
Lower respiratory tract complications during nasal provocation: nonspecific stimulant or specific allergen?

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Background: Allergic rhinitis (AR) is an allergic inflammatory disease in which allergen exposure leads to the appearance of symptoms in sensitized individuals because of histamine liberation from nasal mucosal mast cells. Comorbidity of this disease with allergic asthma is common. Therefore, the one airway one disease theory has been put forward. Lower respiratory tract provocation tests with both nonspecific (methacholine) and specific stimulants (allergen) have yielded positive results in nonasthmatic patients with AR. However, not enough research is available to demonstrate whether there is a response in the lower respiratory tract during nasal provocation tests (NPTs) performed to evaluate only nasal airway in these patients.

Objectives: To determine if the lower respiratory tract was affected as a result of NPTs with nonspecific and specific stimulants in nonasthmatic patients with AR and to determine the frequency of lower respiratory tract obstruction due to NPT with nonspecific and specific stimulants.

Methods: Thirty-six participants were enrolled in the study between November 2005 and January 2006 (18 AR patients and 18 healthy control subjects). Patients underwent 2 sessions of NPT. The first session was performed with nasal methacholine as a nonspecific stimulant, and the second session was performed with nasal *Olea europaea* extract as a specific stimulant. The control group underwent only nonspecific nasal provocation with methacholine. Basal nasal opening and nasal pressures were evaluated spirometrically by rhinomanometric measurements and basal respiratory function tests in both groups before methacholine nasal provocation. Whether or not nasal provocation was achieved, spirometric measurements were performed in all patients and controls after NPTs.

Results: NPTs with methacholine resulted in a similar frequency of nasal provocation in the patient and control groups ($P = .63$). However, the mean methacholine dose was lower in patients with AR ($P = .049$). There was a decrease in parameters of asthma, including the ratio of forced expiratory volume in 1 second to forced vital capacity ($P = .04$), peak expiratory flow ($P = .01$), and forced expiratory flow between 25% and 75% ($P = .004$), as a result of NPTs with methacholine in the patient group. However, NPTs with allergen did not cause a change in lower respiratory tract obstruction criteria.

Conclusions: Lower respiratory tract obstruction can occur after NPTs with nonspecific stimulants; therefore, tests performed with specific allergens can be regarded as safer.

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INTRODUCTION

Allergic rhinitis (AR) is a disease characterized by allergic inflammation.¹ In AR, histamine liberation from nasal mucosal mast cells follows allergen exposure in sensitive patients and symptoms develop because of the influence of histamine on its nasal mucosal receptors.¹ As is indicated in the Allergic Rhinitis and Its Impact on Asthma (ARIA) consensus statement, AR shows comorbidity with allergic asthma. It is known that nonspecific and specific stimulant exposure to the lower respiratory tract in these patients affects the lower respiratory tract even if asthma symptoms are lacking.^{2–4} This

finding supports the one airway one disease theory.⁵ However, not enough research is available to evaluate the lower respiratory tract effects of diagnostic nasal exposure to nonspecific and/or specific stimulants especially in nonasthmatic patients with AR.

In this study, we aimed to determine if the lower respiratory tract was affected as a result of nasal provocation tests (NPTs) with nonspecific and specific stimulants in nonasthmatic patients with AR. Furthermore, we aimed to observe the frequency of lower respiratory tract obstruction due to NPTs with nonspecific and specific stimulants as a complication and therefore the difference in adverse effects of the 2 types of stimulants.

MATERIALS AND METHODS

Study Participants

Thirty patients with seasonal AR were enrolled in the study during the routine follow-up of seasonal respiratory allergy at our outpatient clinic between November 2005 and January 2006. The diagnosis of seasonal AR was based on history,

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physical examination, and laboratory findings in all patients. Allergic rhinitis was diagnosed on the basis of symptoms, including nasal itching, sneezing, obstruction, and discharge longer than a month during pollen season, and a positive skin prick test (SPT) result according to the ARIA consensus statement.⁶

Participants with seasonal AR who fulfilled the inclusion and exclusion criteria participated in this study. All the patients with seasonal AR selected for this study were sensitive only to *Olea europaea* pollen. The control included individuals without any disease, including allergic states, in their history. Allergic disease was also excluded with negative SPT results in controls. The study was performed in autumn and winter, out of the pollen season, to avoid natural allergen provocation. Before the study, informed consent for the described procedures was obtained from all participants. The study was approved by the local ethics committee of our hospital.

Study inclusion criteria were (1) diagnosis of seasonal AR for at least 1 year before the study; (2) no history of use of oral and/or nasal antihistamines, corticosteroids, or other treatment that may suppress SPT and NPT reactivity such as histamine₂-receptor blockers, decongestants, or antidepressants for at least 2 weeks before SPT and NPT; and (3) normal air flow and pressure values in nasal airway and normal respiratory function test results before NPT. Exclusion criteria were (1) positive allergen SPT results except for *O europaea* and grass or cereal pollen such as mite and mold; (2) allergic asthma diagnosed according to a previous consensus report⁷; (3) an additional disease that can lead to nonspecific nasal and/or bronchial hyperreactivity such as cystic fibrosis, nasal septal deviation, nasal polyposis, or immune deficiency; (4) evidence of abnormal physical findings and viral and/or bacterial infection in the upper and/or lower airway at the time of both the first evaluation, if symptoms of allergic diseases appeared, as seen during the nasal examination; (5) tobacco smoking; and (6) refusal to be enrolled in the study.

Study Design

Patients with seasonal AR underwent 2 NPT sessions. The first session was performed with nasal methacholine as a nonspecific stimulant, and the second session was performed with nasal *O europaea* extract as a specific stimulant. There was an interval of 1 week between the 2 sessions to avoid interaction between the 2 tests. The control group underwent only nonspecific nasal provocation with methacholine.

Basal nasal opening and nasal pressures were evaluated spirometrically by rhinomanometric measurements and basal respiratory function tests in both the patient and control groups before methacholine nasal provocation. Then methacholine was applied to the nasal cavity in increasing doses until clinical and laboratory positive NPT results were obtained. Cases in which 8 mg/mL (4-mg dose) of methacholine failed to produce positive NPT results were considered not to be provoked.⁸ Whether or not nasal provocation was

achieved, spirometric measurements were performed in all patients and controls after NPT.

NPTs with *O europaea* were performed only in the patient group at least a week after this procedure. Basal nasal opening and nasal pressures were remeasured spirometrically by rhinomanometric measurements, and basal respiratory function tests were performed in both the patient and control groups before specific allergen NPTs. If these data were not different from the previous data, then the procedures mentioned were performed.

Specific allergen NPT was performed with *O europaea* extracts in the doses and with the method mentioned herein. Cases in which nasal obstruction could not be achieved with the provocative dose of 4,000 U.RAST/mL were considered to be unprovoked. After allergen-specific NPTs, all patients with seasonal AR underwent spirometric measurements.

Skin Prick Test

The SPT was performed according to European Academy of Allergology and Clinical Immunology guidelines to support the diagnosis of allergy and to determine the allergen or allergens in the etiology of the disease.⁹ Six grasses (*Holcus lanatus*, *Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, *Poa pratensis*, and *Dactylis glomerata*), cereals (*Hordeum vulgare*, *Avena sativa*, *Secale cereale*, and *Triticum sativum*), weeds (*Artemisia vulgaris*, *Urtica dioica*, *Taraxacum vulgare*, and *Plantago lanceolata*), tree pollens (*O europaea*, *Fraxinus excelsior*, *Ulmus scabra*, *Alnus glutinosa*, and *Quercus robur*), and *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* allergen extracts were used in the SPTs. Standardized histamine (1.7 mg/mL) and standard negative prick solution (sodium chloride, 9 mg/mL; phenole, 4 mg/mL; and glycerol, 563 mg/mL) (Allergopharma Ltd, Reinbek, Germany) were used as positive and negative controls, respectively. A wheal that measured 3 mm or more in the areas of histamine and allergen extract application was accepted as a positive SPT result. The SPT reactivity in all study participants was positive only against allergens with seasonal pollination (grass and cereal extracts, weed extract, and tree pollen extract) that lead to seasonal AR.

Nasal Provocation Test

To achieve equilibrium of the nasal mucosa with the environmental conditions of the laboratory, participants were allowed to rest for 15 minutes. Symptom scores and nasal airflow and pressure values were measured with a rhinomanometry device (Betterflow ZAN 100 USB rhinomanometry; ZAN Ferraris Respiratory, Messgeraete GmbH, Northern Bavaria, Germany) and recorded initially as baseline.

Nonspecific NPT With Methacholine

All patients with seasonal AR and control subjects were challenged with methacholine. Methacholine bromide (Sigma, St Louis, MO) was insufflated into each nostril by using metered-dose spray bottles that contained incremental concentrations (0.5, 1, 2, 4, 8, and 16 mg/mL); these insufflations delivered 0.025, 0.5, 1, 2, 4, and 8 mg of methacho-

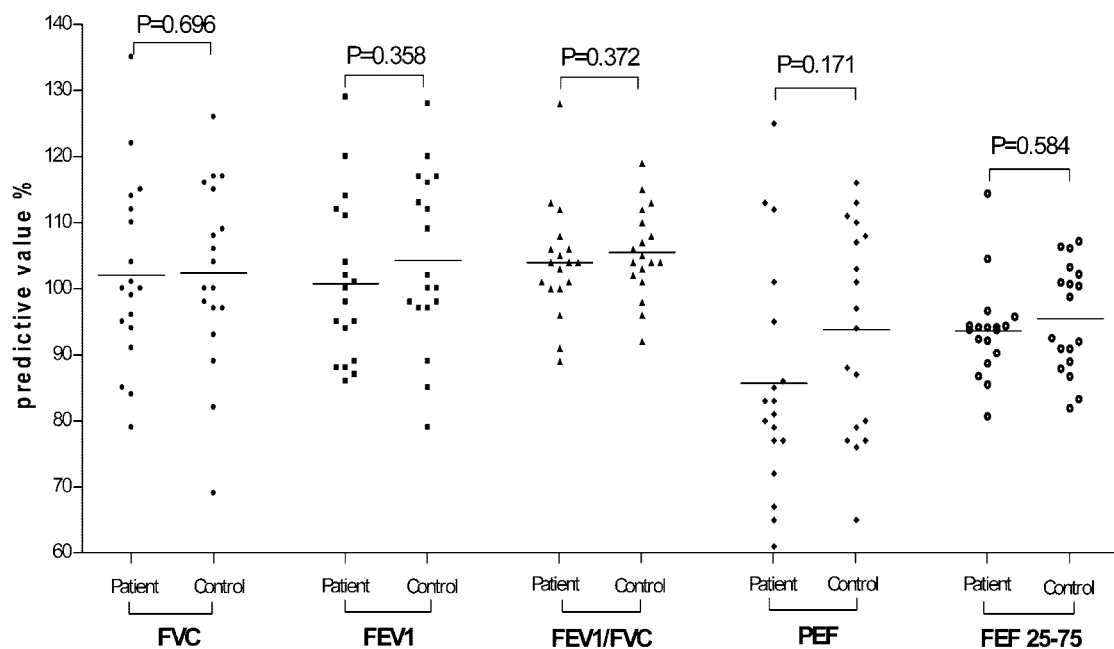


Figure 1. Comparisons of basal respiratory function test results of patients and control subjects guided by the expected predictive respiratory function test results. FEF 25-75 indicates mean forced expiratory flow between 25% and 75%; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow.

line, respectively. At each insufflation, nasal reaction obtained was recorded according to a symptom score technique. Study participants were challenged with incremental doses of the methacholine until a composite symptom score of 6 or more accompanied with a 20% or more decrease in nasal airflow and a 20% or more increase in nasal pressure. The clinical criteria for a positive NPT result included symptom evaluation through use of a semiquantitative method similar to that used by others.^{10,11} Briefly, 3 major nasal symptoms (sneezing, rhinorrhea, and nasal obstruction) were recorded 10 minutes after nasal challenge, according to a scale ranging from 0 (no symptoms) to 3 (severe). A total nasal symptom score was calculated by adding these scores; the maximum possible score was 9 for each participant. A composite symptom score of 6 or more was the clinical criterion for a positive response to the NPT. In addition, a 20% or more decrease in nasal airflow and a 20% or more increase in nasal pressure were the laboratory criteria for a positive response to NPT.

Specific NPT With Allergen

Only patients with seasonal AR were challenged with specific allergen. Soluble *O europaea* pollen extract (Anallergo; Via delgi Alfani, Firenze, Italy) was used for specific NPTs. Specific allergen extract was insufflated into each nostril by using the same metered-dose spray bottles that contained incremental concentrations of allergen (150, 450, 1,350, and 4,000 U.RAST/mL); these insufflations delivered 75, 225, 675, and 2,000 U.RAST of allergen, respectively. The same evidence as for the NPT with methacholine was used as the criterion of positive response for this NPT.

Spirometry

Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), peak expiratory flow (PEF), and mean forced expiratory flow between 25% and 75% (FEF_{25%-75%}) were measured by spirometry (Betterflow ZAN 100 USB spirometry). Spirometry was performed under supervision with the participant in a sitting position and wearing a nose-clip, following the suggestions of the American Thoracic Society.¹² Patient-specific FVC, FEV₁, PEF, FEV₁/FVC, and FEF_{25%-75%} and the percentage of the predicted values based on the participant-specific sex, age, height, and weight were recorded as spirometric values. These spirometric evaluations were made at the beginning of the study and shortly after positive response to the NPT with methacholine and allergen.

Statistical Analysis

The results were analyzed with SPSS statistical software for Windows version 10.0 (SPSS Inc, Chicago, IL) on a personal computer with the Windows XP system. The χ^2 test was performed to evaluate the difference in the NPT response rates between the groups. The Mann-Whitney *U* test was performed to compare the results of rhinomanometric and spirometric measurements and the provocative doses of methacholine and *O europaea* extract between and among the groups.

RESULTS

Thirty-six individuals participated in the study (18 in the patient group and 18 in the control group). Of the 18 participants in the patient group, 12 were female and 6 male. Of the

18 control subjects, 11 were female and 7 male. The mean $\pm$ SD age was 37.94 ± 8.27 years in the patient group and 33.44 ± 8.58 years in the control group. In the patient group, the mean $\pm$ SD age of the disease was 6.69 ± 6.38 years. No statistically significant difference was found in the demographic characteristics of the patient and control groups.

Initial nasal airflow and pressures were in the normal ranges in both the patients with seasonal AR and controls, and no statistically significant difference was found between the 2 groups ($P = .86$ and $P = .84$, respectively). Nasal provocation was achieved in 16 patients with seasonal AR (88.9%) and 15 control subjects (88.3%). No statistically significant difference was found in the rates of nasal provocation with methacholine in the patient and control groups ($P = .63$). The mean $\pm$ SD nasal provocative methacholine total dose was 0.19 ± 0.03 mg in the patient group and

0.43 ± 0.09 mg in the control group. The nasal provocative methacholine dose was significantly lower in the patient group when compared with the control group ($P = .049$).

No statistically significant difference was found between the patient and control groups when basal respiratory function tests were compared, guided by the expected predictive respiratory function tests for their age, sex, height, and weight (Figure 1). When the comparison was performed independently to the predictive respiratory function tests, basal FVC, FEV₁, PEF, and FEF_{25%-75%} values of the patient group were significantly lower than those of the control group (Figure 2).

Evaluation of the respiratory function test results in the patient group after nasal provocation with methacholine revealed that FEV₁/FVC (percent predicted), PEF, and FEF_{25%-75%} values decreased significantly. Results are displayed in Figure 3. Similarly, in the control group, eval-

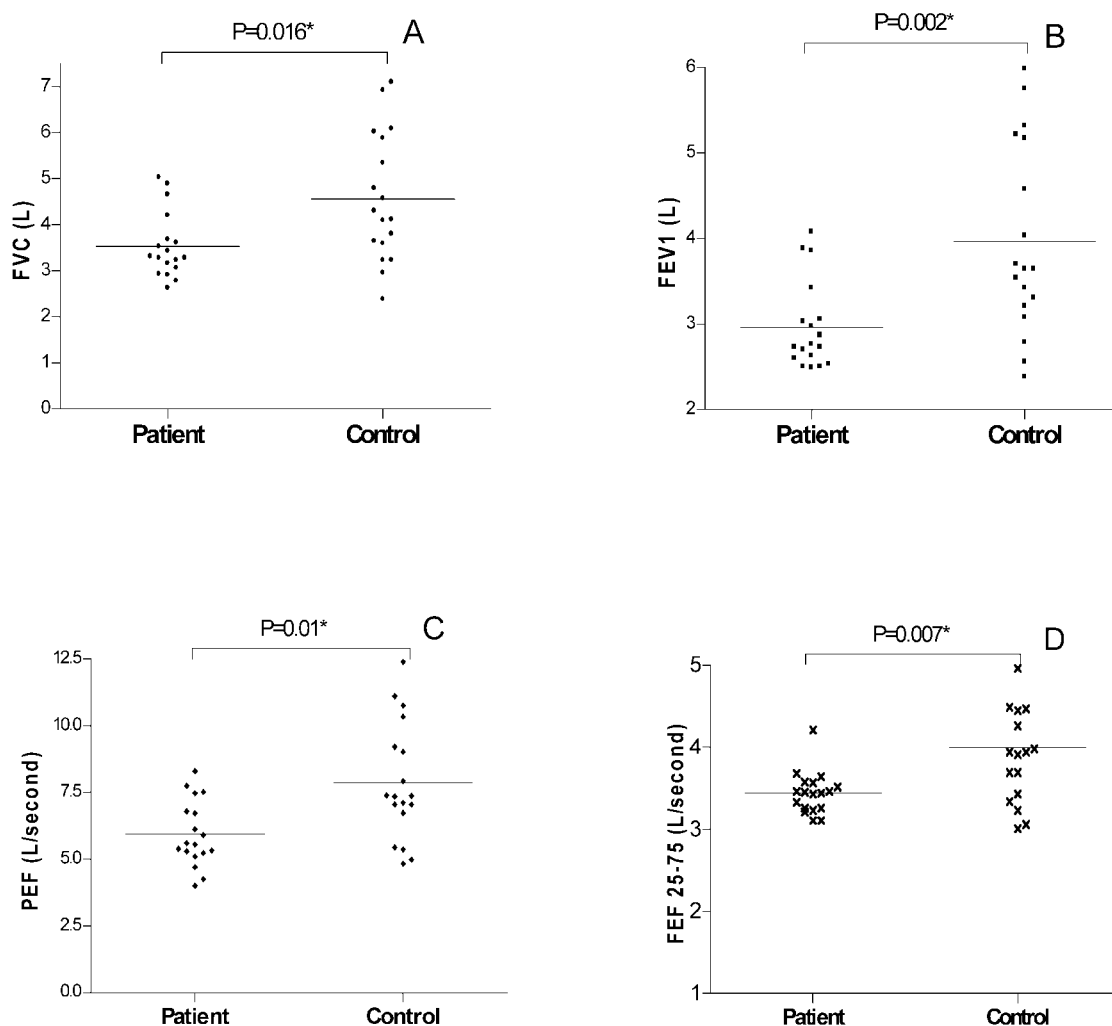


Figure 2. A, Comparison of basal forced vital capacity (FVC) values of patients and control subjects. B, Comparison of basal forced expiratory volume in 1 second (FEV₁) values of patients and control subjects. C, Comparison of basal peak expiratory flow rate (PEF) values of patients and control subjects. D, Comparison of basal mean forced expiratory flow between 25% and 75% (FEF_{25%-75%}) values of patients and control subjects.

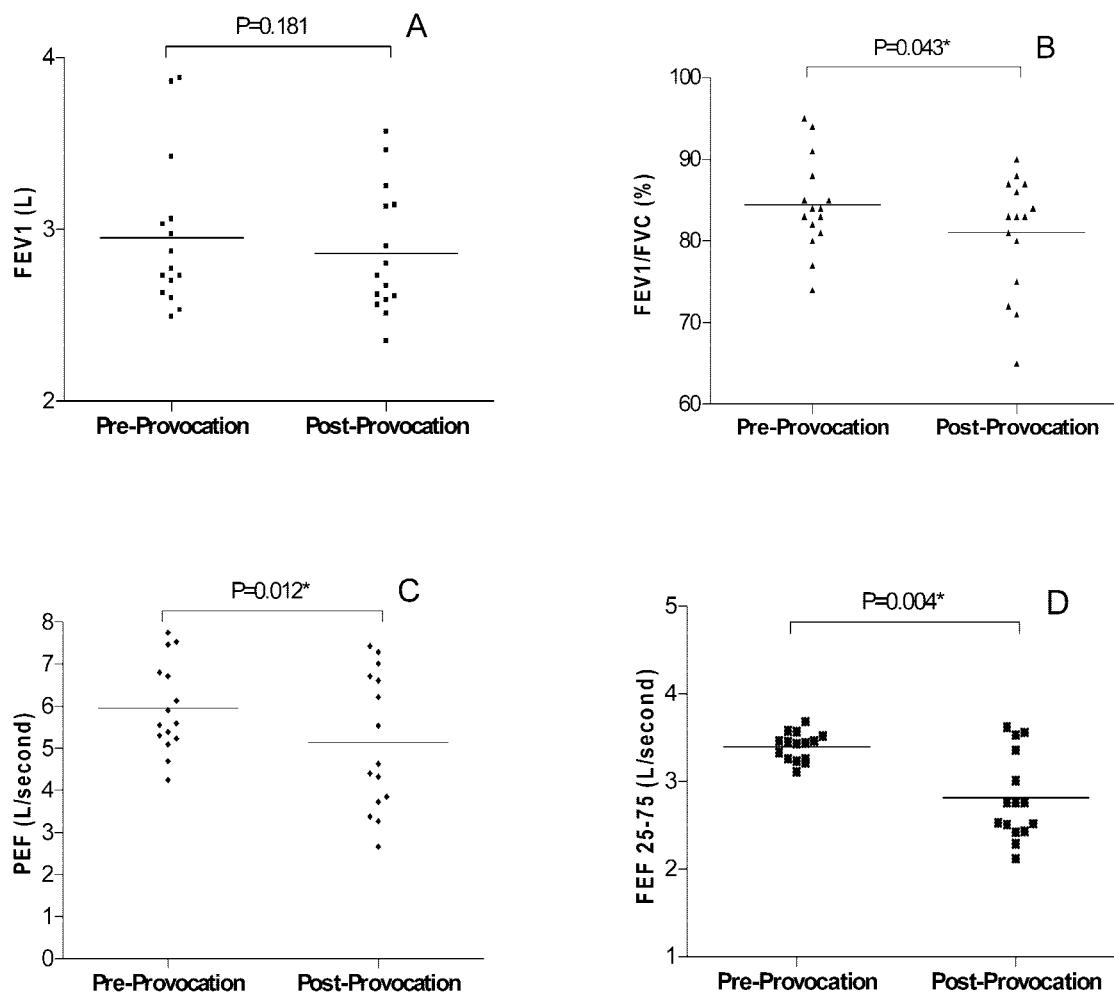


Figure 3. A, Comparison of forced expiratory volume in 1 second (FEV₁) values of study patients before and after provocation by methacholine. B, Comparison of ratio of FEV₁ to forced vital capacity (FVC) (percent predicted) values of study patients before and after provocation by methacholine. C, Comparison of peak expiratory flow (PEF) values of study patients before and after provocation by methacholine. D, Comparison of mean forced expiratory flow between 25% and 75% (FEF_{25%-75%}) values of study patients before and after provocation by methacholine.

uation of the respiratory function test results after nasal provocation with methacholine revealed that FEV₁/FVC (percent predicted) and PEF values decreased significantly. Results are displayed in Figure 4. A statistically significant difference was not detected when respiratory function test results after nasal provocation were compared with the basal values in AR patients with specific sensitivity to *O europaea* allergen who underwent provocation with nasal *O europaea* extract (Figure 5).

DISCUSSION

The patient and control groups can be considered homogeneous and equal, considering that the demographic data did not differ. Therefore, the only and most important difference between the patient and control groups is the presence or absence of allergic disease.

We observed a similar rate of nasal provocation with methacholine in the patient and control groups. This finding was attributable to the nasal obstruction caused by methacholine as a nonspecific stimulant for both groups. Methacholine caused provocation in 88.8% and 83.3% of the participants with seasonal AR and controls, respectively. When the mechanism of action of methacholine is considered, this finding can be explained by the direct stimulation of nasal mucosal glands by methacholine causing hypersecretion.^{13,14} Despite this idea, contradictory theories exist about the pathogenesis of this effect of methacholine. Generally, it is accepted that endogenous histamine is produced only by the stimulation and degranulation of mast cells or basophils, but the secretory activity of nasal glands stimulated by methacholine does not influence the histamine levels in nasal lavage.^{13,14}

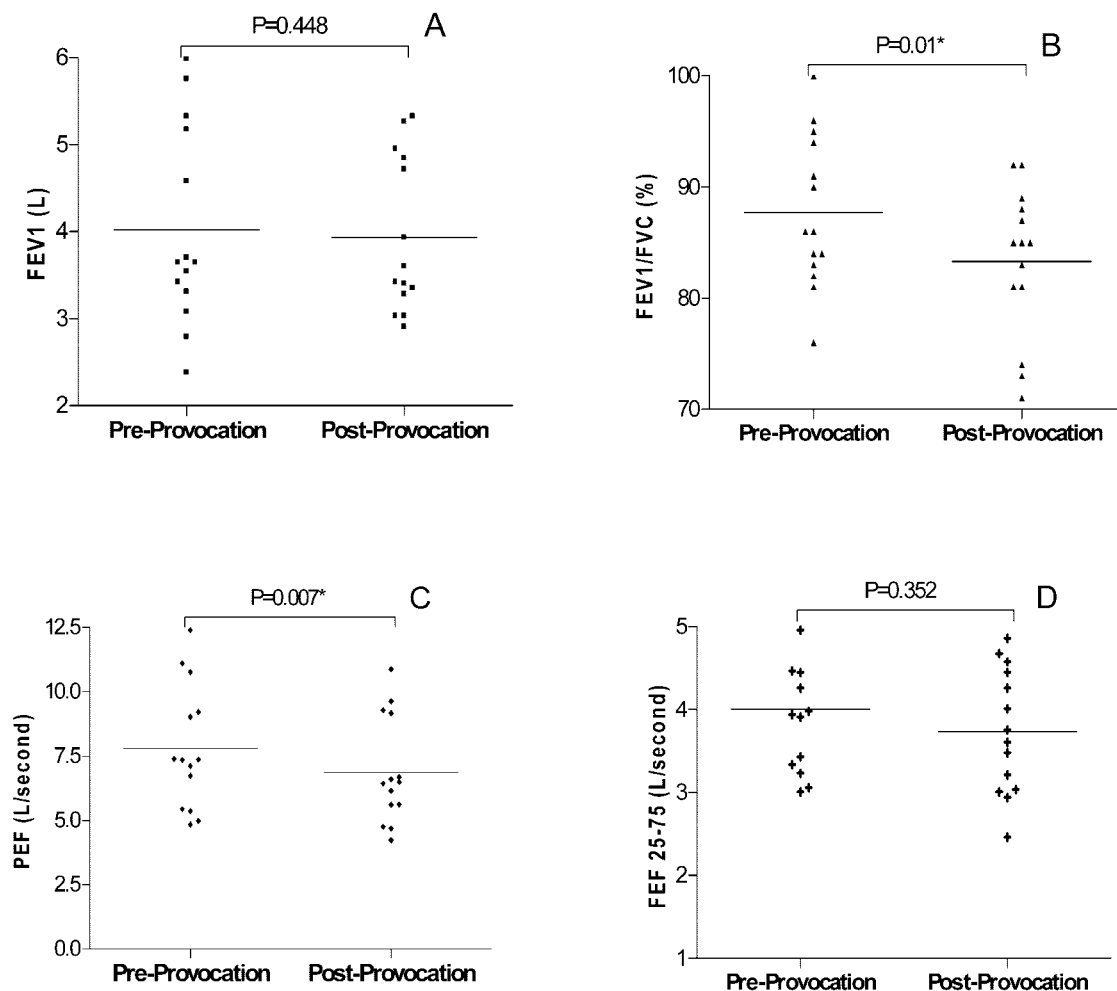


Figure 4. A, Comparison of forced expiratory volume in 1 second (FEV₁) values of control subjects in before and after provocation by methacholine. B, Comparison of the ratio of FEV₁ to forced vital capacity (FVC) (percent predicted) values of control subjects before and after provocation by methacholine. C, Comparison of peak expiratory flow (PEF) values of control subjects before and after provocation by methacholine. D, Comparison of mean forced expiratory flow between 25% and 75% (FEF_{25%-75%}) values of control subjects before and after provocation by methacholine.

Does methacholine cause its effects by secretion of histamine from mast and basophil cells or does it cause hypersecretion and secondary nasal obstruction via its influence on muscarinic receptors? Barody et al¹⁵ did not detect any histamine release in nasal mucosa after stimulation with a 5-mg dose of methacholine in their study. On the contrary, Jacobi et al¹⁶ detected increased histamine levels in nasal lavage with methacholine in patients with allergic rhinitis. Additionally, previous literature reports the presence of tryptase in nasal lavage after specific allergen challenge, but no similar data are available after methacholine.¹⁶⁻²⁰ Therefore, nasal obstruction caused by methacholine is mediated by cholinergic receptors independent of mast cell degranulation. This result explains the similar frequency of nasal obstruction in both groups. Methacholine is a β -methyl derivative of acetylcholine that is resistant to pseudocholines-

terase. It causes bronchoconstriction and nasal obstruction via muscarinic receptors not by aggravation of histamine release from mast cells.²¹ Furthermore, in this study, tryptase was detected in nasal lavage only after allergen challenge not with methacholine provocation. This finding suggests the absence of influence of methacholine on mast cells causing degranulation. Thus, the similar frequency of nasal provocation with methacholine may be attributed to its effects via muscarinic receptors in both groups.

In our study, although the frequency of response to methacholine was similar in patients with seasonal AR and control subjects, patients with seasonal AR were provoked at lower doses of methacholine when compared with the control cases. This result demonstrates that patients with seasonal AR are more sensitive to methacholine when compared with the controls. Therefore, nasal mucosa is hyperreactive to nonspe-

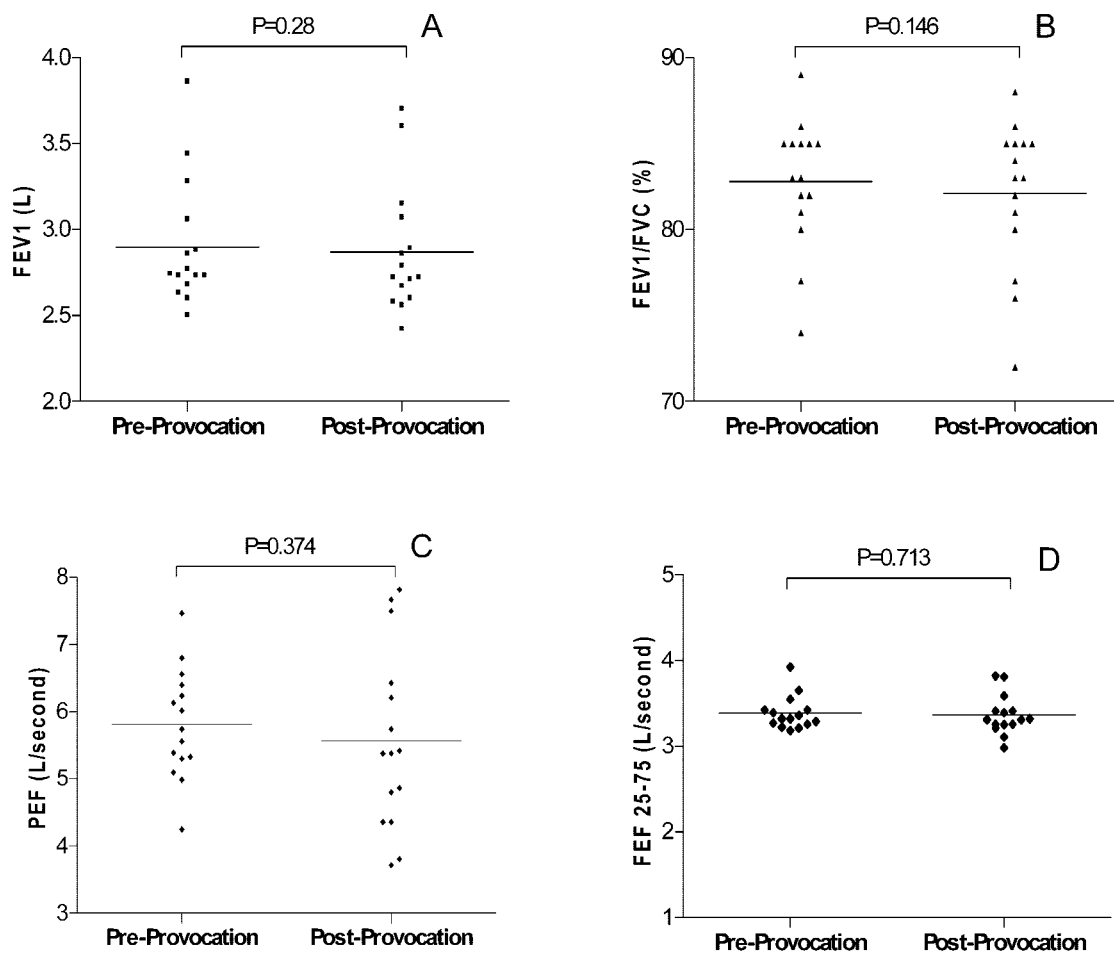


Figure 5. A, Comparison of forced expiratory volume in 1 second (FEV₁) values of study participants before and after provocation by *Olea europaea* extract. B, Comparison of the ratio of FEV₁ to forced vital capacity (FVC) (percent predicted) values of study participants before and after provocation by *O. europaea* extract. C, Comparison of peak expiratory flow (PEF) values of study participants before and after provocation by *O. europaea* extract. D, Comparison of mean forced expiratory flow between 25% and 75% (FEF_{25%-75%}) values of study participants before and after provocation by *O. europaea* extract.

cific stimulants such as methacholine in patients with seasonal AR. Nevertheless, an increase in the secretory response was detected with methacholine and histamine in patients with rhinitis when compared with the patients without rhinitis in a study by Corrado et al.²² In a study by Marquez et al,¹² nasal provocation response to methacholine in nonsymptomatic patients with AR was evaluated. NPTs with methacholine were performed on both patients and healthy controls. The study showed that nonsymptomatic patients with AR responded to methacholine at lower doses than healthy controls. A previous study demonstrated that AR patients were much more sensitive to nonspecific stimulants, such as methacholine, histamine, and phenolamine, than controls.²³ It is evident that all these studies are concordant with our study. As supported by this previous research, patients with seasonal AR respond to nonspecific stimulants at much smaller doses; therefore, patients with seasonal AR have nasal mucosal hyperreactivity.

Our patients with seasonal AR had no history or finding of asthma. Basal respiratory function test results in patients with seasonal AR and control subjects were not different from predictive values, indicating that our patients with seasonal AR did not have manifested asthma. However, comparison of respiratory function test results in patients with seasonal AR and control subjects demonstrated a tendency to develop an obstruction. In other words, although patients with seasonal AR did not have manifest asthma, their respiratory function test results were worse than those of the healthy controls. The reviews by Togias²⁴ and Vignola and Bousquet²⁵ stated that nonasthmatic patients with AR had inflammatory changes in lower airways and hyperreactivity.²⁴ This previous literature explains the decreased respiratory function test results in our patients with seasonal AR when compared with the controls even though they are nonasthmatic. Furthermore, in a study by Cuttitta et al,²⁶ increased bronchial response was identified by bronchial provocation tests with nonspecific stimulants in

nonasthmatic children with AR when compared with healthy children. Evaluation of our study and the previous literature shows that there is an extreme bronchial response to a non-specific stimulant, such as methacholine, even in AR patients without asthma.

Lower airway evaluations after provocation with methacholine nasal challenge demonstrated that valuable indicators of obstruction, namely FEV₁/FVC (percent predicted), PEF, and FEF_{25%-75%} values, deteriorated significantly when compared with the basal values in the patient group. On the other hand, the control group that showed a positive NPT response to methacholine had impairment in FEV₁/FVC (percent predicted) and PEF values. However, the provocative nasal methacholine dose that caused this effect was significantly higher in the control group when compared with the patients with seasonal AR. It was reported previously that lower airway reactivity is seen as a result of NPTs with methacholine, which is a nonspecific stimulant.²⁷ The results of our study are concordant with the results of this study.

Asthma is characterized by an obstruction, and FEV₁, FEV₁/FVC (percent predicted), and PEF are considered to be the main parameters to evaluate bronchial obstruction. Nevertheless, there is an increasing interest in the involvement of small airways in asthma pathogenesis.²⁸ Even though there is no direct parameter capable of assessing small airways, it has been assumed that FEF_{25%-75%} might be considered as a measure of the caliber concerning distal airways.²⁹ Particularly, patients with mild asthma who have a normal FEV₁ may only show impaired FEF_{25%-75%}.³⁰ As also indicated in previous research, lower airways, especially small airways, can be affected in patients with allergic rhinitis by NPT with non-specific stimulants.

We did not detect a significant change from basal values in the respiratory function test results after positive NPT results with *O europaea* allergen in patients with nonasthmatic seasonal AR. These patients had shown lower airway response as a result of NPT with a nonspecific stimulant. However, there was no response in lower airways as a result of allergen-specific NPTs. This finding may be attributable to the local and topical nature of the allergen-specific stimulus and histamine that originates from nasal mucosal mast cells that exert local effects. Additionally, histamine released locally cannot enter the circulation; therefore, it cannot exert systemic effects. Furthermore, histamine is known to be metabolized in a short time after it is liberated with degranulation of mast cells.³¹ This may indicate that the lack of lower airway response after allergen-specific NPT in nonasthmatic patients with seasonal AR may be an expected finding.

In conclusion, lower airway response obtained with use of methacholine that is a nonspecific stimulant was the most important difference between the specific and nonspecific NPTs. This response was not observed after the allergen-specific NPT. Thus, in patients with seasonal AR, allergen-specific NPT has less risk of complication in terms of lower airway effects when compared with the NPT with nonspecific stimulants. Previous literature has reported that lower airway

response can occur after NPTs with methacholine, which may be attributed to systemic absorption of methacholine from nasal mucosa due to spraying during the NPT.²⁷ Although, the ARIA consensus report points out a risk of lower airway sensitivity in nonasthmatic patients with seasonal AR, local histamine liberation as a result of especially allergen-specific NPTs failed to affect lower airways in our patients with seasonal AR.

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