

**DIAGNOSING EXERCISE-INDUCED BRONCHOCONSTRICTION
WITH EUCAPNIC VOLUNTARY HYPERPNEA:
IS ONE TEST ENOUGH?**

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Highlights

1. What is already known about this topic?

Indirect bronchoprovocation testing, specifically eucapnic voluntary hyperpnoea (EVH) is currently recommended for the diagnosis of exercise-induced bronchoconstriction (EIB). However the clinical reproducibility of this methodology has yet to be appropriately established; presenting a potential for misdiagnosis.

2. What does this article add to our knowledge?

This article highlights the need for caution when making a diagnosis of EIB based on a solitary EVH assessment to reduce the potential for misdiagnosis. Indeed when encountering patients with a mild or borderline reduction in lung function post challenge, we recommend that more than one EVH test is performed to exclude or confirm a diagnosis of EIB.

3. How does this study impact current management guidelines?

The application of treatment for EIB in recreational athletes should only be initiated when a diagnosis has been correctly established.

ABSTRACT

Background: In athletic individuals, a secure diagnosis of exercise-induced bronchoconstriction (EIB) is dependent upon objective testing. Indirect bronchoprovocation testing is often employed in this context and eucapnic voluntary hyperpnea (EVH) testing is recommended for this purpose, yet the short-term reproducibility of EVH has yet to be appropriately established. **Objective:** The aim of this study was to evaluate the reproducibility of EVH in a cohort of recreational athletes. **Methods:** A cohort of recreational athletes ($n = 32$) attended the laboratory on two occasions to complete an EVH challenge, separated by a period of 14 or 21 days. Spirometry and impulse oscillometry (IOS) was performed before and following EVH. Training load was maintained between visits. **Results:** Pre-challenge lung function was similar at both visits ($P > 0.05$). No significant difference was observed in maximum change in FEV₁ (Δ FEV_{1max}) post EVH between visits ($P > 0.05$) and test-retest Δ FEV_{1max} was correlated (ICC = 0.81; $r^2 = 0.66$; $P = 0.001$). Poor diagnostic reliability was observed between tests; eleven athletes were diagnosed with EIB (based on Δ FEV_{1max} $\geq 10\%$) at visit 1 and at visit 2. However, only seven athletes were positive at both visits. Whilst there was a small mean difference in Δ FEV_{1max} between tests (-0.6%) there were wide limits of agreement (-10.7 – 9.5%). Likewise, similar results were observed for IOS between visits. **Conclusion:** In a cohort of recreational athletes, EVH demonstrated poor clinical reproducibility for the diagnosis of EIB. These findings highlight a need for caution when confirming or refuting EIB based on a single indirect bronchoprovocation challenge. When encountering patients with mild or borderline EIB, we recommend that more than one EVH test is performed to exclude or confirm a diagnosis.

Key words: Airway dysfunction, Athletes, Eucapnic voluntary hyperpnea, Exercise-induced bronchoconstriction, Indirect bronchoprovocation testing, Reproducibility.

ABBREVIATION LIST

AQUA	Allergy Questionnaire for Athletes
AUC	Area under the curve
A_x	Area of reactance (area integrated from 5Hz to R _F)
BMI	Body mass index
CO₂	Carbon dioxide
EIB	Exercise-induced bronchoconstriction
EVH	Eucapnic voluntary hyperpnea
FEV₁	Forced expiratory volume in one second.
FVC	Forced vital capacity
ICC	Intra-class correlation
IOC-MC	International Olympic Committee-Medical commission
IOS	Impulse oscillometry
LOA	Limits of agreement
MVV	Maximal voluntary ventilation
N₂	Nitrogen
O₂	Oxygen
R	Resistance
R₅	Resistance at 5 Hz
R₂₀	Resistance at 20 Hz
R_F	Resonance frequency

SABA	Short acting beta-2 agonist
SD	Standard deviation
X	Reactance
Z	Impedance
Z₅	Magnitude of impedance at 5 Hz

INTRODUCTION

Exercise-induced bronchoconstriction (EIB) describes the transient airway narrowing that occurs in association with exercise. It is prevalent in both elite and recreational athletes (1) and may impact upon both their respiratory health and athletic performance (2-4). It is now well established that the diagnosis of EIB in athletes should not be based on clinical assessment alone (5-7) since a poor correlation exists between exercise-related symptoms and objective evidence of airway narrowing (8). As a consequence of this dissociation, current guidelines recommend that objective bronchoprovocation testing is employed to secure a diagnosis of EIB (9, 10).

Exercise testing is frequently employed to diagnose EIB. However, whilst an exercise test is ecologically valid and possesses good specificity for diagnosis, it has poor sensitivity and is limited by difficulties in controlling environmental conditions and exercise load and thus, the airway stimulus during a challenge (11). Indeed, poor short-term reproducibility of a laboratory exercise test for the diagnosis of EIB in a non-athletic group has previously been observed, with the conclusion that one test may not be enough to secure a diagnosis (12). Several 'indirect' airway challenges have been developed and recommended as surrogate means for diagnosing EIB. The International Olympic Committee-Medical Commission (IOC-MC) (13) and several other guideline committees strongly endorse the eucapnic voluntary hyperpnea (EVH) challenge in this capacity (9, 10). The EVH challenge uses a compressed, dry gas as the stimulus for provoking bronchoconstriction with controlled hyperpnea.

The EVH test has been used and recommended for screening athletic cohorts for EIB (14, 15); with a positive 'diagnosis' being made from a single provocation test. However, there is sparse data regarding the reproducibility of EVH (16, 17) and the inherent variability in any test has pragmatic implications for evaluating the effectiveness of a diagnostic tool in

screening programmes and interventions. Moreover, the reliability of EVH testing in the population of athletes in whom EVH screening has been advocated (i.e. team squads (15, 18) at amateur or varsity level) has not been established. In this population, the fall in FEV₁ post-challenge can often be borderline (i.e. 10-15% fall) and thus it is important to determine the stability and thus precision of such a result.

We therefore undertook this study with the aim of evaluating the test-retest reproducibility of EVH in a cohort of recreational athletes. We proposed that there would be no difference in airway response following EVH between visits; i.e. EVH would have good test-retest reproducibility. A secondary aim was to evaluate the reproducibility of measures of small airway function utilising impulse oscillometry (IOS) over the same period of time.

MATERIALS AND METHODS

Study population

Thirty-six recreational athletes (training 6 ± 1 hours / week) (male: $n = 31$) from a variety of sporting disciplines; endurance ($n = 22$) (runners, cyclists and triathletes), intermittent high-intensity ($n = 11$) (soccer, rugby and hockey), and non-endurance ($n = 3$) (weightlifters) were recruited to take part in the study. All subjects were non-smokers, free from respiratory, cardiovascular, metabolic and psychiatric disease, and any other significant medical condition except mild asthma. Six subjects had a physician-based diagnosis of mild asthma; all were prescribed a short acting beta-2 agonist (SABA) and two prescribed a regular inhaled corticosteroid.

Experimental design

All subjects were required to attend the laboratory on two occasions separated by a period of either 14 or 21 days. Subjects entered the laboratory 1-hr postprandial at a similar (± 1 h) time of day for each visit. An assessment of respiratory health and evaluation of allergy status was determined via completion of the Allergy Questionnaire for Athletes (AQUA) and aeroallergen skin prick testing. Spirometry and impulse oscillometry (IOS) manoeuvres were performed pre and post an EVH provocation challenge (described below).

Subjects were instructed to maintain their normal diet and physical activity levels throughout the duration of the study and compliance with this regime was assessed by interview. Exclusion occurred if any alteration in training and/or health status/allergen exposure or respiratory tract infection was reported. Subjects were asked to abstain from strenuous physical activity and SABA medication for 24 hrs and inhaled corticosteroid for 72 hrs, respectively, prior to each laboratory visit. All tests and procedures were approved by the

local research ethics committee and all subjects provided written informed consent for experimentation with human subjects.

Atopic Status

Sensitivity to seven common airborne allergens (early blossom tree, mid blossom tree, grass, weed, mould, cat and dust mite) were assessed via skin prick testing (19). Subjects also completed AQUA to assess allergic symptoms (20). An athlete was considered to be allergic if they presented with a positive skin prick test and a positive AQUA score ≥ 5 .

Pulmonary function

Spirometry

Lung function was assessed by forced flow-volume spirometry (MicroLoop ML3535; Cardinal Health, UK) (21). Subjects with airway obstruction at visit 1 ($FEV_1/FVC < 0.7$; FEV_1 % predicted < 0.8) were excluded.

Eucapnic voluntary hyperpnea

A modified version of EVH was performed based on the protocol described previously (17, 22). Briefly, subjects breathed a dry compressed gas mixture (21% O₂, 5% CO₂, balance N₂) at a target ventilation rate equivalent to 85% (baseline $FEV_1 \times 30$) of their predicted maximal voluntary ventilation (MVV) for 6 min. Subjects received real-time visual feedback of their ventilation in order to ensure they maintained the target level. Spirometry was performed in triplicate at baseline and in duplicate at 3, 5, 7, 10 and 15-minute post EVH. Values within 5% were considered acceptable (21). The highest recorded value at each time point was used for analysis. A positive diagnosis for EIB was defined by a fall in FEV_1 of $\geq 10\%$ at two consecutive time points following the EVH challenge in accordance with IOC-MC recommendations (13). Severity of EIB was classified according to the magnitude of reduction in FEV_1 ; mild ($\geq 10\%$ - $< 25\%$), moderate ($\geq 25\%$ - $< 50\%$) or severe ($\geq 50\%$).

Impulse oscillometry technique

Impulse oscillometry measures were obtained (MasterLab IOS System (Erich Jaeger Co., Wurzburg, Germany), in accordance with international recommendations (23), prior to spirometry, immediately pre and post EVH. In brief, subjects performed 30 s of tidal breathing prior to a maximal inspiratory manoeuvre followed by a passive expiratory manoeuvre.

Statistical analysis

It was calculated that a sample size of thirty-two subjects would provide statistical power above 80%, with an alpha level of 0.05. Normally distributed data are expressed as mean (\pm SD) or 95% confidence intervals (CI). Significance was set at $P < 0.05$. A two-sided paired t -test was used to evaluate differences in variables between visits. Pearson's intra-class correlation coefficient (ICC) was calculated using a two-way mixed effect model with the mean single measure reported; ICC ranges from -1 to +1; the latter indicating perfect agreement. Reproducibility was assessed using the method described by Bland and Altman (24) with difference expressed as mean bias (i.e. mean difference between group measures) and upper and lower 95% limits of agreement (LOA). $AUC_{0-15\text{min}}$ was calculated by the trapezoidal method and expressed as percentage fall in FEV_1 . Data was analysed using PASW Statistics 19 statistical software package (SPSS Inc., Version 19, Chicago, IL) and GraphPad Prism Version 5.0 (GraphPad Software, San Diego, California, USA).

RESULTS

Thirty-two athletes (male: $n = 28$) completed the study. One athlete was excluded at the initial visit on the basis of resting airway obstruction and three athletes were excluded due to illness. Subjects' characteristics are presented in Table 1.

Baseline characteristics and pre-challenge lung function

Eighteen athletes (56%) were atopic to skin prick tests and eighteen (56%) had a positive (≥ 5) AQUA questionnaire. Thirteen athletes (41%) with a positive AQUA questionnaire were also atopic and therefore considered allergic. Exercise associated respiratory symptoms (e.g. cough, wheeze, dyspnea etc.) were reported by ten athletes (31%).

At baseline, all pulmonary function measures were within normal predicted limits with no evidence of airflow obstruction (Table 2). Resting spirometric variables were similar between visits except for FEV₁/FVC ($P < 0.01$). All IOS measures were similar between visits.

Short-term reproducibility ($n = 32$)

Similar ventilation rates were achieved at both visits (visit 1: 113 ± 25 L·min⁻¹; visit 2: 119 ± 25 L·min⁻¹) ($P = 0.08$). Target ventilation was achieved at visit 1 (84.6%) and visit 2 (90.4%) respectively. Eleven athletes (34% of cohort) were diagnosed with EIB at visit 1 (mild: $n = 10$; moderate: $n = 1$) and eleven athletes at visit 2 (mild: $n = 9$; moderate: $n = 2$). Seven athletes were positive at visit 1 and visit 2 (endurance athletes $n = 5$; intermittent high-intensity athletes $n = 1$; non-endurance athletes $n = 1$). Seventeen subjects (75%) were negative on both occasions.

In those with a previous physician diagnosis of asthma ($n = 6$), four (66%) were positive at visit 1 (mild: $n = 3$; moderate $n = 1$) and three (50%) at visit 2 (mild: $n = 2$; moderate: $n = 1$). Two were positive on both occasions (mild: $n = 1$; moderate $n = 1$). A diagnosis of asthma

therefore provided a positive and negative predictive value of 33% and 94% respectively, for the diagnosis of EIB at either visit.

Eight athletes with allergy (25%) were positive at visit 1 and six (19%) were positive at visit 2. Four athletes with allergy (13%) were positive on both occasions. Allergy therefore provided a positive and negative predictive value of 67% and 82% respectively, for the diagnosis of EIB at either visit.

No difference was observed in maximum change in FEV₁ (Δ FEV_{1max}) post EVH between visits (visit 1: -9.8%; visit 2: -10.4%) ($P = 0.51$) [95% CI: 6.5-7.8] and test-retest Δ FEV_{1max} was correlated (ICC = 0.81; $r^2 = 0.66$; $P = 0.001$) (Figure 1). Although there was only a small bias in Δ FEV_{1max} between tests (-0.6%) the data exhibited wide limits of agreement (-10.7 - 9.5%) (Figure 2). In addition, no difference was observed for AUC_{0-15 min} % fall in FEV₁ between visit 1 (98.3%) and visit 2 (107.0%) ($P = 0.33$) (Figure 3) and test-retest was correlated (ICC = 0.85; $r^2 = 0.73$; $P = 0.001$). No difference was observed for resting %FEV₁ predicted between the day of the positive test ($98.7 \pm 12.2\%$) and the day of the negative test ($98.8 \pm 14.0\%$) ($P = 0.97$). In addition, no difference was observed when both tests were either negative ($P = 0.66$) or positive ($P = 0.16$).

Non-asthmatic athletes (n = 26)

When excluding mild asthmatics from the analysis ($n = 6$) no difference was observed in maximum change in FEV₁ (Δ FEV_{1max}) post EVH between visits (visit 1: -8.2%; visit 2: -9.0%) ($P = 0.41$) [95% CI: 6.3-8.0] and test-retest Δ FEV_{1max} was correlated (ICC = 0.66; $r^2 = 0.43$; $P = 0.001$). Although there was only a small bias in Δ FEV_{1max} between tests (-0.9%) the data exhibited wide limits of agreement (-11.0 - 9.3%). In addition, no difference was observed for AUC_{0-15 min} % fall in FEV₁ between visit 1 (78.2%) and visit 2 (88.5%) ($P = 0.29$) and test-retest was correlated (ICC = 0.73; $r^2 = 0.53$; $P = 0.001$).

Impulse oscillometry ($n = 32$)

No difference was observed in any of the IOS variables post EVH between visits ($P > 0.05$) (Table 3). Whilst significant correlations were observed between visits for R_5 (ICC = 0.89; $r^2 = 0.80$); R_{20} (ICC = 0.80; $r^2 = 0.64$); X_5 (ICC = 0.62; $r^2 = 0.39$); Z_5 (ICC = 0.89; $r^2 = 0.80$); R_F (ICC = 0.94; $r^2 = 0.89$) and A_X (ICC = 0.94; $r^2 = 0.87$) ($P = 0.001$), all variables exhibited wide limits of agreement.

DISCUSSION

In a cohort of recreational athletes, EVH demonstrates poor diagnostic test-retest reproducibility over a short-term period of assessment. This finding has implications for the clinical utility and application of EVH as a bronchoprovocation challenge in the diagnosis of EIB; specifically when it is utilised in a population of recreationally recreational athletes with mild reductions in lung function following a challenge. Moreover it highlights the need for caution when EVH is employed as a screening tool for EIB in a comparable population.

Reproducibility is important in characterising the diagnostic utility of a test. A high level of short-term, clinically relevant, test-retest reproducibility is vital for clinicians to adopt a diagnostic procedure. This is particularly pertinent in the context of EIB where the differential diagnosis for exercise-associated dyspnea is broad (25) and several conditions mimic transient airflow narrowing, e.g. exercise-induced laryngeal obstruction (26). The implications of over and under-diagnosis of EIB in elite athletes have previously been raised (6). For example, a false-positive/negative diagnosis has implications on health (e.g. unnecessary medication) and performance (e.g. reduced ability) respectively.

Our findings indicate that whilst there was a good correlation in the change in FEV₁ (i.e. fall in lung function) between visits, a Bland-Altman plot, which is a clinically relevant assessment of reproducibility (24), revealed wide limits of agreement. Specifically, in the current study, fifteen athletes (47%) were diagnosed with EIB at either visit 1 (Δ FEV₁ -17.9 ± 9.0%) or visit 2 (Δ FEV₁ -19.0 ± 9.6%) but only seven athletes (22%) were diagnosed positive at both visits. This was despite strict regulation of training and environmental conditions between visits and similar ventilation during the challenge. Therefore when applying a threshold of ≥10% fall in FEV₁ for the diagnosis of EIB (27) the inherent variability in the test between visits raises significant clinical diagnostic implications; particularly in athletes

presenting with mild EIB. Indeed, in the small number of athletes ($n = 4$) with a fall of $\geq 20\%$ in FEV₁, their EVH test remained positive at both visits.

Variability in bronchial responsiveness has been previously observed with direct bronchoprovocation challenges (28-30). However as airway hyper-responsiveness to pharmacologic agents such as methacholine differ from hyperresponsiveness to exercise or osmotic agents (31) and do not infer the presence of inflammatory cells or mediators (32), indirect bronchoprovocation challenges such as exercise or surrogate challenges are more appropriate for the diagnosis of EIB (33).

Previous research evaluating EVH indicates good reproducibility in the maximum fall in FEV₁ following the challenge (16, 17). Argyros and colleagues (17) observed no difference in the degree of bronchoconstriction over a 6-week period following EVH. However, the magnitude of the fall in FEV₁ ($-27 \pm 11\%$) was much greater than in the present study ($-10 \pm 8\%$) and they did not calculate limits of agreement. Stadelmann and colleagues (16) demonstrated similar findings with limits of agreement at 6% for Δ FEV₁ between challenges. Although the average reduction in FEV₁ was equivalent to the current study, the time interval between tests was highly variable and evidence of training load maintenance was not reported. Furthermore, as the population consisted of highly competitive swimmers, a unique pathophysiological basis to the development of EIB (i.e. airway injury) (34) may be apparent and thus differ from the population of the present study. Good reproducibility has previously been established for indirect tests such as the dry powder mannitol challenge in asthmatic children (35) but this again has not been evaluated in a recreationally athletic cohort.

In keeping with our findings, recent evidence has highlighted the potential diagnostic pitfalls of performing a one-off exercise test for the diagnosis of EIB in subjects with possible asthma symptoms. Anderson and colleagues (12) found that 89 of 373 (24%) individuals tested positive following an exercise challenge (based on a $\geq 10\%$ fall in FEV₁) at one of two visits.

Similar findings were apparent in a study of asthmatics 'screened' using a dry gas exercise test with a diagnostic cut-off of 15% fall in FEV₁ (36). The authors concluded that more than one test may be required to exclude or confirm EIB (12). The findings from the present study suggest this recommendation with EVH testing.

The reason for the poor reproducibility is not clear however environmental factors and/or physiological variation impacts significantly on variability (37). Airway calibre fluctuates throughout the day and over short-term periods (38). Airway hyperresponsiveness in elite swimmers assessed by EVH and methacholine has previously been shown to reduce over a short-term period as a result of a reduction in training load (34).

In the current study, no difference was observed in baseline FEV₁ and ventilation achieved during EVH remained unchanged between visits. Moreover, in those athletes who tested positive on only one occasion, no difference was observed in FEV₁ on the day of the positive versus the negative test.

A secondary aim of this study was to evaluate the stability of small airway function determined by impulse oscillometry (IOS). The latter provides an alternative method for measuring and monitoring lung function and is advantageous in that the test is non-effort dependent and potentially evaluates small airway function. The technique has previously been found to be an acceptable measure to supplement spirometry following EVH (39-41). The current study indicates that IOS variables appear to exhibit a similar degree of variability to spirometry following EVH, and as such does not support the notion of superiority when employed to assess airway calibre in this setting.

Methodological considerations / future research

The current study focused on assessing EVH utility in a population of individuals with few symptoms, as per AQUA questionnaire assessment. Moreover, the fall in FEV₁ post-test was

mild with few subjects demonstrating moderate or severe degrees of bronchoconstriction. For example, when athletes were positive at only one visit, an average fall in FEV₁ of 10.5% (range: 4.9 - 19.1%) was observed (i.e. mild). The population of positive individuals could therefore be viewed as 'borderline' positive for a diagnosis of EIB. Accordingly, the findings of the current study are applicable specifically to individuals presenting a mild severity (i.e. $\geq 10\%$ - $< 25\%$ fall in FEV₁) reduction in lung function. This population is clinically relevant and is frequently reported in screening studies (6, 18, 42, 43), but by the nature of the cut-off values are often classified as 'borderline' positive.

The optimum diagnostic 'cut-off' for a diagnosis of EIB is not clear. The current value of $\geq 10\%$ has been recommended since early use of the test and translated from both asthmatic and non-asthmatic populations (17, 22, 44). In the current study, the mean fall in negative athletes following EVH was a $5.7 \pm 2.5\%$ and thus comparable with prior literature (15). In contrast to the proposed bronchodilatory response observed following exercise (45) the 'normal' population mean response to EVH appears to be bronchoconstriction. Indeed, the provocative stimulus to the airways during EVH is highly potent and further work is needed to determine an appropriate cut-off specifically in endurance athletes who generally achieve high ventilation rates.

Conclusion

Eucapnic voluntary hyperpnea is currently recommended as a key bronchoprovocation challenge for the diagnosis of EIB in athletes. This study demonstrates poor diagnostic reproducibility over a short-term period of assessment in recreational athletes, when a cut-off value $\geq 10\%$ fall in FEV₁ is employed. Accordingly, the findings indicate the need for caution when clinicians make a diagnosis based on a solitary EVH assessment and suggest that further assessment and/or surveillance is considered. Therefore when encountering patients with a mild or borderline reduction in lung function post challenge, we recommend that more than

one EVH test is performed to exclude or confirm a diagnosis. This is important to minimise the chance of misdiagnosis and thus mistreatment.

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OP was involved in the conception and design of the study, acquisition, interpretation of data, drafting and critical revision of manuscript; and final approval of the version to be published.

LA was involved in the conception and design of the study, acquisition, interpretation of data, drafting and critical revision of manuscript; and final approval of the version to be published

JH was involved in the conception and design of the study, acquisition, interpretation of data, drafting and critical revision of manuscript; and final approval of the version to be published

GUARANTOR STATEMENT

OP confirms full responsibility for the content of the manuscript, including data and analysis.

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TABLE HEADINGS

Table 1: Subject clinical characteristics.

Definitions of abbreviations: **BMI**, body mass index; **AQUA⁺**, Allergy Questionnaire for Athletes (positive scores above ≥ 5).

Table 2: Baseline pulmonary function.

Definitions of abbreviations: **FEV₁**, Forced expiratory volume in 1st; **FVC**, Forced vital capacity; **R₅**, resistance at 5 Hz; **R₂₀**, resistance at 20 Hz; **X₅**, reactance at 5Hz; **Z₅**, magnitude of impedance at 5 Hz; **R_F**, resonance frequency; **A_X**, area of reactance (area integrated from 5Hz to R_F); **EVH**, eucapnic voluntary hyperpnea.

Table 3: Impulse oscillometry variables post eucapnic voluntary hyperpnoea between visits.

Definitions of abbreviations: **R₅**, resistance at 5 Hz; **R₂₀**, resistance at 20 Hz; **X₅**, reactance at 5Hz; **Z₅**, magnitude of impedance at 5 Hz; **R_F**, resonance frequency; **A_X**, area of reactance (area integrated from 5Hz to R_F).

Table 1.

Variables	
Sex (M:F)	28 : 4
Age (years)	28 ± 7
Height (cm)	178.3 ± 6.5
Weight (kg)	81.4 ± 12.0
BMI (kg•m ⁻²)	25.6 ± 3.3
Training (hrs•wk ⁻¹)	6 ± 1
Physician diagnosed asthma	6/32 (19%)
Self-report symptoms	10/32 (31%)
AQUA ⁺	18/32 (56%)
Atopic	18/32 (56%)

Data presented as Mean ± SD. *n* = 32.

Table 2.

Baseline pulmonary function		
	Visit 1	Visit 2
FEV ₁ (L)	4.45 ± 0.75	4.40 ± 0.76
FEV ₁ (% predicted)	101.5 ± 11.8	100.3 ± 12.6
FVC (L)	5.51 ± 0.83	5.56 ± 0.75
FVC (% predicted)	105.6 ± 11.9	106.8 ± 10.9
FEV ₁ /FVC (%)	80.9 ± 6.0	78.99 ± 7.37**
R ₅ (kPa•L ⁻¹ •s ⁻¹)	0.29 ± 0.09	0.27 ± 0.06
R ₂₀ (kPa•L ⁻¹ •s ⁻¹)	0.25 ± 0.06	0.24 ± 0.05
X ₅ (kPa•L ⁻¹ •s ⁻¹)	-0.10 ± 0.10	-0.08 ± 0.02
Z ₅ (kPa•L ⁻¹ •s ⁻¹)	0.30 ± 0.09	0.29 ± 0.06
R _F (Hz)	11.69 ± 4.44	11.68 ± 3.55
A _X (Hz. kPa•L ⁻¹ •s ⁻¹)	0.32 ± 0.44	0.25 ± 0.17

Data presented as Mean ± SD. *n* = 32, **denotes different from visit 1 (*P*<0.01).

Table 3.

Impulse oscillometry variables		
	Visit 1	Visit 2
R_5 (kPa•L ⁻¹ •s ⁻¹)	0.36 ± 0.11	0.37 ± 0.13
R_{20} (kPa•L ⁻¹ •s ⁻¹)	0.29 ± 0.10	0.27 ± 0.07
X_5 (kPa•L ⁻¹ •s ⁻¹)	-0.11 ± 0.07	-0.13 ± 0.08
Z_5 (kPa•L ⁻¹ •s ⁻¹)	0.38 ± 0.12	0.38 ± 0.15
R_F (Hz)	16.15 ± 5.95	16.02 ± 5.98
A_X (Hz. kPa•L ⁻¹ •s ⁻¹)	0.73 ± 0.87	0.78 ± 1.04

Data presented as Mean ± SD. *n* = 32