

# Smoking and bronchial responsiveness in non-atopic and atopic young adults

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## Abstract

**Background** – Smoking may influence the response of the lungs to other inhaled substances. A study was undertaken to assess the effect of the interaction between smoking and the immunoresponse to common aeroallergens (atopy) on bronchial responsiveness.

**Methods** – A random sample was selected from the general population census of five areas of Spain (Albacete, Barcelona, Galdakao, Huelva, and Oviedo). A total of 1169 (35%) subjects completed a face-to-face respiratory questionnaire, a methacholine bronchial responsiveness challenge, and underwent measurements of total and specific serum IgE levels to mites, pets and moulds. A survival model (Weibull) was used to examine the methacholine dose-response relation, adjusting for bronchial obstruction.

**Results** – Smokers showed greater bronchial responsiveness than never smokers ( $p < 0.05$ ) at any dose of methacholine, but only among non-atopic individuals. Atopy had a large effect on responsiveness at low levels of methacholine, but smoking did not increase responsiveness in atopic subjects. There were no differences in intensity or cessation of smoking between atopic and non-atopic subjects, suggesting that smoking self-selection does not fully explain these results.

**Conclusions** – The association between smoking and bronchial responsiveness varies with atopy, which may be explained by different immunological and/or inflammatory effects of smoking on atopy.

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Keywords: smoking, atopy, bronchial responsiveness.

Smoking influences the response of the lungs to other inhaled substances. Workers who smoke are at higher risk of developing asthma against novel aeroallergens – for example, in fish processing – than non-smokers<sup>1,2</sup> and, in the epidemics of asthma in Barcelona, smoking increased the likelihood of reacting to soybean.<sup>3</sup> Similarly, in a study on a middle aged and elderly general population<sup>4</sup> smoking and skin reactivity to common aeroallergens showed an interactive effect on bronchial responsiveness. Inflammation of the mucosa and increasing lung permeability secondary to smoking could augment the access of antigens to immunocompetent cells.<sup>5</sup>

By contrast, smokers were not found by Halonen *et al* to have a higher frequency of skin reactivity to common aeroallergens,<sup>6</sup> although a possible self-selection bias of smoking could be the reason.<sup>7</sup> Another conflicting result was the negative relation between smoking and the appearance of allergic alveolitis<sup>8</sup> which might be due, in part, to a potential immunosuppression of smoking.<sup>9</sup>

A study was undertaken to assess the effect of the interaction between smoking and atopy on bronchial responsiveness in a general population study of young adults in five areas of Spain. Bronchial hyperresponsiveness has been related to the development of chronic obstructive pulmonary disease, and it provides a reliable way of measuring asthma in population studies.<sup>7</sup>

## Methods

### STUDY POPULATION

A sample of 16 884 subjects aged 20–44 years was randomly selected from the general population census from the five areas of Spain (Albacete, Barcelona, Galdakao, Huelva, and Oviedo) that participated in the European Community Respiratory Health Survey with a screening questionnaire on respiratory symptoms.<sup>10,11</sup> A random subcohort of 3310 subjects (approximately 20% of the initial sample) was invited to complete a face-to-face respiratory questionnaire, to undergo spirometric tests and a dose-response methacholine challenge test, and for measurement of total and specific serum IgE levels. A complete bronchial responsiveness assessment was obtained in 1169 (35.3%) subjects. The study protocol was approved by the Institutional Review Board of the participating centres and patients gave written informed consent.

### SMOKING AND ATOPY

Smoking was measured using a standard questionnaire on status, daily number of cigarettes smoked, and duration in years.<sup>10</sup> Total serum IgE and specific serum IgE levels against *Dermatophagoides pteronyssinus*, cat dander, timothy grass, *Cladosporium*, and *Parietaria* were measured by the CAP method (Kabi Pharmacia, Uppsala, Sweden) in a central laboratory. Values were expressed in kU/l. Specific IgE levels were treated as dichotomous variables using a cut-off value of 0.35 kU/l and atopy was defined as a positive response to any of the five common aeroallergens.

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Table 1 Descriptive statistics by atopy and sex

	Women		Men	
	Non-atopic (n = 456)	Atopic (n = 102)	Non-atopic (n = 412)	Atopic (n = 199)
Geometric mean of total IgE (kU/l)	22.6	88.7*	28.4	113.4*
Mean age (years)	32.1	30.1*	32.4	30.4*
Mean baseline FEV <sub>1</sub> (l)	3.13	3.20	4.16	4.31*
Mean FEV <sub>1</sub> (% predicted)	109.6	107.2	105.2	106.5
Never smokers (%)	41.3	35.3	25.7	28.6
Past smokers (%)	12.3	20.5	14.6	13.1
Current smokers (%)	46.4	44.1	59.7	58.3
Mean number of cigarettes/day	17.8	20.7	17.9	17.5
Mean number of years smoking	15.9	14.1	16.2	15.4
Bronchial responsiveness (%)†	17.9	20.4	11.4	20.6*

FEV<sub>1</sub> = forced expiratory volume in one second.

\* p < 0.05 versus non-atopic individuals of same sex.

† Decrease in FEV<sub>1</sub> of >20% from saline FEV<sub>1</sub> with a cumulative dose of 5.117 μmol methacholine.

#### BRONCHIAL RESPONSIVENESS

Subjects underwent baseline spirometric tests (Biomedin, Padova, Italy), performing at least three acceptable manoeuvres (repeatable within 5% or 100 ml) to measure forced expiratory volume in one second (FEV<sub>1</sub>).<sup>12</sup> Bronchial responsiveness was measured by methacholine inhalation challenge. Standard methacholine solutions (Hoffman La Roche, London, UK) were prepared by the Department of Pharmacy of one of the participating centres. After inhaling nebulised saline, each methacholine dose was administered using a Mefar dosimeter according to the European Community Respiratory Health Survey protocol.<sup>10</sup> The methacholine challenge test was performed only if the best FEV<sub>1</sub> after saline inhalation was more than 90% of the baseline FEV<sub>1</sub> value. Increasing concentrations of methacholine from 0.195 mg/ml to 100 mg/ml were sequentially inhaled. Individuals without symptoms of asthma followed a shortened protocol in which concentrations of methacholine were quadrupled, whereas individuals reporting symptoms of asthma used the full protocol in which concentrations were doubled. The challenge was terminated when the FEV<sub>1</sub> fell by more than 20% from the best saline FEV<sub>1</sub> or after 1 mg (5.117 μmol inhaled) of methacholine had been given.

#### DATA ANALYSIS

To assess how smoking and atopy may affect bronchial responsiveness, adjusting for confounding variables such as age, sex, total IgE, and percentage predicted FEV<sub>1</sub>, standard logistic regression methods were used. Adjustment for basal FEV<sub>1</sub> and FEV<sub>1</sub>/VC (vital capacity) did not improve the adjustment of predicted FEV<sub>1</sub>. Models were also adjusted for geographical area since bronchial responsiveness, atopy, and response rate varied between centres. In the logistic regression models bronchial responsiveness was considered as a dichotomous variable – that is, the presence or absence of bronchial responsiveness when the provocative concentration causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>) was <100 mg/ml.

To examine the methacholine dose-response relationship, making full use of all the available information, we also applied methods of sur-

vival analysis,<sup>13</sup> in particular the Weibull model described in detail elsewhere.<sup>14,15</sup> In this method the different concentrations of methacholine are viewed as chronological times to an event, thus permitting the incorporation of information on the whole dose-response relationship between concentrations of methacholine and hazard of responding. This method also allows incorporation of information on the degree of response (the different magnitude in the fall of FEV<sub>1</sub>) by using a simple interpolation that incorporates the differences between the short and the full methacholine challenge protocols.<sup>14</sup> The Weibull model allows calculation of the concentration of methacholine at which a percentage of the population responded, which is given by  $\exp(\beta) \times [-\log(1-p)]^{-\sigma}$  (where  $\beta$  and  $\sigma$  correspond to the location and scale parameter of the model). This simple relationship provides the basis for comparing the concentration needed to achieve a given percentage response in different groups of individuals, and to define the relative concentrations (generally known as relative percentiles or RP). In cases where there is no difference with respect to responsiveness both  $\beta_1$  and  $\sigma_1$  (the differences in location and scale parameters between the groups) will be zero and all the relative percentiles will be identically equal to 1. If only  $\sigma_1 = 0$  (that is, same scale for both groups) then  $RP = \exp(\beta)$ . In this case, if  $\beta_1 < 0$ , then  $RP < 1$ , which will indicate that the exposure has the effect of proportionally compressing the concentrations needed for a percentage of the individuals to respond. If the groups are different in both location and scale, then  $\sigma \neq 0$  and the relative percentiles will not be proportional. Positive (negative) values of  $\sigma_1$  will determine whether the relative percentiles will increase (decrease) with cumulative percentage of responders.

Estimation of parameters was achieved by maximum likelihood methods using the statistical package EGRET (Seattle, Washington, USA).

#### Results

Atopic individuals of both sexes had higher levels of total IgE, were younger, and had a higher baseline FEV<sub>1</sub> than non-atopic individuals. Differences in baseline FEV<sub>1</sub> by atopy disappeared after adjusting for age (table 1). Smoking was very common, with 46% of women and 59% of men being current smokers. Smoking frequency, intensity, and duration did not vary with atopy. Bronchial responsiveness was more frequent in atopic subjects.

Table 2 Frequency of bronchial responsiveness by smoking, atopy, and sex

Sex	Smoking history	Non-atopic		Atopic	
		No.	(%)*	No.	(%)*
Women	Never	188	(12.8)	36	(25.0)
	Past	56	(14.3)	21	(14.3)
	Current	212	(23.6)	45	(20.0)
Men	Never	106	(4.7)	57	(21.1)
	Past	60	(8.3)	26	(30.7)
	Current	246	(17.9)	116	(18.1)

\* Percentage in each group with bronchial responsiveness.

Table 3 Adjusted† odds ratio (and 95% confidence intervals) of bronchial responsiveness by smoking, atopy, and sex

Sex	Smoking history	Non-atopic	Atopic
Women	Never	1.0	1.0
	Past	1.15 (0.46 to 2.84)	0.34 (0.08 to 1.77)
	Current	1.89 (1.06 to 3.29)*	0.58 (0.18 to 1.89)
Men	Never	1.0	1.0
	Past	1.45 (0.39 to 5.43)	1.91 (0.64 to 5.64)
	Current	3.52 (1.33 to 9.33)*	0.97 (0.40 to 2.39)

† Adjusted by area, age, total IgE, and percentage predicted FEV<sub>1</sub>.  
\* p<0.05.

Table 4 Mean (SE) estimates of regression and scale coefficients from multivariate Weibull model for concentrations of methacholine provoking a bronchial response

Variable	Regression	Scale
Constant‡	2.842	0.181
Atopy	0.025 (0.111)	0.088 (0.041)*
Past smoking	-0.028 (0.077)	
Current smoking	-0.128 (0.048)*	
Atopy and past smoking	-0.093 (0.137)	
Atopy and current smoking	0.184 (0.094)†	
Age (years)	0.005 (0.003)	
Male	-0.087 (0.050)	
Log of total IgE (kU/ml)	-0.043 (0.013)*	
FEV <sub>1</sub> (% predicted)	0.024 (0.003)*	

\*p<0.05; †p=0.051.

‡Reference category comprises non-atopic women aged 32 who had never smoked with IgE=36 kU/ml and percentage predicted FEV<sub>1</sub>=107%.

In non-atopic individuals of both sexes bronchial responsiveness occurred more frequently in current smokers than in both never and past smokers (p<0.01; table 2). However, bronchial responsiveness did not vary with smoking status in atopic individuals. The association between current smoking and bronchial responsiveness in non-atopic individuals and the lack of association in atopic individuals remained after adjusting for area, age, total IgE, and percentage predicted FEV<sub>1</sub> (table 3). The high variability of the odds ratio for past smoking in atopic men could be explained by the small size of this group.

Given that the association between smoking and bronchial responsiveness with atopy was similar in both sexes, and to increase the statistical power, the dose-response survival curve was put in a common model for men and women (table 4). Current smoking for the non-atopic individuals had a negative coefficient, indicating that smokers required a lower concentration of methacholine to respond than non-smokers. By contrast, a positive coefficient for the interaction between atopy and current smoking indicated that atopic non-smokers did not differ from atopic never smokers. This

implies a negative interaction between smoking and atopy. Differences between past smokers and never smokers were not statistically significant. Subjects with high total IgE levels required a lower concentration of methacholine to respond, even after controlling for specific IgE levels, while the reverse pattern was seen for age and high percentage predicted FEV<sub>1</sub> values. Adjustment for geographical area did not modify these results. The scale coefficient varied with atopy – specifically, the scale of the atopic individuals was significantly higher than that of the non-atopic individuals. This indicates that the dose-response relation between responsiveness and methacholine was steeper in non-atopic subjects.

Table 5 presents relative concentrations of methacholine by smoking and atopy which were computed by applying the coefficients in table 4. Among non-atopic individuals current smokers responded at concentrations of methacholine about 12% lower than never smokers (RP=0.88) at any of the population response rates. Atopic individuals, either smokers or non-smokers, responded at lower levels than non-atopic non-smokers for a population response rate up to 10%. Above this the differences became non-significant. In addition, smoking status had no effect in atopic subjects.

## Discussion

Increased bronchial responsiveness was seen in smokers compared with non-smokers, but only in non-atopic subjects. Atopy had a significant effect on responsiveness at low doses of methacholine which was not increased by smoking in atopic individuals. There are two possible explanations for the observed interrelations between smoking, atopy, and bronchial responsiveness: self-selection of smokers, or biological antagonism between atopy and smoking.

Self-selection has frequently been used as an explanation for the lack of association between asthma and smoking in cross-sectional studies,<sup>9</sup> and may also be applied to our findings on bronchial responsiveness. Thus, subjects with bronchial responsiveness or atopy tend never to take up smoking, and smokers who develop bronchial responsiveness tend to give up smoking. However, our data suggest that it is unlikely that these biases account totally for the present results. The intensity and duration of current smoking was no higher in non-atopic subjects than in atopic subjects, and the frequency of

Table 5 Concentration of methacholine (C) needed to provoke a bronchial response† in predefined percentage of individuals. Relative percentiles (RP) adjusted for atopy and current smoking

Percentage of population	No atopy			Atopy			
	Non-smoking C*	Smoking		Non-smoking		Smoking	
		C	RP (95% CI)**	C	RP (95% CI)**	C	RP (95% CI)**
1	7.5	6.6	0.88 (0.80 to 0.97)	5.1	0.68 (0.46 to 0.94)	5.4	0.72 (0.56 to 0.93)
5	10.0	8.8	0.88 (0.80 to 0.97)	7.9	0.79 (0.64 to 0.96)	8.4	0.84 (0.71 to 0.99)
10	11.4	10.0	0.88 (0.80 to 0.97)	9.6	0.84 (0.72 to 0.98)	10.1	0.89 (0.78 to 1.00)
15	12.3	10.8	0.88 (0.80 to 0.97)	10.8	0.88 (0.79 to 1.00)	11.4	0.93 (0.82 to 1.03)

\* Reference category comprises women aged 32 with IgE=36 kU/ml, predicted FEV<sub>1</sub>=107%.

† Decrease in FEV<sub>1</sub> of >20% from post-saline FEV<sub>1</sub>.

\*\* Relative percentiles and 95% confidence intervals adjusted for age, sex, total IgE, and predicted FEV<sub>1</sub>.

past smoking in men was the same in atopic and non-atopic individuals. In addition, bronchial responsiveness in past smokers did not differ from that in never smokers.

It can also be argued that atopic individuals who smoked had less "severe" symptoms than those who did not smoke. We examined the interaction between atopy and smoking by reported lifetime history of asthma and also by reported respiratory symptoms during the previous 12 months. A negative interaction was present in both symptomatic and non-symptomatic subjects (data not shown). This is an important finding since the perception of symptoms would be the likely cause of self-selection. The low response rate is unlikely to explain our results, as we have argued previously,<sup>14</sup> and adjustment for area indicated that differences in bronchial responsiveness and atopy between centres also did not account for our findings.

Changes in the immunological or inflammatory function due to smoking may explain the observed antagonism between smoking and atopy. Smoking reduces the immune receptor activity of alveolar macrophages and alters T cell function by increasing the T helper/suppressor ratio.<sup>16,17</sup> These changes could explain the "protective" effect of smoking in some rare immunological diseases such as allergic alveolitis.<sup>16</sup> However, smoking increases the amount of allergen in contact with the immune system<sup>5</sup> which could compensate for the immunosuppression and explain the higher risk of occupational asthma to certain allergens in smokers.<sup>1-3</sup>

The present findings were obtained using both classical logistic regression analysis with PC<sub>20</sub> as the outcome and the newly proposed survival analysis.<sup>14,15</sup> Both methods used the same inferences. However, survival analysis is well suited to the dose-response nature of the methacholine challenge test, incorporating the complexities of the way in which different variables may interact to affect bronchial responsiveness. Thus, atopy seems to have a greater effect at low levels of airway responsiveness whereas smoking among non-atopic individuals is equally effective at all levels of airway responsiveness. The effect of atopy at low doses of methacholine is probably explained by the fact that severe asthmatic individuals, who responded at low doses, had a high risk of being atopic.

The relation of smoking to bronchial responsiveness has been found in many population studies,<sup>7</sup> although it is more prominent in subjects aged over 45 years.<sup>18</sup> Young age was attributed by Rijcken *et al*<sup>19</sup> to be the cause of a non-significant association between smoking and bronchial responsiveness in a Dutch population, which confirmed previous clinical studies in young subjects of mean ages 21 and 27.<sup>20,21</sup> We did find an association between bronchial responsiveness and smoking in our relatively young population (mean age 31), but only in non-atopic individuals. Differences in the proportion of atopic individuals in different studies could explain the divergent results on the role of smoking in bronchial responsiveness.

The negative interaction between smoking and atopy on bronchial responsiveness may be relevant to the fact that the role of smoking in the occurrence and clinical history of asthma is unclear.<sup>21-25</sup> Knowledge of the underlying mechanism of the present findings may help to improve understanding of the interaction between environmental and constitutional factors in the aetiology of asthma, as well as providing new information for pathological models of chronic obstructive pulmonary disease.

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