

Improved bronchodilator effect of deep inhalation after allergen avoidance in asthmatic children

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Background: In healthy adults and children, deep inhalation (DI) is able to reverse induced bronchoconstriction. This ability is impaired in asthma, but the reasons are still to be elucidated. **Objectives:** This study investigated whether the bronchodilator effect of DI during methacholine-induced bronchoconstriction can be improved by allergen avoidance in asthmatic children, and its relationship with airway inflammation.

Methods: The effect of DI on methacholine-induced bronchoconstriction was studied at the beginning and the end of a 3-month allergen avoidance period at high altitude in 14 allergic asthmatic children who had severe asthma attacks. Changes in airway caliber were inferred from the respiratory resistance (Rrs) measured by a forced oscillation technique. Results were related to the percentage of eosinophils in induced sputum and compared with those obtained in 9 age-matched nonasthmatic children.

Results: In asthmatic subjects, DI had no significant effect on methacholine-induced increase in Rrs before ($P = .62$) but significantly reversed it after ($P < .01$) allergen avoidance. However, the ability of DI to reverse a methacholine-induced increase in Rrs tended to remain less in asthmatic than nonasthmatic children even after allergen avoidance ($P = .05$). In the asthmatic children, the percentage of eosinophils in induced sputum was decreased at the end of the allergen avoidance period ($P < .001$), without any significant correlation between sputum eosinophils and airway responsiveness to methacholine or effect of DI.

Conclusion: A short period of allergen avoidance may improve the ability of DI to reverse induced bronchoconstriction in some asthmatic children. This effect is associated, yet not correlated, with a reduction in airway inflammation. (*J Allergy Clin Immunol* 2004;114:505-11.)

Key words: Respiratory resistance, forced oscillation technique, eosinophils, methacholine challenge

In healthy and mildly asthmatic adults, induced bronchoconstriction is totally or partially reversed by taking a deep inhalation (DI), but this effect is blunted or

Abbreviations used

DI: Deep inhalation
PD₃₀: Dose of methacholine causing a 30% increase of respiratory resistance above baseline
Rrs: Respiratory resistance
Zrs: Input impedance of the respiratory system

even absent in patients with moderate to severe asthma.¹⁻⁴ Mechanisms proposed to explain this impairment in asthma include an involvement of lung periphery in the constrictor response,^{1,5,6} a reduced interdependence between airways and lung parenchyma, and a reduced airway smooth muscle stretching during tidal breathing.⁷ Furthermore, the observations that DI has a bronchoconstrictor effect during spontaneous asthma³ and less bronchodilator effect during late than early asthmatic responses to allergen⁸ suggest a role for airway inflammation in the pathogenesis of the phenomenon. However, studies attempting to relate the effect of DI to the presence of airway inflammatory cells in the airways yielded inconsistent results.^{9,10} One reason for discrepancy among studies may be that the effect of DI likely reflects both chronic (airway wall remodeling) and acute (cell infiltration and edema) inflammatory changes, which may coexist in various combinations within individuals with long-standing asthma.

Allergen avoidance has been shown to be effective in reducing or even ablating asthma symptoms in children with allergic asthma with recurrent severe attacks.^{11,12} This beneficial effect may be already discernible after few months and is associated with a reduction in airway inflammation,¹² suggesting reversal of acute inflammatory changes in subjects with relatively small chronic changes. Therefore, allergen avoidance in children may represent a suitable model to investigate the relationships between airway mechanics and inflammation, avoiding the confounding effects of drug treatments.

In this study, the effect of DI on airway caliber was assessed before and during methacholine-induced bronchoconstriction in a group of asthmatic children (8-14 years) sensitized to house dust mite at the beginning and the end of a 3-month period in an allergen-free environment (1756 m altitude). The results were related to changes in airway inflammation as assessed by sputum eosinophils.

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TABLE I. Anthropometric and functional data of children before allergen avoidance*

Subject	Sex, M/F	Age, y	Height, cm	Body mass index, kg/m ²	FEV ₁ , †% predicted‡	Baseline Rrs, † cm H ₂ O·L ⁻¹ ·s
Asthmatic children						
1	F	14	154	19.8	103	3.1
2	M	14	160	27.3	107	1.8
3	M	14	161	17.7	109	1.5
4	M	14	157	16.6	99	2.9
5	M	8	132	16.1	92	3.1
6	M	9	134	20.0	112	4.4
7	M	11	142	19.9	97	3.7
8	M	14	160	26.6	110	2.1
9	M	9	149	18.9	92	4.1
10	M	12	158	18.0	92	2.5
11	M	9	136	14.1	97	3.2
12	M	13	159	17.4	115	2.5
13	F	14	152	30.3	115	3.7
14	F	13	159	27.7	104	2.4
Mean ± SE		12 ± 0.6	151 ± 3	20.7 ± 5.1	103 ± 2	2.9 ± 0.2
Nonasthmatic children						
15	M	9	130	15.4	98	3
16	M	10	135	18.1	102	3.5
17	M	12	136	19.5	106	4
18	F	13	160	20.7	108	3.5
19	M	14	160	21.9	101	3.1
20	F	7	157	21.1	105	2.8
21	M	13	126	15.7	98	4.2
22	M	12	150	19.1	106	2.6
23	M	10	149	19.8	108	4
Mean ± SE		11.1 ± 0.7	145 ± 4	19.0 ± 2.3	104 ± 1	3.4 ± 0.2

*None of the differences between asthmatic and non-asthmatic children were statistically significant.

†Before allergen avoidance in asthmatic children.

‡From reference 31.

METHODS

Subjects

The study was conducted in 14 asthmatic and 9 age-matched nonasthmatic children (Table I). The asthmatic children were all sensitized to house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) as assessed by a positive skin prick test and RAST (Pharmacia, Uppsala, Sweden). They had intermittent asthma but had required multiple hospitalizations during the last previous years, usually between October and January, because of severe asthmatic attacks. For this reason, they were enrolled in an allergen avoidance program on the basis of a residency at 1756 m above sea level in the Italian Alps. After discharge from the last hospitalization, all children had been treated with inhaled steroids, which were progressively tapered down to 100 µg budesonide or 50 µg fluticasone propionate twice daily and eventually stopped 4 weeks before the allergen avoidance program, which was started in September. None of the children included in the study had a severe asthma attack in the previous 6 months, and their asthma had been well controlled during the last month with short-acting β₂-agonists on demand only.

The study was approved by the local Ethics Committee, and both children and parents gave informed consent.

Study design

The asthmatic children were admitted to the high-altitude hospital (Istituto Pio XII) in September. On admission, they underwent routine clinical examination and spirometry (Biomedin, Padua, Italy) following the American Thoracic Society guidelines.¹³ After 2 days

of observation and a 24-hour abstention from bronchodilators, the eligible children were studied for the effect of DI on respiratory resistance (Rrs) at baseline and during methacholine-induced bronchoconstriction. Three months later (December), all asthmatic children were re-evaluated with an identical protocol. At the beginning and the end of study, sputum was induced and eosinophils were enumerated to assess changes in airway inflammation. The nonasthmatic children were studied on a single occasion, following the same protocol but without sputum induction.

Measurements

A forced oscillation technique (Oscillink; Datalink, Montpellier, France) was used to measure the input impedance of the respiratory system (Zrs). The acquisition period lasted 17 seconds, during which pseudorandom pressure oscillations were applied at the mouth. The children breathed through a mouthpiece connected to a Lilly-type heated pneumotachograph (Hans-Rudolph model 3813; Hans-Rudolph, Kansas City, Mo) connected to a magnetic reluctance differential pressure transducer (Validyne, ±2 hPa; Validyne Engineering, Northridge, Calif). Pressure and flow signals were low-pass filtered at 30.4 Hz by using Bessel-type fifth order (30 dB/octave) analogue filters and digitized simultaneously at a sampling rate of 128 Hz with 12-bit resolution. Fourier coefficients of pressure and flow were computed to obtain Zrs and its real (Re[Z]) and imaginary (Im[Z]) parts. Noisy data were eliminated by filtering out Zrs values lying outside 3 SD above or below the mean. The system was calibrated before each study by using a mechanical analogue consisting of a brass tube filled with bundle of 30 pipelets (20 cm long, 2 mm internal diameter) and araldite glue between pipelets.¹⁴ Measurements were

taken while children were sitting with their head in a natural position, supporting their cheeks and mouth floor by their hands, and wearing a nose clip. Immediately after each acquisition period, the time course of respiratory flow, tidal volume, $Re[Z]$, and $Im[Z]$ were displayed on a computer screen. Recordings showing marked irregular breathing or signs of glottic closure were discarded. Rrs was calculated as the average $Re[Z]$ between 12 and 16 Hz.¹⁵

Respiratory movements were monitored by a respiratory inductive plethysmograph (Respirace; SensorMedics, Yorba Linda, Calif) calibrated by single posture method for 30 to 50 tidal breaths.¹⁶ Thoracic and abdominal signals were processed in a direct current mode and summed to obtain changes in lung volume, continuously displayed. This allowed detection of sighs or nonrequested deep breaths, evaluation of the magnitude of DI when requested, and verification that data acquisition for impedance measurements was started after tidal breathing at functional residual capacity had resumed.

Bronchial challenge and effect of DI. On each study day, Rrs was measured at control both before (baseline) and immediately after a DI. When Rrs had returned to baseline values, the methacholine challenge was started. Solutions of methacholine were prepared by adding distilled water to dry powder methacholine chloride (Laboratorio Farmaceutico Lofarma, Milano, Italy). A dosimeter (Mefar, Brescia, Italy) with 1-second actuations was used to deliver aerosols (10 μ L per actuation) during quiet tidal breathing. The subjects inhaled double-increasing doses of methacholine starting from 10 μ g by using 2 methacholine concentrations (1 and 10 mg/mL) with appropriate numbers of breaths. A 3-minute interval was allowed between dose increments. Measurements of Rrs were taken before and 1 minute after each methacholine dose. When an increase of Rrs >30% from baseline had been achieved, the subject was asked to take a DI, and Rrs was again measured immediately after. The dose of methacholine causing a 30% increase of Rrs above baseline (PD_{30}) was calculated by linear interpolation between the last 2 points of the dose (log)-response curve. At the end of the methacholine challenge, 200 μ g salbutamol was administered by a metered dose inhaler, and subjects left the laboratory only after Rrs had returned to within 10% of baseline value.

Sputum induction and analysis

Sputum was induced by inhalation of hypertonic saline, as described by Pin et al.¹⁷ Briefly, a hypertonic saline solution was nebulized with an ultrasonic nebulizer (UN202; Technomedica, Verona, Italy) for 4 periods of 5 minutes each. The starting concentration was 4% NaCl delivered for a total of 10 minutes, followed by 5% NaCl for a further 10 minutes. Before hypertonic saline inhalation, all subjects were premedicated with inhaled salbutamol (200 μ g) to prevent bronchoconstriction. After each period of nebulization, the children rinsed their mouths and then coughed sputum into a Petri dish. In the laboratory, the Petri dish was placed in a dark background to identify better the sputum portion in the watery saliva. The sputum portion from lower respiratory tract was selected by visual inspection, suspended in dithiothreitol for 30 minutes at 37°C, and then centrifuged at 1300 rpm for 10 minutes at room temperature. The pellets were washed in PBS and suspended in paraformaldehyde 4% at room temperature for 15 minutes to fix the cells. Thereafter, the cells were washed in PBS, centrifuged at 1000 rpm for 5 minutes, suspended in physiological solution, counted, and transferred onto polylysinate slides.

Slides were fixed in alcohol 95%, stained with hematoxylin-eosin, and counted by 2 independent observers who were unaware of the purposes of the study. A differential cell count was obtained by counting at least 400 cells, and the percentage of eosinophils was

calculated after exclusion of epithelial cells to correct for salivary contamination.

Statistical analysis

Baseline characteristics of asthmatic and nonasthmatic children were compared by unpaired Student *t* test. Three-factor and 2-factor repeated-measures ANOVA with Duncan post hoc test was used to assess for significant differences in the effects of DI, methacholine, and allergen avoidance within asthmatic children, and also between asthmatic and nonasthmatic children. The relationships between eosinophils and functional data were tested by Pearson correlation analysis. *P* values <.05 were considered statistically significant. Data are presented as means $\pm$ SDs unless otherwise indicated.

RESULTS

On arrival at the high-altitude hospital, after 1 month of steroid avoidance, all children were asymptomatic with normal lung function (Table I). Baseline Rrs were not significantly different between asthmatic and nonasthmatic children (Table I) either before or after allergen avoidance (*P* = .5 for both comparisons). Within the asthmatic children, baseline Rrs were not significantly different before and after allergen avoidance (*P* = .08). During the whole period of allergen avoidance, none of the children had respiratory infections or required antiasthma drugs.

Effect of DI at control

Before methacholine, Rrs of asthmatic children was increased significantly after DI both in September (from 2.9 ± 0.8 cm H₂O·L⁻¹·s to 3.4 ± 1.1 cm H₂O·L⁻¹·s; *P* < .001) and in December (from 3.1 ± 0.9 cm H₂O·L⁻¹·s to 3.9 ± 1.0 cm H₂O·L⁻¹·s; *P* < .001). The increment of Rrs after DI observed in asthmatics tended to be greater in December than September (*P* = .08) and was significantly greater (*P* < .05) than that observed in nonasthmatic children (from 3.4 ± 0.6 cm H₂O·L⁻¹·s to 3.8 ± 0.6 cm H₂O·L⁻¹·s; *P* < .05; Fig 1, upper panel).

Effect of DI during induced bronchoconstriction

At methacholine endpoint, Rrs of asthmatic children did not change significantly after DI in September (from 4.4 ± 1.3 cm H₂O·L⁻¹·s to 4.3 ± 1.4 cm H₂O·L⁻¹·s; *P* = .62) but decreased significantly in December (from 4.8 ± 1.1 cm H₂O·L⁻¹·s to 4.5 ± 0.7 cm H₂O·L⁻¹·s; *P* < .01). There was a highly significant difference between the changes in Rrs after DI observed in nonasthmatic children (from 4.9 ± 0.8 cm H₂O·L⁻¹·s to 4.1 ± 0.7 cm H₂O·L⁻¹·s; *P* < .001) and in asthmatic children in September (*P* < .001) but only a tendency to be less in December (*P* = .05; Fig 1, lower panel).

Effects of methacholine before and after allergen avoidance

At methacholine endpoint, Rrs was increased in asthmatic children by $50\% \pm 9\%$ in September and $57\% \pm 14\%$ in December. These values were not significantly different from each other (*P* = .79) or from those

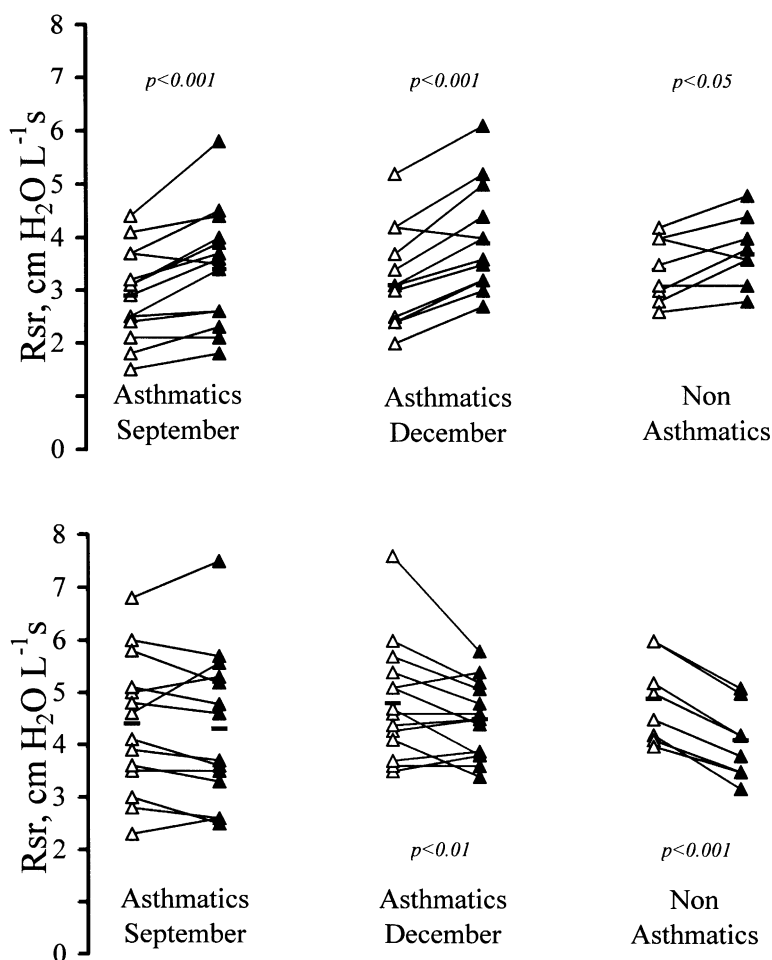


FIG 1. Effects of deep inhalation on Rrs at baseline (upper panel) and during methacholine-induced bronchoconstriction (lower panel) before (September) and after (December) allergen avoidance in 14 asthmatic children and 9 age-matched nonasthmatic children. Open and closed triangles indicate measurements before and after deep inhalation, respectively.

induced in nonasthmatic children ($44\% \pm 7\%$; $P > .09$). Both the maximum dose of methacholine and the Rrs $\log PD_{30}$ were lower in asthmatic than in nonasthmatic children in September, but not in December (Table II).

Airway inflammation

The percentage of eosinophils in induced sputum decreased from 9.5 ± 2.4 in September to 3.8 ± 1.4 in December ($P < .001$), but no significant correlation ($P > .35$) was found between the effects of DI during methacholine-induced bronchoconstriction and the percentage of eosinophils, either before or after allergen avoidance (Fig 2). Similarly, no correlations were found between PD_{30} and percentage of eosinophils ($P > .20$; data not shown).

DISCUSSION

The major findings of this study are that (1) the ability to reverse methacholine-induced bronchoconstriction by DI

was impaired in allergic school children with intermittent asthma with recurrent severe attacks, but (2) it was improved after 3 months of allergen avoidance, and (3) this improvement was associated, even if not statistically correlated, with a reduction of sputum eosinophils.

Most studies attempting to relate pulmonary function with severity of disease or airway inflammation have been largely disappointing, because no or very weak relationships were almost invariably found.¹⁸ The reason for this likely stems from the complexity of factors modulating airway caliber *in vivo*, which may be present in different combinations among individuals. Furthermore, airway responsiveness as assessed by the dose-response curve to any constrictor substance is difficult to interpret in mechanistic terms, because it is determined by the interaction between agonist and receptors, and also by the balance between the airway smooth muscle force and the opposing forces.¹⁹ In the last decade, the effect of DI on airway caliber has been proposed as a new approach to study airway mechanics. It has been reported that DI has

TABLE II. Airway responsiveness to methacholine

	Maximum dose, μg (log)	Rrs PD ₃₀ , μg (log)
Nonasthmatic subjects	2.581 $\pm$ 0.210	2.326 $\pm$ 0.176
Asthmatic subjects		
September	2.153 $\pm$ 0.514*	1.831 $\pm$ 0.496*
December	2.302 $\pm$ 0.524	2.073 $\pm$ 0.533

* $P < .05$ versus nonasthmatic group. Data are means $\pm$ SDs.

a bronchodilator effect during induced bronchoconstriction in normal subjects,^{1,2} which is reduced or even absent in asthmatic subjects, depending on the severity of disease.^{3,4} Moreover, DI has a bronchoconstrictor effect in patients with severe asthma during spontaneous attacks.³ These differences have been related to the involvement of lung periphery⁵ or to the uncoupling between airways and lung parenchyma,²⁰ which may be related to the severity of disease and to airway inflammation. Indeed, DI had a bronchoconstrictor effect in chronically obstructed subjects with more airway inflammation⁹ and a blunted bronchodilator effect in asthmatics during late-phase reaction to inhaled allergen.⁸ By contrast, multiple DIs were unable to shift the dose-response curve to methacholine in asthmatic subjects, and this was not related to the presence of inflammatory cells in the airways.¹⁰ These inconsistencies between studies attempting to relate effect of DI and airway inflammation may also be explained by large interindividual differences.

The allergen avoidance program developed for children with recurrent severe asthma attacks provided the opportunity to investigate the effect of DI on airway mechanics in a longitudinal fashion over a relatively short period, thus allowing minimization of the confounding effects of interindividual differences in chronic airway remodeling and pharmacologic treatments.

The findings of the current study show that asthmatic children, unlike nonasthmatic children of same age, were unable to dilate their constricted airways consistently, and this difference was highly significant before allergen avoidance. This inability might have contributed to the severity of asthmatic attacks, because the lack of bronchodilator effect of DI was found to be associated with excessive airway narrowing.²¹ After 3 months of allergen avoidance, the ability to dilate methacholine-constricted airways by DI was improved in asthmatic children, although it tended to remain less than in nonasthmatic children, because a bronchodilator effect was still absent in several of them. It can be speculated that in some children, the length of the allergen avoidance period was insufficient to recover a bronchodilator effect of DI, but it cannot be excluded that some asthmatic children were unable to dilate constricted airways independent of allergen exposure, and this difference was undetected in this study because of the small sample size. Nonetheless, the airway responsiveness, as assessed by PD₃₀, tended to decrease with allergen avoidance to values not significantly different from those for nonasthmatic children.

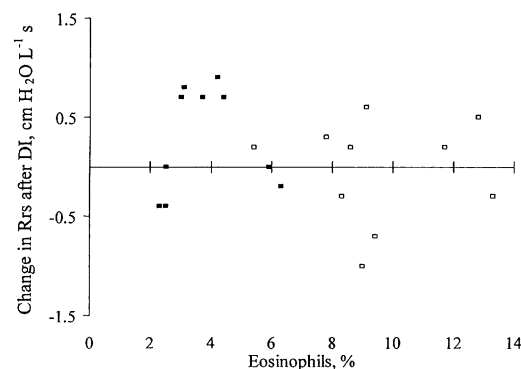


FIG 2. Individual changes of Rrs after DI during methacholine-induced bronchoconstriction versus induced sputum eosinophils before (open squares) and after (closed squares) allergen avoidance.

These changes were associated with a significant reduction of eosinophilic airway inflammation, but neither PD₃₀ nor the bronchodilator effect of DI was correlated with eosinophil numbers either before or after allergen avoidance. Therefore, the effects of allergen avoidance on airway inflammation and lung mechanics seem to reflect 2 parallel events without a direct causal relationship. The ensuing discussion analyzes some mechanisms that may explain an improved bronchodilator effect of DI after allergen avoidance and are likely to be independent of the numbers of inflammatory cells in the airways.

According to the theory proposed by Froeb and Mead,²² the bronchodilator effect of DI is the result of a prevalence of airway over parenchymal hysteresis. As airway hysteresis increases with airway smooth muscle contraction,²³ the bronchodilator effect of DI should increase with induced bronchoconstriction. The similarity of increase in Rrs induced by methacholine before and after allergen avoidance may be taken as suggesting similar levels of airway smooth muscle activation. However, it cannot be excluded that a thicker airway mucosa before than after allergen avoidance might have reduced the amount of methacholine reaching the airway smooth muscle, thus causing less airway smooth muscle activation and shortening. If this were the case, the similarity of response to methacholine before and after allergen avoidance could be explained by the effect of wall thickness on the luminal area,²⁴ and the blunted bronchodilator effect of DI before allergen avoidance by a decreased airway smooth muscle hysteresivity.⁷ The lack of relationship between changes in the effect of DI and airway inflammation, as assessed from sputum analysis, makes this mechanism less likely than others. An alternative explanation for a different effect of DI is that the response of the more peripheral airways, which may result in an increase of parenchymal hysteresis,⁵ was less after than before allergen avoidance. However, measurement of pulmonary hysteresis requires positioning of an esophageal balloon, which was not feasible in the current study.

The magnitude of response to constrictor stimuli is limited by the force of interdependence between airways and lung parenchyma.²⁰ Had allergen avoidance reduced peribronchial inflammation, the external load acting on airway smooth muscle would have increased, thus attenuating airway responsiveness and reducing airway smooth muscle stiffness as a result of an increase in tidal stretching.⁷ This mechanism would explain the improved bronchodilator effect of DI after allergen avoidance, independent of the relative hysteresis of airways and lung parenchyma.

Subepithelial fibrosis may be present in childhood asthma,^{25,26} thus increasing airway wall stiffness. Had allergen avoidance reduced subepithelial fibrosis, the airways would have become easier to distend with the increase in lung volume, thus explaining the improved bronchodilator effect of DI. However, a thinner basement membrane would have reduced the internal load of airway smooth muscle,²⁷ thus resulting in a greater responsiveness to methacholine.²⁸

In this study, Rrs was measured by a forced oscillation technique that required 17 seconds of data acquisition. *In vitro*, allergen sensitization with serum from asthmatic subjects increases the velocity of airway smooth muscle shortening.²⁹ It is therefore possible that the differences in the effect of DI between nonasthmatic and asthmatic children, as well as between before and after allergen avoidance, observed in the current study reflect a different time course of renarrowing after DI.

Finally, some possibilities exist that altitude by itself could have changed airway physiology in different ways. First, tidal volume might have been transiently increased in the first few days before full adaptation to altitude was achieved. The effect of breathing at increased mean lung volume could explain the tendency for Rrs to be less at the beginning than at the end of study. However, an increased tidal volume would have increased tidal stretching of airway smooth muscle, which should have resulted in a greater bronchodilator effect of DI, which was not the case in this study. Second, altitude might have increased extravascular water in the lung before full adaptation. In theory, peribronchial edema would result in a decreased stretching of airways by the surrounding lung parenchyma, thus possibly explaining the inability of DI to dilate constricted airways before allergen avoidance. However, liquid loading has been shown to increase pulmonary resistance and airway responsiveness to methacholine without altering the bronchodilator effect of DI during induced bronchoconstriction.³⁰

In conclusion, whatever the underlying mechanism, the current study demonstrates that in asthmatic children with severe asthmatic attacks, a 3-month period of allergen avoidance can improve the ability of DI to dilate constricted airways. It can be speculated that continuous allergen exposure may impair the ability to dilate constricted airways with DI and contribute to the severity of asthma attacks in allergic children.

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