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Effects of allergen exposure on methacholine and AMP-induced air trapping in pollen-sensitive subjects

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Received 9 September 2010; accepted 22 November 2010

KEYWORDS

Adenosine 5'-
monophosphate;
Allergens;
Air trapping;
Airway responsiveness;
Methacholine

Summary

Background: The effect of pro-inflammatory stimuli on bronchoconstrictor-induced air trapping has not been studied.

Objective: To determine the effect of natural allergen exposure, a pro-inflammatory stimulus, on methacholine- and adenosine 5'-monophosphate (AMP)-induced air trapping.

Methods: Airway responsiveness to methacholine and AMP before and during the pollen season was obtained in 25 subjects with pollen allergy and in 10 healthy controls. The response was expressed by the sensitivity (PC₂₀ value) and by the slope and intercept of the FVC values recorded at each step of the challenge against the corresponding FEV₁ values.

Results: The slope and intercept FVC *versus* FEV₁ values for both methacholine and AMP were significantly higher in subjects with pollen allergy than in healthy controls. In the group with pollen allergy, both methacholine and AMP PC₂₀ values decreased significantly during the pollen season. However, the mean (95% CI) slope FVC *versus* FEV₁ values for methacholine were 1.00 (0.84–1.16) before the pollen season and 0.99 (0.86–1.12, *P* = 0.90) during the pollen season. Similar results were obtained with AMP.

Conclusions: Although the air trapping induced by both methacholine and AMP is significantly greater in subjects with pollen allergy than in healthy controls, natural allergen exposure is associated with a selective increase in airway sensitivity without concomitant changes in bronchoconstrictor-induced air trapping. These findings suggest that the information provided by the bronchoconstrictor-induced change in FEV₁ and FVC is not equivalent and may be complementary.

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Introduction

Airway hyperresponsiveness can be defined as the tendency of the airways to narrow too easily and too much in response to a wide variety of provoking stimuli.¹ Therefore, airway responsiveness can be studied by constructing concentration-response curves to pharmacological bronchoconstrictors, and the response must be characterized by at least two abnormalities: a leftward shift (increased sensitivity) and an upward displacement (excessive airway narrowing) of the dose-response curve. However, during routine bronchoprovocation procedures, the response is generally expressed as the provocation concentration of agonist that caused a decrease in forced expiratory volume in 1 s (FEV₁) of 20% (PC₂₀ or sensitivity), whereas the potential for excessive airway narrowing is only exceptionally identified^{2–4} owing to the inherent risks of provoking an excessive decline in FEV₁.

Clinically and for research purposes, airway responsiveness is measured by bronchial challenge, usually with methacholine or histamine.⁵ Both agonists predominantly induce bronchoconstriction though a direct effect on airway smooth muscle. In contrast, adenosine 5'-monophosphate (AMP) acts indirectly, causing primed mast cell degranulation and the release of pro-inflammatory mediators (histamine and leukotrienes) with subsequent smooth-muscle contraction.⁶ Since mast cells are believed to play a predominant role in atopic asthma, the bronchial response to AMP may be a more direct marker of allergic airway inflammation than direct bronchoconstrictors.^{7,8}

On the other hand, it has long been appreciated that during bronchoconstriction induced by acetylcholine or methacholine, residual volume (RV) increases^{9,10} and forced vital capacity (FVC) decreases¹¹ significantly. The conventional explanation for why the FVC decreases during induced bronchoconstriction is that change in the smooth muscle tone causes an increase in air trapping. Assuming that during induced bronchoconstriction total lung capacity (TLC) remains constant,¹² this air trapping could be measured by the dose-dependent decline in FVC. Furthermore, because lung volume is a major determinant of the bronchoconstrictor response,¹³ it has been suggested that changes in FVC may give relevant additional information as they correlate with the maximal degree of airway narrowing¹⁴ and may reveal information about the underlying asthma pathogenesis that is not apparent from the changes in FEV₁.¹⁵ All these previous investigations were performed with direct bronchoconstrictor agents (histamine, acetylcholine or methacholine), but little information is available on AMP-induced air trapping.¹⁶

There is convincing evidence that, in sensitized subjects with pollen-induced rhinitis or asthma, natural exposure to pollen during the season provokes an increase in airway responsiveness to both methacholine^{17,18} and AMP.^{19,20} However, the influence of natural antigenic exposure on the magnitude of air trapping, as measured by the decrease in FVC remains to be further documented.

The aim of this study was to determine the effect of a pro-inflammatory stimulus, such as allergen exposure, on methacholine- and AMP-induced air trapping. To this end, we performed methacholine and AMP inhalation challenges

in patients with pollen-induced allergic rhinitis associated or not with mild asthma both before and during the pollen season.

Subjects and methods

A total of 35 subjects volunteered for this study. Twenty-five patients with seasonal allergic rhinitis, with or without mild asthma were recruited from the allergy clinic of our institution. Asthma was identified by the presence of asthmatic symptoms plus airway hyperresponsiveness with a methacholine PC₂₀ of less than 8 mg/ml ($n = 8$) if the FEV₁/FVC was 70% or greater or an improvement of the FEV₁ from predicted of 15% or greater after 200 µg of inhaled salbutamol if the FEV₁/FVC was less than 70% ($n = 2$). Subjects with allergic rhinitis were defined as those individuals with a characteristic history of seasonal rhinitis during the pollen season and no history of asthma.

Skin-prick tests showed positive response (≥ 3 mm wheal diameter) to grass and/or *olea europea* and/or *parietaria judaica* pollens in all of the subjects. Seven subjects were also sensitized to perennial allergens, although they had no symptoms out of the pollen season. A control group of 10 healthy nonatopic subjects was also studied. These subjects were recruited from volunteers in the laboratory and among students. Selection criteria for this group included no history of asthma, allergic rhinitis, atopic eczema, or other relevant disease.

All 35 subjects were nonsmokers, had baseline FEV₁ $\geq 70\%$ predicted and FEV₁/FVC $\geq 60\%$, and no subjects had a history of chronic bronchitis, emphysema, or respiratory tract infections during the 4 weeks before the study. The study protocol was approved by the local ethics committee, and written informed consent was obtained from all the participants.

Study design

The study was performed between January and June 2009. Patients were first evaluated between mid-January and the end of February (pre-seasonal evaluation), before the pollen season had begun in Valencia, Spain. During this period, patients had 3 laboratory visits. At the first visit, all patients were evaluated for suitability and spirometry was performed. At each of the next 2 visit (3–7 days apart), spirometry and concentration-response studies with either methacholine or AMP were performed on separate days, with the order of challenge randomized. Patients returned to the laboratory at the height of the pollen season (seasonal evaluation) between April and June. During this period, patients attended 2 laboratory visits. At each visit, spirometry and concentration-response studies with either methacholine or AMP were performed.

The pollen season period was estimated according to atmospheric pollen counts obtained in the Valencia area.²¹ In our region, pollen counts between 27 and 60 grains/m³ for grass and between 30 and 345 grains/m³ for *olea europea* were recorded from May to June. Urticaceae (*Parietaria judaica*) pollen levels were high from the end of March to the beginning of July, with pollen counts between 23 and 59 grains/m³.

Pulmonary function

Spirometry was performed using a calibrated pneumotachograph (Jaeger MasterScope; Erich Jaeger, GmbH; Würzburg, Germany) according to standardized guidelines.²² Baseline FEV₁ and FVC were measured until 3 reproducible recordings differing less than 5% were obtained. Reference values were those of the European Community for Coal and Steel.²³

Inhalation challenge tests

Inhalation provocation tests were performed using a modification of the dosimeter method^{24,25} as previously reported.¹⁶ Short-acting inhaled β_2 -agonists were withheld for at least 6 h before each challenge, oral antihistamines for at least 72 h, and nasal topical corticosteroids for at least 2 weeks. No other asthma or rhinitis medications were used. Methacholine and AMP (Sigma–Aldrich Corp, St Louis, MO) were dissolved freshly in 0.9% saline solution to produce doubling concentration ranges of 0.39–100 mg/ml (2–512 mMol/L) for methacholine and 6.25–1600 mg/ml (18–4608 mMol/L) for AMP. Each solution was administered from a jet nebulizer attached to a breath-activated dosimeter (Mefar MB3; Mefar, Brescia, Italy) with an output of 10 μ l per breath. Patients inhaled the aerosolized methacholine and AMP solutions in 5 inhalations from FRC to TLC through a mouthpiece with the nose clipped. Both FEV₁ and FVC were measured 60–90 s after inhalation of each concentration. The FVC maneuver was continued until a plateau in the volume-time curve was achieved by visual inspection, and the minimum duration of the FVC maneuver was 6 s. The test was interrupted when a 20% decrease in FEV₁ from the post-saline solution administration value was recorded or when the highest concentration was administered.

The PC₂₀ was calculated by means of logarithmic interpolation.²⁶ Occurrence of air trapping during airway narrowing was inferred from the linear regression of the FVC values recorded at each step of the challenge against the corresponding FEV₁ values.²⁷ In this analysis, the slope and intercept of FVC *versus* FEV₁ values quantify the amount of air trapping associated with airway narrowing. A low slope and a high interception indicate the occurrence of airway narrowing with a small or no air trapping and *vice versa* (Fig. 1).

Statistical analysis

The primary outcome of the study was the slope FVC *versus* FEV₁ for methacholine. Secondary outcomes were the intercept FVC *versus* FEV₁ for methacholine, slope and intercept FVC *versus* FEV₁ for AMP, and PC₂₀ values. On the basis of previous data,^{16,27} this study had 90% power to detect a change of 0.23 in the slope FVC *versus* FEV₁ at 0.05 significance.

Data were analysed using a standard statistical software package (Instat for Windows version 3.0; GraphPad Software Inc, San Diego, CA). All PC₂₀ values were log-transformed before analysis and are presented as geometric means with 95% confidence intervals (CIs). All other numerical variables

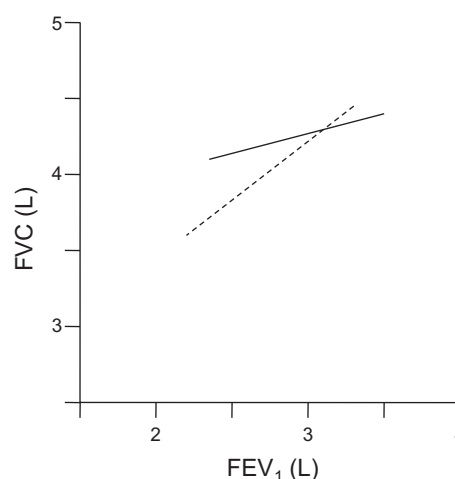


Figure 1 Regression lines between forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁) measured during bronchial challenge with methacholine in a patient with pollen allergy (dashed line) and in a healthy control (solid line). Regression lines were calculated by solving the linear model $y = a + bx$, where a indicates the intercept and b the slope. The slope of the regression line between FVC and FEV₁ was higher and the intercept lower in the patient with pollen allergy due to a greater decline in FVC at each step of FEV₁, indicating, therefore, a greater increase in residual volume and air trapping.

are reported as arithmetic means with 95% CIs. Comparison of the characteristics between groups was performed using unpaired t tests or the Fisher exact test, as appropriate. Changes in PC₂₀, slope and intercept FVC vs FEV₁ values were evaluated using paired t tests within each group and unpaired t tests to compare differences between groups. Correlations were evaluated using the Pearson correlation coefficient; P values are 2-sided, and $P < 0.05$ was considered statistically significant.

Results

The clinical and pulmonary function data for subjects with pollen allergy and healthy volunteers are presented in Table 1. Healthy controls were younger than patients with pollen allergy, but the two groups were similar with regard to sex and pulmonary function. In subjects with pollen allergy, the FEV₁ and FVC decreased from a mean value of 2.97 L (95% CI, 2.62–3.32 L) and 4.09 L (95% CI, 3.68–4.50 L), respectively, out of season to 2.95 L (95% CI, 2.61–3.29 L) and 4.02 L (95% CI, 3.63–4.42 L), respectively, during the pollen season, although the differences were not significant ($P = 0.67$ for FEV₁ and $P = 0.22$ for FVC).

Methacholine and AMP sensitivity in and out of pollen season

Methacholine and AMP PC₂₀ values were significantly lower ($P = 0.012$ for methacholine and $P = 0.014$ for AMP) in subjects with pollen allergy during the pollen season than that in control subjects (Table 2). Out of the pollen season,

Table 1 Subject characteristics^a.

Characteristics	Pollen allergy ^b	Healthy	P
Subjects, No	25	10	
Age, yr	50.7 (47.0–54.5)	32.1 (22.6–41.6)	<0.01
Male/female gender, No	12/13	5/5	0.97
Duration of symptoms, yr	14.5 (10.7–18.3)		
FEV ₁ , % predicted	105.9 (99.6–112.3)	108.6 (99.1–118.1)	0.63
FVC, % predicted	121.3 (116.0–126.6)	114.9 (100.7–128.9)	0.26

Abbreviations: FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity.

^a Data are presented as mean (95% CI) unless otherwise indicated.

^b A total of 15 subjects had allergic rhinitis alone and 10 had concomitant asthma.

methacholine PC₂₀ values were also significantly lower in subjects with pollen allergy than in healthy controls ($P = 0.03$), whereas AMP PC₂₀ values were similar in the two groups ($P = 0.16$, Table 2).

In the overall group of subjects with pollen allergy, AMP PC₂₀ values decreased significantly (Table 2) during the pollen season compared with the values found out of season ($P = 0.007$); the mean (95% CI) reduction was -1.4 doubling concentrations (-0.4 to -2.5 doubling concentrations). Methacholine PC₂₀ values were also lower during the pollen season than out of season, but differences did not reach statistical significance ($P = 0.07$); the mean (95% CI) reduction was -0.5 doubling concentrations (-1.0 – 0.1 doubling concentrations). Although natural pollen exposure was associated with greater changes in AMP PC₂₀ than methacholine PC₂₀ values, differences did not reach statistical significance, the mean difference being 0.9 doubling concentrations (95% CI, -0.2 – 2.1 doubling concentrations, $P = 0.09$).

Methacholine- and AMP-induced air trapping in and out of pollen season

Slope FVC versus FEV₁ values for methacholine and AMP were significantly higher in subjects with pollen allergy than in healthy controls (Table 2) both during the pollen season ($P = 0.0004$ for methacholine and $P = 0.03$ for AMP) (Fig. 2) and out of season ($P = 0.001$ for methacholine and $P = 0.04$ for AMP). Values for intercept FVC versus FEV₁

obtained with either methacholine or AMP were significantly lower in subjects with pollen allergy than in healthy controls (Table 2) both during the pollen season ($P = 0.01$ for methacholine and $P = 0.02$ for AMP) (Fig. 3) and out of season ($P = 0.01$ for methacholine and $P = 0.03$ for AMP).

In the overall group of subjects with pollen allergy, changes in the slope and intercept FVC versus FEV₁ for methacholine during the pollen season were not significant ($P = 0.90$ for the slope and $P = 0.60$ for the intercept, Table 2 and Fig. 4). These changes were also not significant for AMP ($P = 0.74$ for the slope and $P = 0.55$ for the intercept, Table 2 and Fig. 5). In addition, pollen-induced change in the slope and intercept FVC versus FEV₁ was similar for both bronchoconstrictor agents, the mean difference being -0.02 (95% CI, -0.29 – 0.26 , $P = 0.90$) for the slope and -0.29 L (-1.08 – 0.51 L, $P = 0.47$) for the intercept FVC versus FEV₁.

Changes in the slope and intercept FVC versus FEV₁ induced by natural pollen exposure were not significant in both the 15 nonasthmatic subjects with allergic rhinitis and the 10 subjects with mild asthma (Table 3). Pollen-induced changes in either the slope and intercept FVC versus FEV₁ with methacholine were not significantly different between the group with allergic rhinitis alone and the group with mild asthma, the mean difference being 0.10 (95% CI, -0.28 – 0.48 , $P = 0.59$) for the slope and 0.07 L (95% CI, -0.34 – 0.47 , $P = 0.71$) for the intercept. Changes in either the slope and interception FVC versus FEV₁ with AMP were also not significantly different between the two groups, the mean

Table 2 Airway sensitivity and air trapping in subjects with pollen allergy and healthy controls.

Variables	Pollen allergy		Healthy
	Out of season	During season	
PC ₂₀ methacholine, ^b mg/ml	21.5 (10.5–44.2)	15.3 (7.1–33.0)	75.9 (53.5–107.4)
PC ₂₀ AMP, ^b mg/ml	839.4 (481.9–1462.2)	307.6 (137.4–690.2)	1563.1 (1479.2–1600.0)
Slope FVC versus FEV ₁ ^a			
Methacholine	1.00 (0.84–1.16)	0.99 (0.86–1.12)	0.52 (0.30–0.75)
AMP	0.75 (0.59–0.92)	0.72 (0.59–0.85)	0.44 (0.15–0.73)
Intercept FVC versus FEV ₁ , ^a L			
Methacholine	1.20 (0.87–1.54)	1.32 (0.83–1.81)	3.46 (1.87–5.04)
AMP	2.14 (1.58–2.70)	1.97 (1.53–2.41)	3.94 (2.38–5.50)

Abbreviations: AMP = adenosine 5'-monophosphate.

^a Data are given as mean (95% confidence interval).

^b Data are given as geometric mean (95% confidence interval).

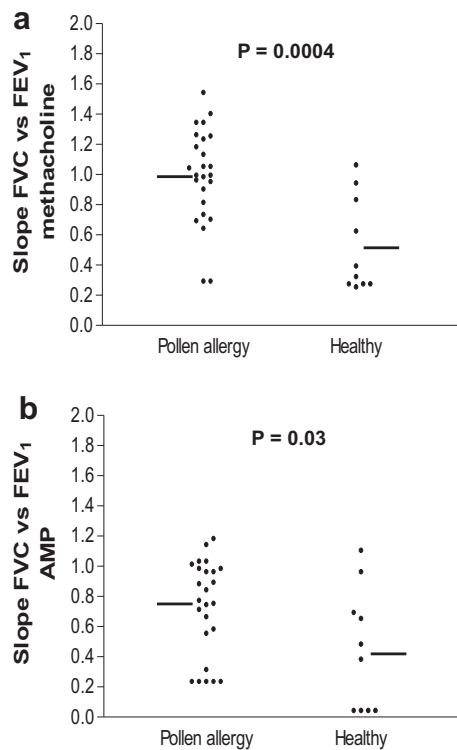


Figure 2 Individual values for the slope of FVC recorded at each step of the challenge against the corresponding FEV₁ values (slope FVC *versus* FEV₁) obtained with (a) methacholine and (b) AMP in subjects with pollen allergy during the pollen season and in healthy controls. Horizontal lines indicate means.

difference being -0.09 (95% CI, -0.49 – 0.31 , $P = 0.63$) for the slope and 0.06 L (95% CI, -1.29 – 1.40 , $P = 0.93$) for the intercept.

Correlations

No significant correlation was found between the slope or intercept FVC *versus* FEV₁ values obtained with methacholine and with AMP. Furthermore, seasonal changes in either the slope or intercept FVC *versus* FEV₁ with methacholine and AMP were not significantly related.

Discussion

The results of the present study have confirmed that in subjects who are pollen-sensitive and have allergic rhinitis associated or not with mild asthma, natural exposure to pollen increases airway sensitivity (decrease in PC₂₀ values) to both methacholine and AMP. The new findings are that, in these pollen-sensitive subjects, natural pollen exposure is not associated with significant modifications in the methacholine- and AMP-induced changes in FVC, and that even during a period of absence of natural allergen exposure, the air trapping induced by each bronchoconstrictor agent, as reflected by changes in FVC, is significantly greater in subjects with pollen allergy than in healthy controls. Furthermore, allergen-induced modifications in air trapping in response to each agonist were not significantly related.

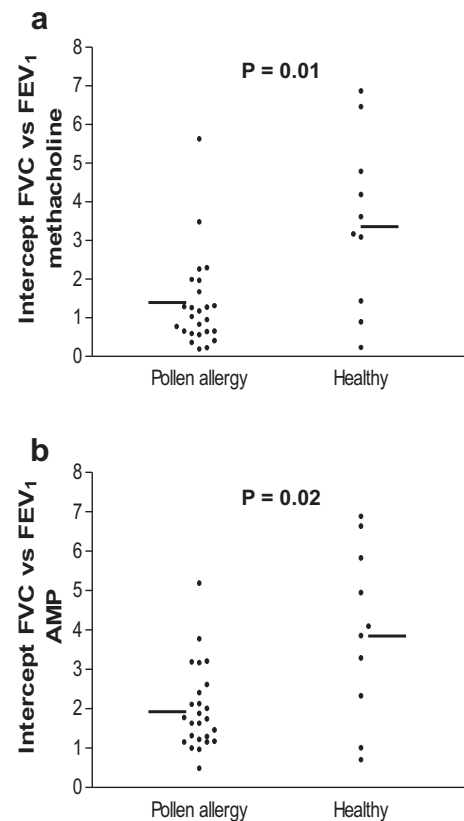


Figure 3 Individual values for the intercept of FVC recorded at each step of the challenge against the corresponding FEV₁ values (intercept FVC *versus* FEV₁) obtained with (a) methacholine and (b) AMP in subjects with pollen allergy during the pollen season and in healthy controls. Horizontal lines indicate means.

These results suggest that airway sensitivity to direct and indirect bronchoconstrictor agents may be increased in response to pro-inflammatory stimuli, such as allergenic exposure, without concomitant modifications in bronchoconstrictor-induced changes in FVC.

Earlier studies have already shown that, in subjects with pollen-induced allergic rhinitis or asthma, natural pollen exposure during the pollen season significantly increases airway sensitivity to methacholine^{17,18} and AMP.^{19,20} The present study confirms and extends these previous observations. In addition, our results demonstrate that, in this group of subjects with pollen allergy, methacholine sensitivity is significantly increased compared with healthy controls, both during the pollen season and out of pollen season. AMP sensitivity is also increased during the period of natural pollen exposure, but AMP PC₂₀ values out of the pollen season are similar to those in healthy controls. These findings are consistent with the suggestion that the response to AMP may reflect acute inflammatory changes in the bronchial mucosa, whereas methacholine responsiveness might be predominantly dependent on more persistent changes on the bronchial tree.⁷

Our results clearly demonstrate that, in pollen-sensitive subjects, natural allergen exposure is not associated with modifications in the methacholine- and AMP-induced air

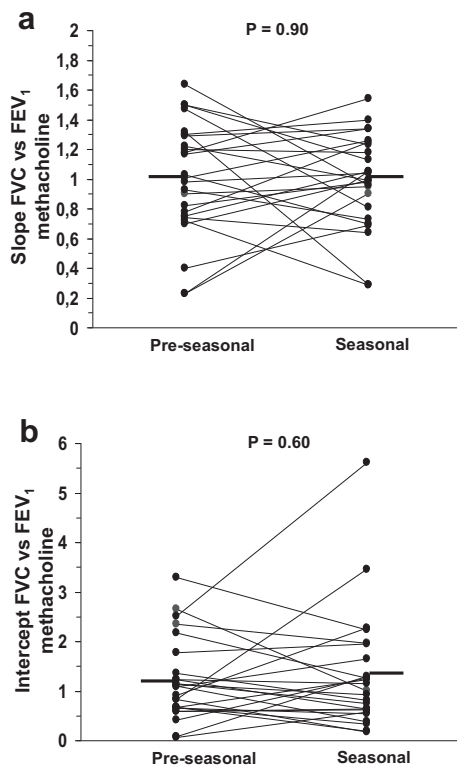


Figure 4 Individual values for the (a) slope FVC versus FEV_1 and (b) intercept FVC versus FEV_1 obtained with methacholine before and during the pollen season. Horizontal lines are arithmetic means.

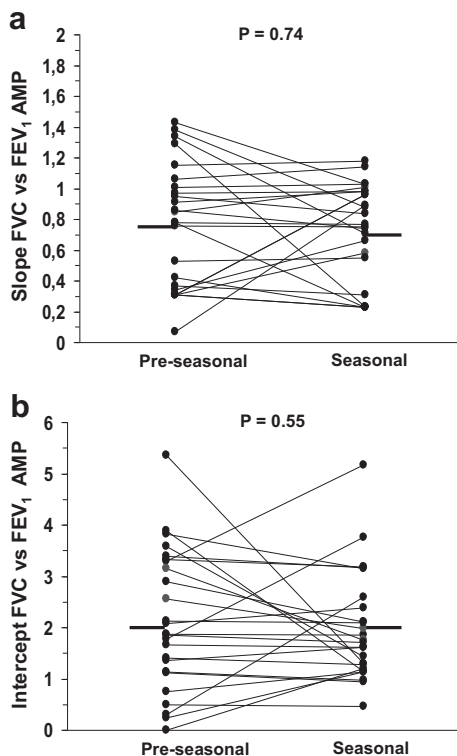


Figure 5 Individual values for the (a) slope FVC versus FEV_1 and (b) intercept FVC versus FEV_1 obtained with AMP before and during the pollen season. Horizontal lines are arithmetic means.

trapping, as reflected by changes in FVC. These findings confirm and extend previous observations²⁸ showing that, in subjects with seasonal allergic rhinitis the mean change in FEV_1/FVC ratio at the highest methacholine concentration was not significantly different during the pollen season and out of season. To the best of our knowledge, however, this is the first study designed to determine the effect of natural allergen exposure on AMP-induced air trapping. Our results clearly demonstrate that natural allergen exposure is not associated with significant modifications in AMP-induced air trapping, as reflected by changes in FVC. It has been postulated that methacholine-induced changes in FVC may provide information regarding excessive airway narrowing¹⁴ and, therefore, the results of the present study seem to be inconsistent with those obtained in several previous investigations reporting that, in pollen-sensitive subjects with allergic rhinitis or asthma, natural pollen exposure is associated with significant increases in the maximal degree of airway narrowing.^{18,29} However, we have recently reported that in pollen-sensitive subjects with asthma or allergic rhinitis, the maximal degree of airway narrowing increases during the pollen season, without concomitant modifications in bronchoconstrictor-induced fall in FVC.³⁰ These results suggest that the change in FVC induced by the bronchoconstrictor agent cannot be used as a surrogate estimation of the maximal degree of airway narrowing. However, our results were obtained in patients with mild asthma or allergic rhinitis without asthma, and it could be argued that the effect of natural allergen exposure on methacholine and AMP-induced changes in FVC might be different in subjects with greater asthma severity. This should be investigated in future studies.

Although we do not believe that our findings can be explained by measurement errors, alternative explanations should be considered for differences in methacholine- and AMP-induced changes in FVC between subjects with pollen allergy and healthy controls. It is known that histamine-induced declines in FVC are related to some extent to the baseline airway calibre.³¹ Thus, the greater effect of each bronchoconstrictor on FVC in subjects with pollen allergy compared with healthy controls might be a consequence of differences in baseline FEV_1 . However, we believe that these results are not affected significantly by this factor because FEV_1 values in both populations were similar. In addition, the age was significantly higher in the group with pollen allergy than in healthy controls. Although the influence of this factor on methacholine- and AMP-induced changes in FVC has not been investigated, we cannot reject that differences in the FVC decline might be caused, at least partially, by this factor.

Why would natural allergen exposure significantly increase methacholine and AMP sensitivity, but not the air trapping induced by each agonist? The response to this question may be only speculative. It has been postulated that inflammatory changes in the airways are more important in modulating the calibre of peripheral than of central airways.¹⁵ Furthermore, in subjects with almost normal lung function, as in the present study, the FEV_1 reflects expiratory flow at high-to-mid lung volumes, which are probably determined by the calibre of relatively large airways, whereas the RV (and the subsequent reduction in FVC) is probably determined by closure of smaller

Table 3 Pollen-induced changes^a in air trapping in subjects with allergic rhinitis and in those with mild asthma.

Variables	Allergic rhinitis		Mild asthma	
	Out of season	During season	Out of season	During season
Slope FVC vs FEV ₁				
Methacholine	0.77 (0.54–1.00)	0.87 (0.70–1.04)	1.16 (0.99–1.33)	1.16 (1.02–1.30)
AMP	0.64 (0.44–0.84)	0.61 (0.45–0.78)	0.80 (0.49–1.12)	0.87 (0.68–1.05)
Intercept FVC vs FEV ₁ , L				
Methacholine	1.98 (1.11–2.85)	1.64 (0.93–2.35)	0.92 (0.49–1.35)	0.85 (0.46–1.25)
AMP	2.60 (1.76–3.45)	2.27 (1.60–2.93)	2.05 (1.20–2.91)	1.67 (1.28–2.05)

For abbreviations see Tables 1 and 2.

^a Data are presented as mean (95% confidence interval).

airways.²⁷ If these inferences are correct, the results of the present study would suggest that the consequences of natural allergen exposure are predominantly expressed in large airways.

In conclusion, this study sheds new information on the way in which allergen exposure induces changes in airway responsiveness of subjects with respiratory allergy. In particular, the findings show that natural allergen exposure is associated with a significant increase in airway sensitivity without concomitant changes in bronchoconstrictor-induced air trapping. These data suggest that the information provided by the agonist-induced change in FEV₁ and FVC is not equivalent and may be complementary.

Acknowledgements

The authors wish to thank Regiduria de Sanidad (Sección de Programas de Salud) from the Ayuntamiento de Valencia for providing data on atmospheric pollen counts, and Diater Laboratorios (Madrid, Spain) for help and collaboration. The technical assistance of Rocio Rojas and Ana Lucas is gratefully acknowledged.

Conflict of interest

None declared.

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