Eur Respir J, 1995, 8, 913–921
DOI: 10.1183/09031936.95.08060913

European Respiratory Journal
ISSN 0903 - 1936

Increased maximal airway response to methacholine during seasonal allergic rhinitis in nonasthmatic subjects: relationships with airway wall thickness and inflammation

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Increased maximal airway response to methacholine during seasonal allergic rhinitis in nonasthmatic subjects: relationships with airway wall thickness and inflammation. L-P. Boulet, H. Turcotte, G. Carrier, M. Boutet, M. Laviolette. ©ERS Journals Ltd 1995.

ABSTRACT: This study was carried out to determine whether the increase in airway responsiveness induced by natural antigenic exposure in nonasthmatic subjects is associated with an increase in maximal bronchoconstrictor response (MBR), and if these changes could be due to an increase in airway wall thickness from allergen-induced increase in airway inflammation.

In 11 nonasthmatic subjects with seasonal allergic rhinitis, a methacholine challenge was obtained monthly, during and out of pollen exposure. Each subject had a high-resolution chest tomography in and out of the pollen season, to determine the relative thickness of the right intermediary bronchus over its total diameter (T/D), as well as inflammatory cell counts, apparent basement membrane thickness as an indication of subepithelial fibrosis and epithelial desquamation in bronchial biopsy specimens.

In season, the mean provocative concentration of methacholine producing a 20% decrease in forced expiratory volume in one second (PC_{20}) decreased from 51.5 to 25.8 mg·mL^{-1}, and the maximal post-methacholine fall in forced expiratory volume in one second ($\Delta FEV_{1,max}$) or forced vital capacity (ΔFVC) and the slope of the dose response curve (DRS) increased compared with out of season: $\Delta FEV_{1,max}$ 44±5 *vs* 25±5%; ΔFVC 34±5 *vs* 16±4%; and slope of DRS 14.1±2.8 *vs* 6.9±1.3%/mg·mL^{-1}. No significant change was observed in T/D ratio. The seasonal change in ΔFVC was positively correlated with the $\Delta FEV_{1,max}$ (r_s=0.891) and the change in DRS (r_s=0.909), but not with the change in PC_{20}, nor with changes in bronchial biopsy inflammatory features or T/D ratio.

In conclusion, we found an increase in airway responsiveness and maximal bronchoconstrictor response after natural allergen exposure in nonasthmatic rhinitic patients. However, there was no significant increase in bronchial inflammatory features or large airway wall thickness. This could indicate that the statistical power of this study is limited, or alternatively, that the small airways are more important in determining the physiological responses to pollen exposure.

Eur Respir J., 1995, 8, 913–921.

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Keywords: Airway responsiveness
airway wall thickness
allergic rhinitis
computed chest tomography
maximal airway narrowing

Received: September 7 1994
Accepted after revision March 5 1995

Natural or laboratory exposure to allergens can increase airway response to nonallergic stimuli both in asthmatic and nonasthmatic atopic subjects [1–4]. The mechanisms underlying this change are still unknown, but it is suspected that they may be related to airway inflammation. In allergic asthma, this possibility has been supported by observations showing concomitant increases in airway inflammatory features and responsiveness following acute or long-term exposure to antigens [5–7]. Nonasthmatic subjects with allergic rhinitis also present evidence of airway inflammation, such as an increase in inflammatory cells, although less intense than asthmatic subjects [8]. However, the influence of natural exposure to allergens on maximal bronchoconstrictor response in nonasthmatic subjects with allergic rhinitis remains to be further documented.

Normals and some subjects with mild asthma, contrary to the more severe asthmatics, show a plateau response to inhalation of agonists, indicating limited maximal bronchoconstrictor response [9, 10]. BOONSAWAT *et al.* [11] showed that in subjects with allergic rhinitis and in mild atopic asthmatics, maximal response to methacholine increases after an allergen challenge inducing a late response, and that some of these subjects even lose their plateau. More recently, PRIETO *et al.* [12] reported an increase in maximal bronchoconstrictor response to methacholine in pollen-sensitized asthmatics during natural allergen exposure.

It has been suggested that an increase in airway wall thickness could modify mechanical properties of airways and increase their response to agonists, even with mild or no change in baseline airway calibre [13, 14].

It is possible that airway inflammation increases airway response to agonists by increasing the effect of smooth muscle contraction, or by enhancement of the activity of smooth muscle and interstitial contractile elements [15]. In order to try to evaluate airway wall thickness, we recently used high-resolution chest computed tomography (HRCT) [16]. This technique allowed us to obtain reproducible measurements at the level of large airways, but it made it difficult to adequately evaluate small airway wall thickness.

On the other hand, it has been shown that methacholine-induced changes in forced vital capacity (FVC) may be useful to assess modifications of airway mechanical properties potentially involved with maximal airway response, even in subjects without measurable plateau effect [17]. It would be useful to determine whether changes in FVC correlate with those of maximal bronchoconstrictor response following a proinflammatory stimulus, such as allergen exposure.

The goals of the present study were to determine whether natural antigenic exposure increases the maximal bronchoconstrictor response to methacholine in nonasthmatic subjects, and whether there is a correlation with changes in airway responsiveness to methacholine, and other aspects of airway response, such as airway wall thickness and airway inflammatory features. Our hypothesis was that the increased airway responsiveness induced by natural allergen exposure in nonasthmatic subjects is associated with an increase in maximal bronchoconstrictor response, and that these physiological changes are due to an increase in airway wall thickness following an allergen-induced increase in airway inflammation.

Methods

Subjects

Eleven subjects (8 males and 3 females), nonsmokers, aged 20–36 yrs (mean 28 yrs), with seasonal allergic rhinitis but no past or present history of asthma, took part in a series of studies on the mechanisms of seasonal rhinitis in relation to airway inflammation and volunteered for this evaluation. Upon entering the study, all had a forced expiratory volume in one second (FEV_1) ≥90% and a provocative concentration of methacholine inducing a 20% fall in FEV_1 (PC_{20}) >8 $mg \cdot mL^{-1}$ (geometric mean 25.8 $mg \cdot mL^{-1}$) (table 1).

Table 1. – Subjects' characteristics

Subjects n	11
Age yrs*	28±2
Sex M/F	8/3
Baseline FEV_1 % pred*	112±4
Baseline FVC % pred*	110±3
Baseline PC_{20} $mg \cdot mL^{-1}$†	25.8 (10 to >128)
Positive skin-prick test n	
Tree	9
Grass	9
Ragweed	9
Housedust	7

*: mean±SEM; †: geometric mean, and range in parenthesis. M: male; F: female; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; PC_{20}: provocative concentration producing a 20% decrease in FEV_1; % pred: percentage of predicted.

Skin-prick tests showed wheal and flare responses to tree, grass and/or ragweed pollens in all of the subjects. Seven subjects were also sensitized to housedust, although they had no symptoms, or only minimal ones, out of the pollen season. No medication was taken for at least 3 weeks before the endoscopies in or out of pollen exposure, but short-acting antihistamines and inhaled nasal steroids were allowed after the in-season bronchoscopy, during the week before methacholine challenge. Two subjects used short-acting antihistamines, and 3 used inhaled steroids, both for short periods. However, no inhaled or oral anti-inflammatory drug was used within three months of the study. The study was approved by our local Ethics Committee and all subjects gave a written informed consent.

Study design

The subjects were studied, first during the pollen season, and then out of it, over a period from June to December. For each individual, the pollen season period was estimated according to atmospheric pollen counts obtained in the Quebec area and the types of allergens (pollens) to which the subject was sensitized. The actual pollen counts were not obtained, but an estimate of the period of pollen exposure was used based on historical measurements from the last few years.

Subjects initially had a clinical evaluation, baseline spirometry, a methacholine challenge and allergy skin-prick tests with a battery of 26 common airborne allergens. Every month, during and after the pollen season, a methacholine challenge was obtained, with measurement of the maximal bronchoconstrictor response. Symptoms of rhinoconjunctivitis were recorded for one week before each monthly visit. Out of the pollen season (between mid-September and December, in order to allow a delay of 2 months after the subjects' pollen exposure), and at the peak of symptoms of allergic rhinoconjunctivitis (between June and September), a chest HRCT and a bronchoscopy were performed. The subjects were told to inform by a phone call when their symptoms were at their worst. When their message was received, the two tests were scheduled for within 2 weeks, with the order of their performance depending on hospital appointment availabilities. Since a methacholine inhalation test was performed every month, a measure of airway responsiveness was available from within 2 weeks of both the chest HRCT and the bronchoscopy. Lung volumes were measured by plethysmography out of pollen exposure, at the end of the study, to determine whether there was a correlation between functional residual capacity (FRC) and changes in FVC and FEV_1 following methacholine challenge.

Spirometry and lung volume measurements

Measurements of FEV_1 and FVC were obtained with a spirometer (Vitalograph PFT II) [18], and FRC by body plethysmography, according to the method of DUBOIS *et al*. [19].

Methacholine inhalation tests and maximal bronchoconstrictor response

Airway responsiveness to methacholine, maximal bronchoconstrictor response and the dose-response slope (DRS) were assessed according to methods described previously [20, 21]. Aerosols were generated by a Wright nebulizer at 344 kPa (50 psi) and 8 $L \cdot min^{-1}$ in order to get an output of 0.13 $mL \cdot min^{-1}$. The aerosol was inhaled by tidal breathing, with a face-mask held loosely over the face and a noseclip. Half-doubling concentrations of methacholine (from 0.03 to 128 $mg \cdot mL^{-1}$) were inhaled for 2 min at 5 min intervals, until one of the following occurred: FEV_1 fell ≥50%; a plateau was reached; or the patient felt such discomfort that he wanted to use a bronchodilator. A plateau response was obtained if the FEV_1 was within 5% on at least four successive methacholine half-concentrations. Maximal bronchoconstrictor response ($FEV_{1,max}$) and FVC_{max} were expressed as the maximal percentage fall in FEV_1 ($\Delta FEV_{1,max}$) or in FVC (ΔFVC_{max}) post-methacholine. Airway responsiveness was expressed as the PC_{20} methacholine, obtained from the log dose-response curve. Methacholine DRS was measured as follows: maximal fall in FEV_1 divided by the highest dose of inhaled methacholine or by the first dose of methacholine at which a plateau response was reached. The term "airway response" is used to describe the overall changes in the above parameters.

Symptoms of rhinoconjunctivitis and peak expiratory flow rates (PEFR)

Symptoms of rhinitis (sneezing, nasal obstruction, pruritus and rhinorrhoea) and conjunctivitis (ocular pruritus, swelling, pricking and redness of the eyes) were recorded daily, one week before each monthly visit on a scale of 0 to 5: where 0 indicates no symptoms; 1=very light; 2=light; 3=moderate; 4=severe; and 5=very severe symptoms. The maximum score of the week was retained for each symptom category (rhinitis or conjunctivitis). PEFRs were measured daily with a mini-Wright peak flow meter, on awakening and at bedtime at the same time as symptoms were measured. The best of three consecutive recordings was noted on a diary card.

High-resolution chest tomography

High-resolution chest tomography was performed on a CT Toshiba 900S (Toshiba, Japan). HRCT were always performed at total lung capacity to eliminate variations due to lung volume. Two millimetre thick nonangulated scans were obtained at the level of the intermediary bronchus in inspiration. There was immediate reconstruction with high-spatial frequency algorithm and zooming centred on bronchi. Scans were evaluated at WL-600 and WW 1600. The airways were all measured directly from the CT console with an electronic caliper, with the same window level and window width to ensure that all measurements were carried out under the same conditions. Airway wall thickness was estimated by observing at air-wall-air contrast. The minimal internal diameter of the bronchus was measured. Relative thickness of airways was compared to total diameter (T/D). Interpretation was blind, made by one of the authors (GC), without knowing whether the subject's chest HRCT had been performed in or out of pollen season.

Bronchial biopsies

Biopsy specimens were obtained during a bronchoscopy performed according to published guidelines [22]. We have previously reported the details of our bronchial biopsy sampling technique and analysis [7]. Bronchoscopy was conducted under O_2 5 $L \cdot min^{-1}$ by nasal catheter, following local anaesthesia of the throat, larynx and bronchi with 2 and 4% lidocaine. Vital signs, electrocardiograph and oximetry were recorded throughout the procedure. Using a flexible bronchoscope (Olympus OES 10 fibrescope), 6–8 biopsies were obtained from carinae of the right upper and middle lobes, and segmental bronchi of lower and upper lobes, using conventional forceps.

For light microscopy, routine studies were carried out after fixation in Bouin's solution, and samples were examined using a Leitz microscope. The following light microscopic staining techniques were performed on all sections: haematoxylin and eosin, Masson's trichrome, Giemsa, periodic-acid-Schiff (PAS), PAS with diastase digestion and Weigert. Samples for electron microscopic studies were fixed by immersion in Karnovsky's fluid, washed in cacodylate buffer, osmicated, dehydrated in alcohol and embedded in Epon. Half of the samples were treated "en bloc" with uranyl acetate. Sections were stained with lead citrate and analysed using a Jeol 100 CX electron microscope. All counts and measurements were made blind, without knowledge of the source of the specimen.

Briefly, measurements were made from histological slides using a Leitz microscope combined with a Mocha-Jandel morphometric analytic system. They included basement membrane thickness, percentage of epithelial desquamation, biopsy surface in mm^2, and inflammatory cell count per mm^2 of connective tissue surface, excluding smooth muscle cells and mucous glands. Basement membrane thickness, resulting from collagen deposition underneath the "true" basement membrane and reflecting subepithelial fibrosis, was measured from histological sections stained with Massons' trichrome; as it was focally thickened, nine measurements were made, three in each of thin, intermediate and thickened

areas. The mean of these nine measurements was retained as the basement membrane thickness. The percentage cell desquamation was measured by evaluating the length of the basement membrane denuded of epithelial cells (with or without remaining basal cells) over the total length of the basement membrane.

Analysis

Symptom scores, biopsy cell differentials and PC_{20} were compared in and out of the pollen season by Wilcoxon's or paired t-tests. Biopsy cell differentials were compared by the multivariate test of Hotelling. The seasonal variations (in season minus out of season) of symptom scores, number of cells on bronchial biopsies, the ratio T/D, the slope of the DRS, the methacholine-induced fall in FEV_1, FVC or FEV_1/FVC and the PC_{20} in/out of season, were compared for correlations using Spearman's rank correlation test. The PC_{20} in/out of season refers to the change of PC_{20} in number of double concentrations of methacholine during the pollen season compared to out of the season. Changes in the fall in FVC and in FEV_1 after methacholine were compared to the functional residual capacity, at an out-of-season visit. Results are expressed as mean±SEM.

Results

High-resolution chest tomodensitometry and bronchoscopy were performed within 3.4±2 days of each other at the peak of pollen exposure, and within 2.0±0.7 days out of season. A methacholine challenge was available within 13±2 days of the latest of these two tests during pollen season, and from within 9.1±1.6 days of the most recent set of tests out of season.

Symptoms and pulmonary function tests

Nine subjects developed symptoms of rhinoconjunctivitis during pollen exposure, with mean maximal (±SEM) scores of 2.2±0.4 during the week preceding the "in season" endoscopy, and 1.0±0.4 out of pollen season (p=0.014) (fig. 1a). At the time of enrolment into the study, mean (±SEM) baseline FEV_1 and FVC were 112±4 and 110±3% of the predicted value, respectively. Peak expiratory flows and pre-methacholine FEV_1 values remained within the normal range during the whole study (fig. 1b). Mean FRC measured out of season was 98±9% of the predicted value.

Seasonal changes in methacholine responsiveness

Each subject had from three to seven methacholine challenges during the study (fig. 2). Three subjects had a PC_{20} >128 mg·mL^{-1} on one occasion out of the pollen season, and two of them once during the pollen season. In one subject, all PC_{20} measurements were

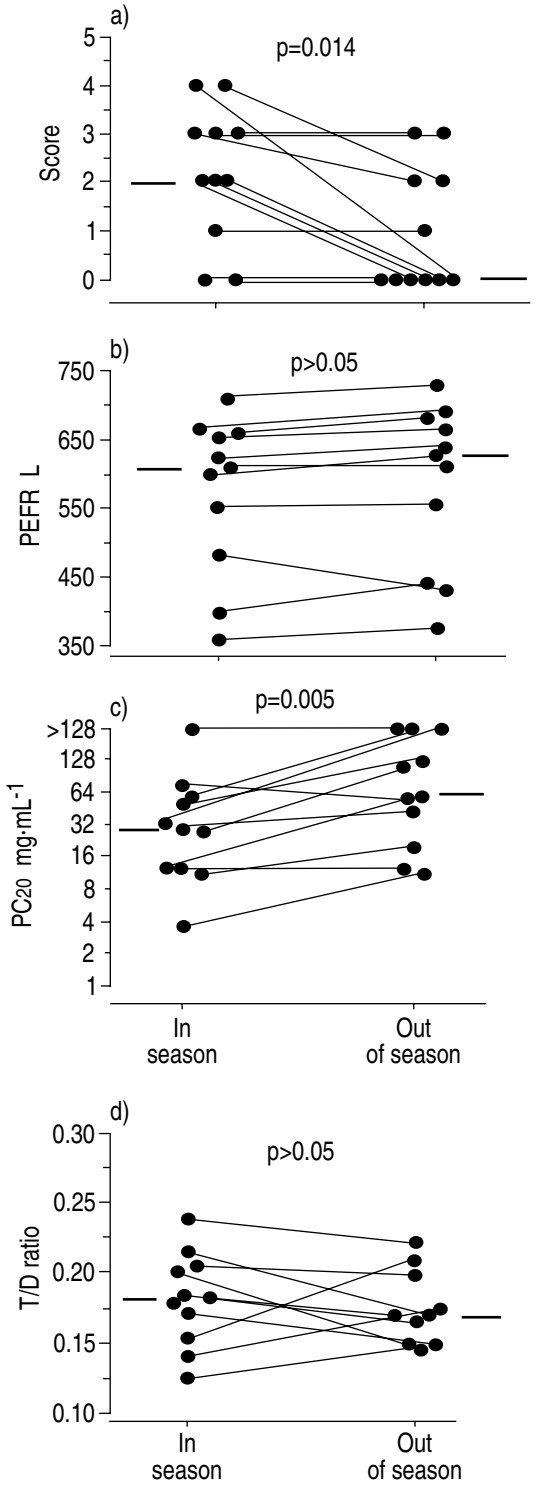

Fig. 1. – Symptoms of rhinitis, peak expiratory flow rates (PEFR), methacholine responsiveness and high-resolution computed chest tomography measured airway wall thickness in and out of the pollen season. Horizontal bar indicates median. PC_{20}: provocative concentration producing a 20% decrease in forced expiratory volume in one second. Horizontal lines indicated median values.

>128 mg·mL^{-1} throughout the study; however, falls in FEV_1 of 18.4, 17.9 and 19.7% were observed with a methacholine concentration of 128 mg·mL^{-1} during the period of pollen exposure. Mean PC_{20} measurements

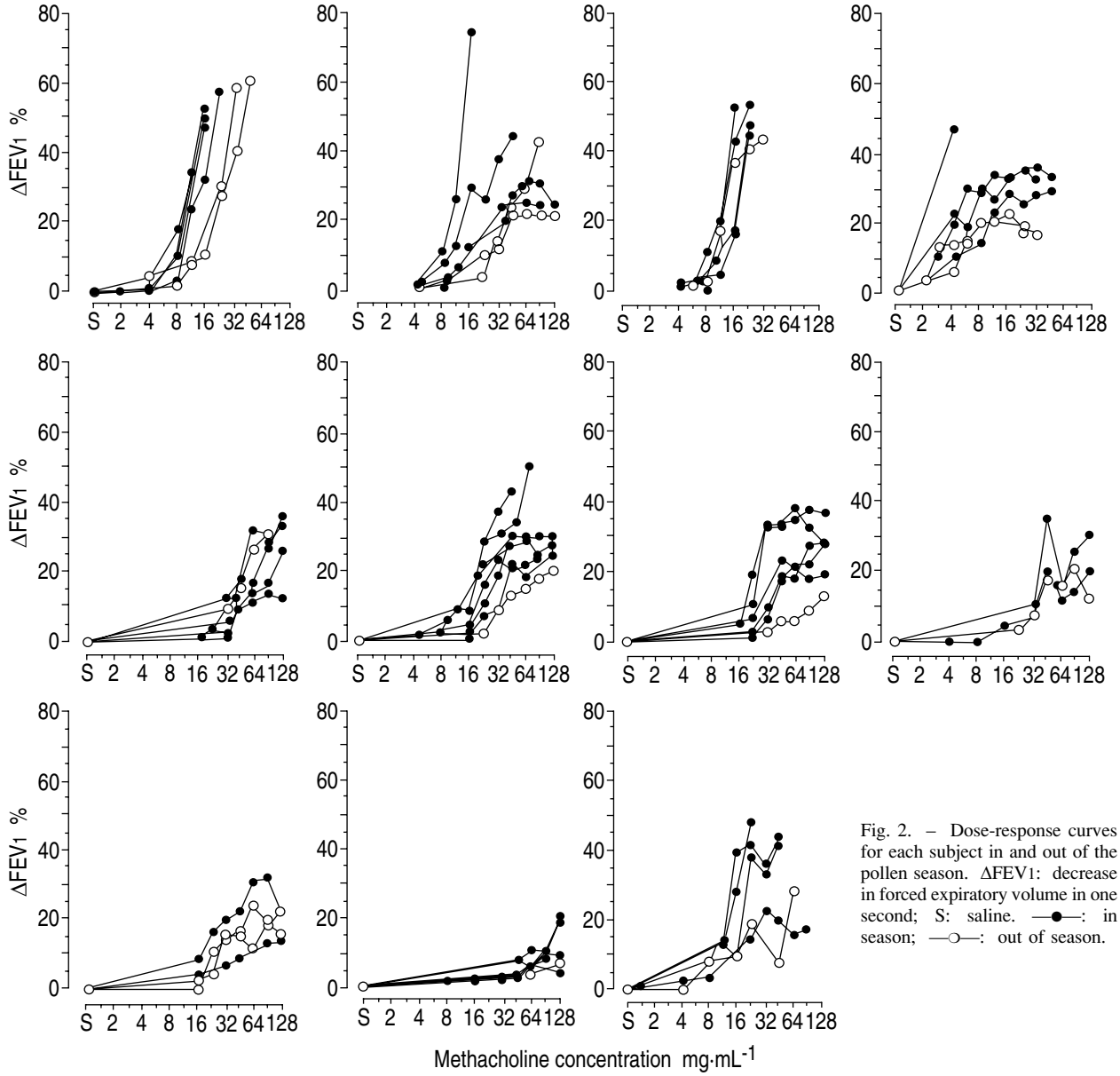

Fig. 2. – Dose-response curves for each subject in and out of the pollen season. ΔFEV1: decrease in forced expiratory volume in one second; S: saline. —●—: in season; —○—: out of season.

closest to the chest tomography were 25.8 mg·mL^{-1} during pollen season and 51.5 mg·mL^{-1} after pollen season (p=0.005) (fig. 1c). Corresponding baseline FEV1 values were 113±5% and 114±5% predicted. Compared with out of season, in season change in PC20 varied from -2.13 to +0.39 doubling concentrations (mean -1.00± 0.28).

Airway wall thickness

There were no overall significant changes in airway wall thickness measured by chest HRCT during or out of the pollen season, with a mean T/D ratio of 0.18± 0.01 and 0.17±0.01, respectively (fig. 1d). In the six subjects for whom a PC20 reduction greater than one doubling concentration of methacholine was observed near the chest HRCT, the mean T/D ratios were 0.20± 0.01 in season and 0.19±0.01 (p>0.05) out of season.

Seasonal changes in maximal bronchoconstrictor response and the DRS

The mean maximal methacholine-induced fall in FEV1 ($\Delta FEV_{1,max}$) obtained in season was 44±5% compared with 25±5% (p=0.002) out of pollen season (fig. 3a). The mean post-methacholine maximal fall in FVC (ΔFVC_{max}) obtained in season was 34±5% compared with 16±4% (p=0.006) out of pollen season (fig. 3b). The mean change in FEV1/FVC ratio at the highest methacholine concentration was not significantly different in the pollen season (0.12±0.02) or out (0.12±0.03) (p>0.05) (fig. 3c). The mean DRS was significantly greater in season (14.1±2.8%/mg·mL^{-1}) compared with out of the pollen season (6.9±1.3%/mg·mL^{-1}; p=0.011) (fig. 3d).

Bronchial biopsy parameters

Bronchial biopsy parameters were not significantly different in or out of the pollen season for the percentage

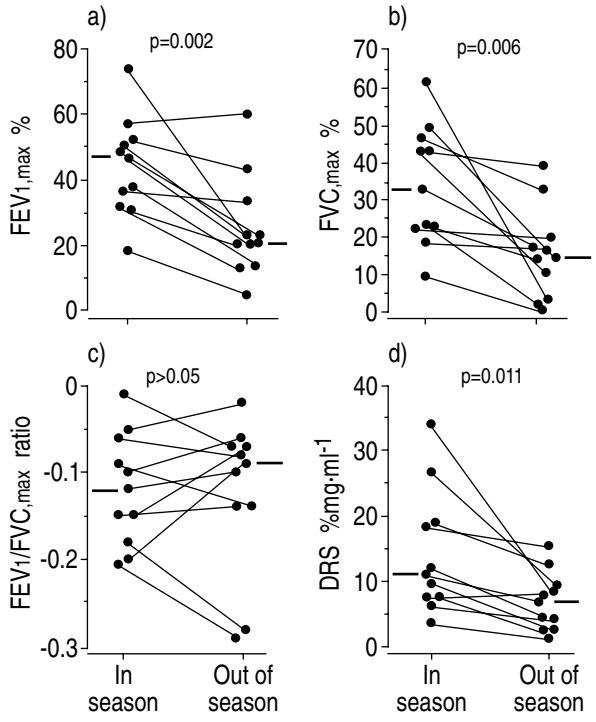

Fig. 3. – Seasonal changes in: a) FEV1; b) FVC; c) FEV1/FVC; and d) DRS in subjects with rhinitis. Horizontal line indicates median. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; DRS: dose-response slope.

Table 2. – Bronchial biopsy parameters in and out of the pollen season

	In season	Out of season
Bronchial epithelium desquamation %+	57±5	57±5
Basement membrane thickness µm+	8.8±0.20	9.2±0.50
Total number of cells*	353.8±62.4	505.0±114.6
Neutrophils	25.8±7.5	27.8±8.9
Eosinophils	25.0±7.7	31.2±11.9
Lymphocytes	239.3±39.1	374.8±77.9
Mast cells	20.1±6.4	14.6±7.0
Plasma cells	11.5±2.6	8.0±1.9
Monocytes and macrophages	12.2±2.1	16.2±4.5

+: mean±SEM; *: cells measured as n·mm^{-2} of connective tissue surface.

desquamation of bronchial epithelium, apparent basement membrane thickness, or for the cell count in the connective tissue (number·mm^{-2} of connective tissue surface): neutrophils, eosinophils, lymphocytes mast cells, plasma cells, monocytes and macrophages, and total number of cells (table 2).

Relationships between seasonal changes in maximal bronchoconstrictor response and airway wall thickness, PC20 or bronchial biopsy findings

There were no significant correlations between the seasonal changes in PC20 and the changes in: 1) methacholine-induced maximal fall in FEV1 or in FEV1/FVC; 2) methacholine-induced maximal fall in FVC; or 3) DRS (p>0.05). The seasonal change in PC20 was not significantly correlated with changes in bronchial biopsy inflammatory cell counts, percentage desquamation of the bronchial epithelium, or the basement membrane thickness.

The seasonal change in methacholine-induced maximal fall in FVC, measured at the visit closest to endoscopies and chest HRCT, was positively correlated with the change in maximal fall in FEV1 (r_s=0.891; p<0.001), and with the change in the DRS (r_s=0.909; p<0.001), but not with the seasonal changes in methacholine-induced FEV1/FVC and T/D, nor with the changes in cell counts, percentage desquamation, and basement membrane thickness on bronchial biopsies (p>0.05).

The change in the DRS was significantly correlated with the difference between the largest maximal fall in FEV1 observed during the pollen season, and the lowest maximal fall in FEV1 observed out of the season (r_s= 0.773; p=0.008). The seasonal change in methacholine-induced maximal fall in FEV1, measured at the visit closest to endoscopies and TDM, was also correlated with the change in the DRS (in/out of the pollen season) (r_s=0.647; p=0.04); it was not correlated with the percentage desquamation and basement membrane thickness on bronchial biopsies, nor with the change in most inflammatory cells, but it was correlated with the change in plasma cells (r_s= -0.674; p=0.032).

There were no significant correlations between the seasonal change in FEV1/FVC and in T/D, DRS, and biopsy parameters. There were no significant correlations between the seasonal variations in T/D and: 1) PC20 in/out season (r_s=0.112; p>0.05); 2) the change in methacholine-induced maximal fall in FEV1 (r_s=-0.491; p>0.05); 3) the change in methacholine-induced maximal fall in FVC (r_s=-0.406; p>0.05); or 4) the change in the DRS.

No significant correlations were found between the seasonal changes in T/D and the following bronchial biopsy parameters: percentage desquamation of bronchial epithelium (r_s=-0.285; p>0.05); basement membrane thickness (r_s=-0.042; p>0.05); or number of mast cells (r_s=0.309; p>0.05). However, the change in T/D was correlated with the seasonal changes in the following bronchial biopsy cell counts: eosinophils (r_s=0.721; p=0.017); neutrophils (r_s=0.697; p=0.024); lymphocytes (r_s=0.721; p=0.017) (fig. 4); plasma cells (r_s=0.842; p=0.0022); and monocytes+macrophages (r_s=0.697; p=0.024).

Relationships between lung volumes and the fall in FVC or FEV1

Baseline FRC measured out of season was not significantly correlated with the methacholine-induced fall in FVC or maximal fall in FEV1 (p>0.05). There was, however, a linear correlation between the FRC and the T/D ratio (r_s=0.721; p=0.03).

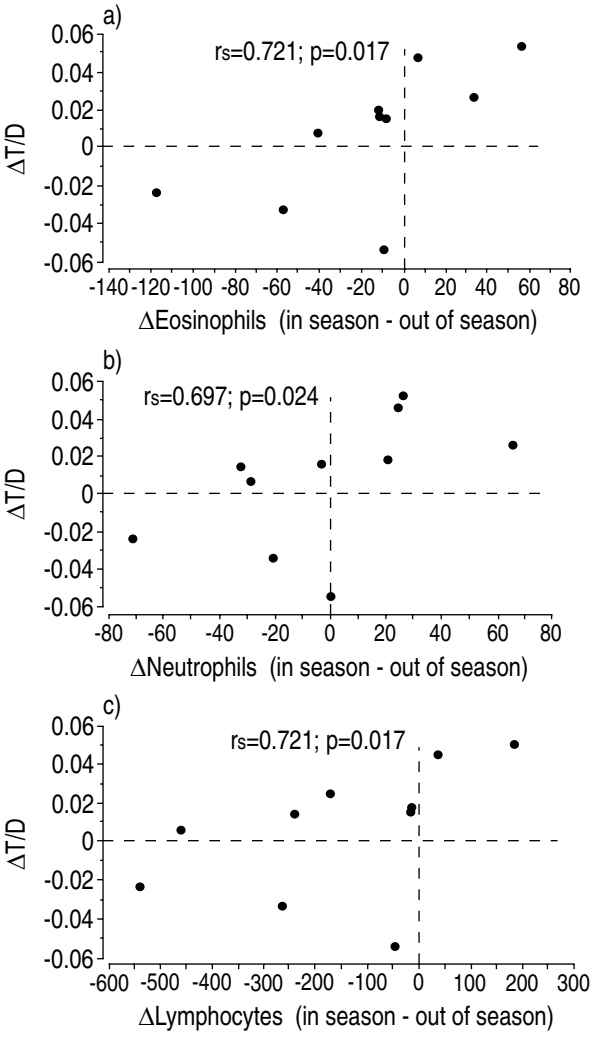

Fig. 4. – Correlations between HRCT-measured airway wall thickness and inflammatory cell counts. A positive correlation was found between seasonal changes in T/D and: a) eosinophil count (p=0.017); b) neutrophil count (p=0.024); or c) lymphocyte count (p=0.017) on bronchial biopsies. HRCT: high resolution computed tomography; ΔT/D: difference in the relative thickness of the airway wall (intermediary bronchus) over its total diameter (in season - out of season).

Discussion

We found that natural exposure to pollen increased airway responsiveness to methacholine, methacholine maximal bronchoconstrictor response and the DRS. Mean large airway wall thickness and inflammatory cell counts on bronchial biopsies were not significantly increased during, compared with after, pollen season.

It has been reported that subjects with allergic rhinitis or mild atopic asthma show an increase in maximal response to methacholine after an allergen challenge-induced late response [11]. This observation has also been reported in subjects with asthma, following natural antigenic exposure [12]. In the present study, we extended these observations and explored the mechanisms of increase of maximal bronchoconstrictor response following natural antigenic exposure.

It has been suggested that factors which may increase airway wall thickness, such as oedema, may heighten bronchial response to agonists [13, 14]. This could be a mechanism by which allergens can increase not only airway responsiveness but also other aspects of airway response, such as maximal bronchoconstrictor response and airway reactivity assessed by the DRS.

Our study, however, suggests some discrepancies between changes in the physiological parameters and either airway inflammatory features or airway wall thickness following allergen exposure. Bronchial biopsies of our subjects with rhinitis showed marked baseline airway inflammation compared with what is found in normal controls. However, there was no significant difference in inflammatory cell counts during and out of the pollen season, whilst we recorded significant increases in symptoms of rhinoconjunctivitis, in airway responsiveness to methacholine, and in maximal bronchoconstrictor response to methacholine.

This discrepancy between the clinical and physiological features observed and the lack of significant change of bronchial biopsy parameters may possibly be explained by different factors. Firstly, it is possible that the state of activation of inflammatory cells was different during pollen exposure, even if their number was not increased. Secondly, the discrepancy may be attributable to spontaneous fluctuations in cellular content of airway wall throughout the year, when there was not enough pollen to influence the inflammatory response. The antigenic exposure seemed, however, quite significant, as it was associated with an increase in airway responsiveness to methacholine up to more than fourfold concentration in some subjects, and caused troublesome symptoms of rhinitis in almost all subjects during pollen exposure. Even in the subject who had an increase in airway responsiveness to methacholine within the asthmatic range during the pollen season, the number of cells in biopsies out of the season as compared with those in season was not significantly changed. It is also unlikely that increased oedema of the airway wall during the pollen season diluted the cellular infiltrate.

Our results suggest that in subjects with allergic rhinitis, as had been reported in asthmatics, changes in airway responsiveness to methacholine and maximal bronchoconstrictor response following natural antigenic exposure are not well correlated [12, 23]. In our study, this was also true for changes in methacholine-induced maximal fall in FVC. This lack of correlation could be partly due to the small number of subjects included in the study. In addition, there seems to be a similar overall pattern of change when we compare $\Delta FEV_{1,max}$ or ΔDRS with airway responsiveness, all parameters increasing during pollen season.

The lack of correlation between measurements of airway response and airway wall thickness measured by HRCT may suggest that the latter is not a major determinant of airway responsiveness maximal bronchoconstrictor response. However, this lack of correlation could be due to the fact that our methods of assessment of airway wall thickness is limited to large airways, mainly the intermediary bronchus. More significant changes

could have occurred at the level of smaller airways, although, as we found previously, chest HRCT is not a very good tool to assess airway wall thickness in peripheral airways [16]. It is possible that the most significant changes in airway wall thickness following allergen challenge occur in small airways, promoting early airway closure following methacholine inhalation, with a secondary early fall in FVC from hyperinflation and increased maximal airway response. Chest HRCT may perhaps fail to detect subtle changes in airway wall thickness, changes that can have a significant influence on airway response.

Among the factors which may influence measurements of airway wall area are airway smooth muscle shortening, lung volume, technical aspects of measurements and other influences, such as changes in airway wall content. It is probable that smooth muscle shortening did not occur in these patients, as they had showed no evidence of bronchoconstriction. Furthermore, to reduce the influence of these factors to a minimum, we always performed HRCT at total lung capacity, as it seemed more reproducible at this lung level, and using the same radiological parameters [16]. It is, however, possible that acute or subacute changes in airway wall area are due to changes in water content.

Our methods may, however, not have been sensitive enough or the changes may have been too small to be detected. The measurement of airway wall thickness in this study was exploratory as, to our knowledge, there were no prior data on measurements of changes in this parameter following natural allergen exposure. We have previously studied stable asthmatic subjects and have found some differences between subgroups of asthmatics when relatively small numbers of patients were studied, as in the present study, and we have had anecdotal evidence that an acute allergen challenge could increase airway wall thickness when measured by HRCT. Therefore, although it was difficult to estimate the statistical power of this exploratory study, we considered that the number of subjects was at least sufficient to observe some trends in changes of radiological parameters.

Finally, increased airway wall thickness may not be the sole factor leading to changes in airway response; it is possible that submucosal tissular changes influence contractile properties of the airways. BRAMLEY *et al*. [24] recently suggested that excessive airway smooth muscle shortening could result from a reduction in tissue elastance following inflammation-induced changes in airway connective tissue matrix elements. This mechanism could explain the increase in maximum bronchoconstrictor response observed in rhinitic as well as asthmatic subjects following allergen exposure.

Although the main site of clinical response in nonasthmatic subjects with allergic rhinitis is in the nose, whereas we measured inflammatory cell numbers in the lower airways, the fact that airway responsiveness increased during the pollen season suggested that this could be related to changes in airway inflammation. The changes in airway responsiveness observed are, however, small, and might also be induced by other factors, such as the amount of methacholine reaching the airways, increased wall permeability, lack of epithelial inhibitory factors, or changes in mucus production. Inflammatory changes, such as airway wall oedema or an increase in cellular infiltrate, could have been too small to be detected by HRTC. As we described previously, even in asthmatic subjects, changes in airway inflammation following natural allergen exposure are of small amplitude [7].

Whilst some authors report a correlation between the inflammatory features on bronchial biopsies and airway response in asthmatics, others do not [25, 26]. This may be due to the heterogeneity of inflammatory and physiological responses among subjects. Other factors could be involved in modifications of airway response, such as the changes in smooth muscle contractile properties, possibly from alterations of subepithelial connective tissue components or airway oedema. If such changes in airway wall water content occurred in our subjects during pollen exposure, it did not translate into an increase in large airway wall thickness. Two of the subjects included in the analysis had no allergic symptoms during the pollen season. They did, however, have positive allergy tests for pollens, and they had their bronchoscopy and high resolution chest tomodensitometry at the peak of their symptoms. The PC_{20} was reduced, in one case, from >128 to 71 $mg \cdot mL^{-1}$, and for the other subject it remained >128 $mg \cdot mL^{-1}$, but with a fall in FEV_1 >19% in season (about 6% out of season). We can reasonably surmise that there was a sufficient exposure to have an effect on the airways, and that the subjects may not have recognized the presence of allergic symptoms or attributed them to a manifestation of rhinitis.

In this study, we looked at the correlation between changes in FVC and methacholine-induced maximal fall in FEV_1. GIBBONS and MACKLEM [17] had previously shown that bronchoconstriction without loss of the plateau effect is associated with a decrease in forced expiratory volume in one second over forced vital capacity (FEV_1/FVC) without decrease in FVC. Subjects whose FVC decreases after methacholine inhalation lose their plateau at progressively increasing lung volume. Therefore, we could estimate that methacholine-induced changes in FVC could be useful to assess modifications of airway mechanical properties potentially involved with maximal airway response, even in subjects without measurable plateau effect.

This study shows that natural antigenic exposure in nonasthmatic subjects can increase not only airway responsiveness to methacholine but also other aspects of airway response, such as maximal bronchoconstrictor response and DRS. Although overall changes were not significant, variations in inflammatory cell counts during allergen exposure were correlated with changes in large airway wall thickness, whilst neither of these two parameters was significantly correlated with changes in airway responsiveness to methacholine. It is, however, possible that allergen-induced inflammatory changes at the level of small airways are more relevant to the observed physiological changes, as suggested by HOGG [27], and this should be further evaluated.

Acknowledgements: The authors are grateful to S. Simard for his help with data analysis, and to L. Schubert for reviewing the manuscript.

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