13]	0.17 [0.17]
Range	0.01-85	0.01-1.42
>0.25 (no.)	67 (21%)	60 (18%)
<0.25 (no.)	254	282
Histamine responsiveness		
DRS [†] [SD]	2.5 [23.7]	2.7 [25.0]
PC ₂₀ < 16.0 mg/ml (no.)	54 (17%)	49 (14%)

SD, Standard deviation; HDM, house dust mite; DRS, dose-response slope.

^{*}Defined as current or former symptoms of asthma (recurrent attacks of dyspnea with wheezing triggered by exposure to allergens, respiratory tract infections, and/or exercise).

[†]Dose-response slope (for explanation, see text).

subjects with former asthma had a negative histamine challenge test response.

The results of the regression analyses for the degree of histamine responsiveness are shown in Table III. In the analysis without adjustment for prechallenge FEV₁, female gender ($p = 0.009$), symptoms of asthma ($p < 0.0001$), increasing number of blood eosinophils ($p = 0.0001$), and house dust mite allergy ($p < 0.0001$) were associated with increasing level of BR. Adjustment for level of FEV₁ showed a significant association between lower level of prechallenge FEV₁ and increasing degree of BR ($p < 0.0001$). Furthermore, with adjustment for prechallenge FEV₁, female gender was not significantly associated with a higher degree of histamine responsiveness.

The results of repeating the analyses after exclu-

TABLE II. Frequency of asthma symptoms, blood eosinophilia, and house dust mite allergy in a population sample of subjects, aged 13 to 23 years, stratified according to presence or absence of nonspecific bronchial hyperresponsiveness

	BHR+	BHR-
Asthma+	73 (86%)	12
Asthma-	30 (5%)	548
Eosinophilia+	45 (35%)	82
Eosinophilia-	58 (11%)	478
HDM+	50 (29%)	122
HDM-	53 (11%)	438

Row percentages are given in parentheses; for definitions see text.

BHR, Bronchial hyperresponsiveness; HDM, house dust mite allergy.

sion of subjects who reported symptoms of asthma are shown in Table IV. Without adjustment for initial level of FEV₁, male gender ($p = 0.007$) and house dust mite allergy ($p < 0.0001$) were associated with an increasing level of responsiveness, whereas no significant association was found between responsiveness and number of blood eosinophils ($p = 0.28$). After adjustment for prechallenge FEV₁, the association between house dust mite allergy and responsiveness remained significant, whereas gender did not significantly influence the level of responsiveness. Furthermore, this analysis revealed not only an association between responsiveness and prechallenge FEV₁ but also a significant association between increasing level of responsiveness and increasing number of blood eosinophils ($p = 0.004$).

Comparable results were obtained when subjects with a prechallenge FEV₁ less than 80% of predicted value ($n = 46$, 35 male subjects) were excluded from the regression models. Analysis of the data with histamine responsiveness defined as a categorical variable (i.e., with a PC₂₀ of 16 mg/ml histamine as the cutoff point) did not change these findings.

All analyses were repeated after inclusion of the FEV₁/FVC ratio, as an index of prechallenge degree of obstruction, in the regression models. As expected, a lower prechallenge FEV₁/FVC ratio was associated with a higher level of histamine responsiveness, whereas the previously observed relation between age, gender, asthma, number of blood eosinophils and house dust mite allergy, and BR was unchanged whether or not prechallenge FEV₁ was included in the regression model. Fi-

TABLE III. Regression analysis of BR to inhaled histamine (dose-response slope*) on level of FEV₁, age, gender, asthma, number of blood eosinophils, and house dust mite sensitivity in a population sample (*n* = 665) of adolescents and young adults

	Not adjusted for FEV ₁			Adjusted for FEV ₁		
	Coeff.	SEM	<i>p</i> Value	Coeff.	SEM	<i>p</i> Value
Prechallenge FEV ₁ (L)				-0.097	0.022	<0.0001
Gender†	-0.075	0.029	0.009	0.029	0.037	0.43
Age (yr)	-0.008	0.005	0.14	0.002	0.005	0.76
Asthma‡	0.726	0.046	<0.0001	0.698	0.046	<0.0001
Blood eosinophils (10 ⁹ /L)	0.376	0.099	0.0001	0.336	0.098	0.0006
HDM-positive§	0.272	0.034	<0.0001	0.280	0.034	<0.0001

Coeff., Coefficient; SEM, standard error of the mean; HDM, house dust mite.

*Dose-response slope = (% fall in FEV₁ + 3) ÷ (μmol histamine administered).

†Males = 1, females = 0.

‡Defined as current or former symptoms of asthma (recurrent attacks of dyspnea with wheezing triggered by exposure to allergens, respiratory tract infections, and/or exercise).

§Positive skin reaction to *D. pteronyssinus* and/or *D. farinae* (i.e., a reaction with a diameter of at least 3 mm).

TABLE IV. Regression analysis of BR to inhaled histamine (dose-response slope*) on level of FEV₁, age, gender, number of blood eosinophils, and house dust mite sensitivity in nonasthmatic adolescents and young adults (*n* = 580) (subjects with former or current asthma excluded from the analysis)

	Not adjusted for FEV ₁			Adjusted for FEV ₁		
	Coeff.	SEM	<i>p</i> Value	Coeff.	SEM	<i>p</i> Value
Prechallenge FEV ₁ (L)				-0.058	0.021	0.005
Gender†	-0.073	0.027	0.007	-0.012	0.035	0.76
Age (yr)	-0.008	0.005	0.07	-0.003	0.005	0.58
Blood eosinophils (10 ⁹ /L)	0.106	0.097	0.28	0.186	0.097	0.004
HDM-positive‡	0.286	0.033	<0.0001	0.294	0.033	<0.0001

Coeff., Coefficient; SEM, standard error of the mean; HDM, house dust mite.

*Dose-response slope = (% fall in FEV₁ + 3) ÷ (μmol histamine administered).

†Males = 1, females = 0.

‡Positive skin reaction to *D. pteronyssinus* and/or *D. farinae* (i.e., a reaction with a diameter of at least 3 mm).

nally, all analyses were repeated with inclusion of smoking habits in the regression models as either a categorical (current and ex-smokers vs life-long nonsmokers) or a continuous (pack-years of smoking) variable; however, this did not change the previously observed impact of various factors on level of BR.

DISCUSSION

Apart from the expected association between symptoms of asthma and BR, this study of a population sample of adolescents and young adults demonstrated a strong association between lower level of prechallenge pulmonary function and higher level of BR to inhaled histamine. Further-

more, a higher number of blood eosinophils and allergy to house dust mite were associated with increased level of responsiveness, irrespective of the prechallenge level of FEV₁, whereas adjustment for baseline FEV₁ abolished the previously observed association between male gender and decreased level of BR. In subjects who did not report current or former symptoms of asthma, adjustment for baseline pulmonary function revealed a significant association between increasing number of blood eosinophils and increasing level of histamine responsiveness.

Nonspecific bronchial hyperresponsiveness is a characteristic finding in patients with symptomatic asthma, and findings in previous studies suggest

that increased BR usually precedes the development of symptomatic asthma.¹¹ Identification of risk factors for increased BR is therefore of utmost importance in order to prevent, if possible, development of symptomatic asthma. IgE-mediated allergy is closely related to both bronchial hyperresponsiveness and asthma.^{2, 17-19} However, the relative importance of specific allergens for the prevalence of hyperresponsiveness and asthma in different parts of the world has not been extensively studied. This study shows not only a high prevalence of sensitization to house dust mites but also a strong association between house dust mite allergy and level of BR, irrespective of the presence or absence of symptomatic asthma. In keeping with findings by Peat et al.²⁰ in Australia, this study indicates that house dust mite allergy, at least in susceptible individuals, may trigger increased BR, and as a result, probably symptomatic asthma.¹⁹ However, as previously reported in studies from other countries,^{20, 21} two thirds of the subjects sensitized to house dust mites had a PC₂₀ greater than 16 mg/ml histamine (Table II), most likely reflecting the multifactorial causes of bronchial hyperresponsiveness and asthma.

In keeping with previous reports,²²⁻²⁴ Bakke et al.²⁵ reported a higher prevalence of hyperresponsiveness to inhaled metacholine among female patients than male patients in a Norwegian population sample. However, as also observed in this study, adjustment for prechallenge level of pulmonary function reversed this association, showing that male patients in general have a higher level of responsiveness than female patients. The absolute FEV₁ is generally higher in male patients than in female patients, which may explain why adjustment for prechallenge FEV₁ reversed the association between gender and histamine responsiveness. However, in challenge tests, change in pulmonary function is expressed as change from baseline and, combined with the lack of difference in level of responsiveness observed in this study, suggests that there may not be a general male-female difference in level of histamine responsiveness.

Blood eosinophilia has long been accepted as an allergic trait.^{26, 27} However, eosinophils are now increasingly being recognized as important effector cells in the inflammatory reaction in asthmatic airways,²⁸ and a relationship between markers of disease activity and eosinophil counts has been observed in patients with both allergic and nonallergic asthma.²⁹ Furthermore, evidence from recent studies suggests that the role of the eosinophil leukocyte in respiratory diseases may go beyond its

role in allergy.³⁰ The finding in this study that number of blood eosinophils is related to level of histamine responsiveness in nonasthmatic subjects with a prechallenge FEV₁ within the reference range, although significant only after adjustment for baseline FEV₁, suggests that an increased number of eosinophils reflects an abnormality, probably inflammation, in the airways of both subjects with and without respiratory symptoms. Future follow-up examinations of this population sample might therefore show blood eosinophilia to be an independent risk factor for development of reversible and nonreversible obstructive lung function impairment.

Geometric factors might at least partly explain the observed relationship between prechallenge pulmonary function and nonspecific BR.^{31, 32} This relation is well documented in smokers with chronic obstructive pulmonary disease³³ and in general population samples of adults,³⁴ whereas conflicting results have been reported among patients with asthma.^{1, 35} This study demonstrates a strong relation of prechallenge pulmonary function to degree of histamine responsiveness, whether or not subjects with former or current asthma were included in the analysis; and, furthermore, this relation was also observed after exclusion of subjects with a prechallenge FEV₁ less than 80% of predicted value from the model. In addition, adjustment for prechallenge FEV₁ to some extent changed the significance of factors potentially related to the level of responsiveness, suggesting that adjustment for prechallenge level of pulmonary function should be made even in subjects who deny respiratory symptoms and those who have pulmonary function within the reference range.

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