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Towards restoring the physiological protection against airway narrowing in asthma : take a deep breath!

Slats, A.M.

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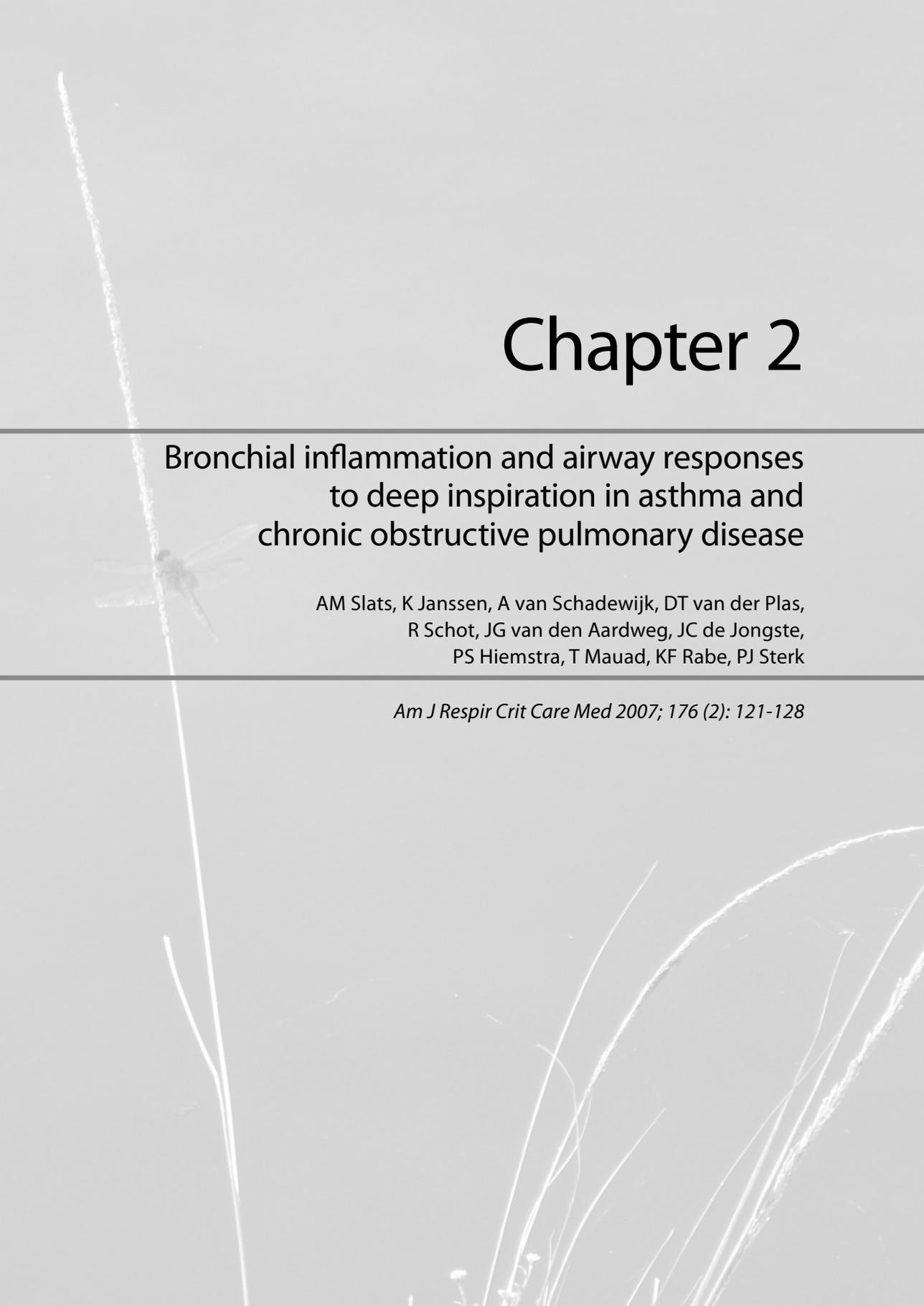
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Chapter 2

Bronchial inflammation and airway responses to deep inspiration in asthma and chronic obstructive pulmonary disease

AM Slats, K Janssen, A van Schadewijk, DT van der Plas,
R Schot, JG van den Aardweg, JC de Jongste,
PS Hiemstra, T Mauad, KF Rabe, PJ Sterk

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Abstract

Rationale: Deep inspirations provide physiologic protection against airway narrowing in healthy subjects, which is impaired in asthma and chronic obstructive pulmonary disease (COPD). Airway inflammation has been suggested to alter airway mechanics during deep inspiration.

Objectives: We tested the hypothesis that the number of bronchial inflammatory cells is related to deep inspiration-induced bronchodilation in asthma and COPD.

Methods: In a cross-sectional study, three modified methacholine challenges were performed in 13 patients with mild, persistent asthma, 12 patients with mild to moderate COPD, and 12 healthy control subjects.

Measurements and Main Results: After a 20-minute period of deep inspiration avoidance, inhalation of methacholine was followed by either one or five deep inspirations, or preceded by five deep inspirations. The response to deep inspiration was measured by forced oscillation technique. Inflammatory cells were counted within the lamina propria and airway smooth muscle area in bronchial biopsies of patients with asthma and COPD. The reduction in expiratory resistance by one and five deep inspirations was significantly less in asthma (mean change \pm SD: -0.5 ± 0.8 and -0.9 ± 1.0 cm H₂O/L/s, respectively) and COPD ($+0.2 \pm 1.1$ and $+0.4 \pm 1.0$ cm H₂O/L/s, respectively) as compared with healthy subjects (-1.5 ± 1.3 and -2.0 ± 1.2 cm H₂O/L/s, respectively; $p = 0.05$ and $p = 0.001$, respectively). In asthma, this was related to an increase in mast cell numbers within the airway smooth muscle area ($r = 0.73$; $p = 0.03$), and in CD4+ lymphocytes in the lamina propria ($r = 0.61$; $p = 0.04$).

Conclusions: Inflammation in the airway smooth muscle bundles and submucosa of bronchial biopsies is positively associated with impaired airway mechanics during deep inspiration in asthma, but not in COPD. Clinical trial registered with www.clinicaltrials.gov (NCT 00279136).

Introduction

Airway hyperresponsiveness is a key feature of asthma¹ and is also frequently present in patients with chronic obstructive pulmonary disease (COPD)². Deep breaths play a major role in modulating airway responsiveness. In healthy subjects, deep breaths reduce the level of pharmacologically induced airways obstruction (bronchodilation)³, whereas prohibition of taking deep breaths enhances the reaction to a bronchoconstrictor agent⁴. Furthermore, deep breaths taken before bronchial challenge reduce the consequent airways obstruction (bronchoprotection)^{5,6}. Thus, deep inspirations provide physiologic protection against airway narrowing.

In asthma, it has been shown that these beneficial effects of deep inspiration are impaired^{5,7,8}, and that deep inspirations may even enhance obstruction during exacerbations⁹. Several studies have demonstrated that the bronchodilatory effect of a deep inspiration is also reduced in COPD^{10,11}, which may be related to parenchymal damage^{12,13}. Understanding of the pathologic processes that lead to impairment of this protective mechanism against airway narrowing is required for attempts to restore it, and thereby advancing treatment in asthma and COPD.

Both asthma and COPD are characterized by airway inflammation, although the predominant inflammatory cell profiles are different^{14,15}. Indeed, inflammation of the airways has been suggested to influence airway mechanics by inducing airway remodeling and thereby increasing airway wall thickness¹⁶. This could result in reduced strain transmission from the parenchyma to the airways during deep inspiration or altered responses of the airway wall to the stretch imposed on it¹⁷. Anti-inflammatory treatment improves deep inspiration-induced bronchodilation in asthma, suggesting a role of airway inflammation as contributive to this mechanism¹⁸⁻²⁰. Although a relationship between airway responses to deep inspiration, without pharmacologically induced airway narrowing, and inflammatory cell counts in sputum has been shown twice^{21,22}, this has not yet been shown for inflammatory cells within bronchial biopsies. We hypothesized that the number of inflammatory cells in the airway smooth muscle bundles and lamina propria of bronchial biopsies of patients with asthma and COPD is related to impaired airway responses to deep inspiration.

The aim of the present study was to examine airway responses to deep inspiration in relation to the number of inflammatory cells in the airway smooth muscle bundles and bronchial submucosa in patients with asthma and COPD. Because a difference has been found in the response of the airways to either one or five deep inspirations^{6,23}, we aimed to examine this relationship under both circumstances. We used the forced oscillation technique to examine the resistance of the respiratory system (respiratory resistance), as this technique allows the continuous recording of deep inspiration-induced changes, and for a longer period of time after deep inspiration as compared to spirometry. Some of the results of this study have been previously reported in the form of an abstract^{24,25}.

Methods

Subjects

The complete methods are provided in the online supplement of this article: <http://ajrccm.atsjournals.org/cgi/data/176/2/121/DC1/1>. For this study, we enrolled 13 nonsmoking atopic patients with intermittent and mild persistent asthma (Global Initiative for Asthma [GINA] steps 1 and 2; provocative concentration of methacholine producing a 20% fall in FEV₁ [PC₂₀ methacholine] < 8 mg/ml)¹, 12 patients with mild to moderate COPD (Global Initiative for Chronic Obstructive Lung Disease [GOLD] I and II²⁶; > 10 pack years; FEV₁ reversibility to salbutamol < 12% of predicted), and 12 nonsmoking, healthy subjects (< 2 pack years; PC₂₀ methacholine > 16 mg/ml). All patients were clinically stable, and had not used inhaled or oral corticosteroids within 3 months before the study. The institutional review board for human studies approved the protocol, and the subjects gave their written, informed consent before entering the study.

Study design

The study had a cross-sectional design. Baseline clinical and functional assessments were performed, divided over 2 days, including medical history taking, skin prick test, spirometry with reversibility testing, and a standard methacholine challenge. In the second phase, three modified (single-dose) methacholine challenges were performed⁵ (see the online supplement). During the first challenge, the single dose of methacholine capable of producing a 20% reduction in FEV₁ was established while the bronchodilator response to one deep inspiration (slow inspiration to total lung capacity followed by a passive exhalation) was measured (**Figure 1A**). During the following single-dose challenges, the inhalation of this dose of methacholine was either preceded by (bronchoprotection; **Figure 1B**) or followed by (bronchodilation; **Figure 1C**) five consequent deep breaths in randomized order. The resistance of the respiratory system (respiratory resistance) was measured continuously during the breathing maneuvers using a forced oscillation device (Woolcock Institute, Sydney, Australia)⁸ with an applied oscillation frequency of 8 Hz and an amplitude of ± 1 cm H₂O (see the online supplement). Within 1 week, a bronchoscopy was performed and six bronchial biopsies were taken in the patients with asthma and COPD. The healthy subjects were not included in the biopsy study, because we aimed to examine the relationship between inflammation and the impaired effect of a deep inspiration within these disease groups.

Bronchoscopy, immunohistochemistry, and image analysis

Bronchoscopy was performed according to a standardized and validated protocol in our laboratory²⁷. Disposable forceps (radial edge; Boston Scientific, Boston, MA) were used to take six biopsy specimens at the (sub)segmental level. A total of 4 biopsies were fixed for 24 hours in 4% neutral-buffered formaldehyde, processed, and embedded in paraffin. From paraffin-embedded tissues, 4- μ m-thick sections were cut, and hematoxylin and eosin staining was used to evaluate overall bronchial architecture. Sections of two biopsies per subject, selected

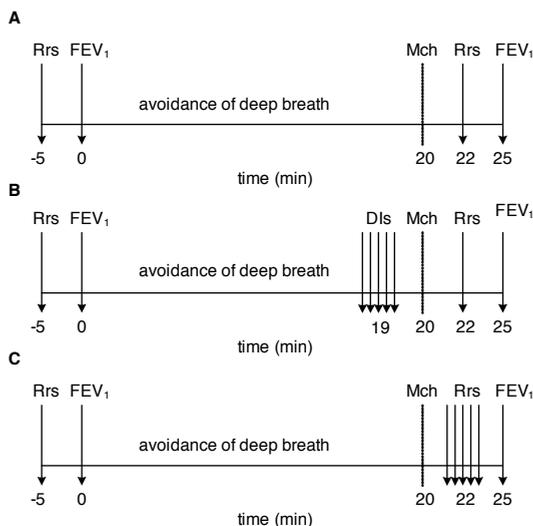


Figure 1. Single-dose challenge measurements.

This figure describes the three different single-dose methacholine (Mch) challenges. The line shows the time in minutes, and the arrows show the number of deep breaths taken. Baseline measurements of respiratory resistance (Rrs) and FEV₁ were followed by a period of 20 minutes with deep-breath avoidance. **(A)** Mch inhalation was followed by Rrs measurement with one deep breath and FEV₁ measurement to determine whether this dose could reduce FEV₁ by at least 20%. **(B)** Mch inhalation preceded by five deep inspirations (DIs), followed by Rrs measurement with one deep breath. **(C)** Mch inhalation followed by Rrs measurement with five deep breaths.

on morphologic quality criteria (intact reticular basal membrane and submucosa without crushing artifacts, large blood clots, or only epithelial scrapings), were stained and analyzed. Sections were incubated at room temperature with monoclonal antibodies directed against CD3, CD4, CD8 (T lymphocytes), EG2 (eosinophils), AA1 (tryptase-positive mast cells), CD68 (macrophages), and NE (neutrophils). Digital images from the stained sections were obtained, and fully automated cell counts (KS400; Carl Zeiss B.V., Sliedrecht, The Netherlands) were performed in the lamina propria (at least 0.125 mm²) by a validated method²⁸. The number of tryptase-positive mast cells in the airway smooth muscle bundles were automatically counted in a manually selected airway smooth muscle area (at least 0.1 mm²)²⁹ using serial sections stained for -smooth muscle actin and myosin to identify the airway smooth muscle area. Positively stained cells were expressed as the number of cells per 0.1 mm².

Analysis

Respiratory resistance was measured during 60 seconds of tidal breathing, followed by one or five slow, deep breaths to total lung capacity, and another minute of tidal breathing. Deep inspiration-induced bronchodilation was expressed as the difference between the mean resistance of all data points of three tidal breaths after and of three tidal breaths before the deep inspiration^{30,31}, which was calculated separately for inspiratory resistance and expiratory

resistance. The latter was done because respiratory resistance fluctuates during tidal breathing due to volume and flow differences between inspiration and expiration, and may be affected differently by deep inspiration maneuvers³². Bronchoprotection by deep inspirations was expressed as the difference in the increase in resistance by methacholine when either five or no deep inspirations were taken before methacholine inhalation. The outcome parameters were log transformed to obtain a normal distribution. The differences between the three groups were analyzed using analysis of variance, with Tukey's honestly significant difference test as post hoc analysis or Kruskal-Wallis test. Within-group differences were analyzed by two-tailed paired t tests or Wilcoxon ranks test. Spearman's rank correlation coefficient was used to explore associations between inflammatory cell counts and deep inspiration-induced changes in respiratory resistance. We used SPSS version 12.01 (SPSS, Inc., Chicago, IL) for all analyses. Statistical significance was associated with p values less than 0.05. Sample size estimation and details on the analysis are given in the online supplement.

Results

Functional parameters

The patient characteristics are given in Table 1. PC₂₀ methacholine (geometric mean \pm SD in doubling dose) was significantly lower in patients with asthma (1.0 ± 1.5 mg/ml) and COPD (2.15 ± 1.8 mg/ml) as compared with that in healthy control subjects (50.1 ± 1.3 mg/ml; $p < 0.001$). Patients with COPD were significantly older, had smoked, and their lung function was significantly more impaired than the patients with asthma and healthy control subjects (**Table 1**).

Table 1. Patient characteristics

Variable	Patients with Asthma	Patients with COPD	Healthy Control Subjects
Sex, male/female	5/8	8/4	2/10
Age, yr	23.8 \pm 5.7	57.9 \pm 7.5 ^{††}	32.8 \pm 13.8
BMI, kg/m ²)	22.9 \pm 2.1	26.3 \pm 3.3 ^{††}	22.2 \pm 3.4
Pack years	0.04 \pm 0.1	38.9 \pm 15.6 ^{††}	0.33 \pm 0.8
Post-salb FEV ₁ % pred	103.9 \pm 11.1	78.6 \pm 13.9 ^{††}	107.4 \pm 12.6
Post-salb FEV ₁ /FVC, %	87.0 \pm 6.4	60.9 \pm 7.6 ^{††}	85.6 \pm 8.3
PC ₂₀ methacholine, mg/ml	1.0 \pm 1.5	2.2 \pm 1.8	50.7 \pm 1.3 ^{††}
Single-dose methacholine, mg/ml	3.3 \pm 1.4	10.0 \pm 1.9 [†]	72.5 \pm 1.5 ^{††}
Fall in FEV ₁ , % (single-dose methacholine challenge)	29.7 \pm 8.0	23.5 \pm 2.8	26.8 \pm 7.6

Data are expressed as mean \pm SD, except for sex (number), PC₂₀ methacholine (geometric mean \pm SD in doubling doses). Analysis of variance, *post hoc* Tukey's honestly significant difference test. ^{*} $p < 0.05$ Healthy control subjects vs. patients with COPD. [†] $p < 0.05$ Patients with asthma vs. those with COPD. ^{††} $p < 0.05$ Healthy control subjects vs. patients with asthma.

Single-dose methacholine challenges

FEV₁ dropped more than 20% from baseline in all subjects by the single dose of methacholine (mean % fall in FEV₁ ± SD: 29.7 ± 8.0%, 23.5 ± 2.8%, and 26.8 ± 7.6% for asthma, COPD, and healthy control subjects, respectively), which was not significantly different between the groups

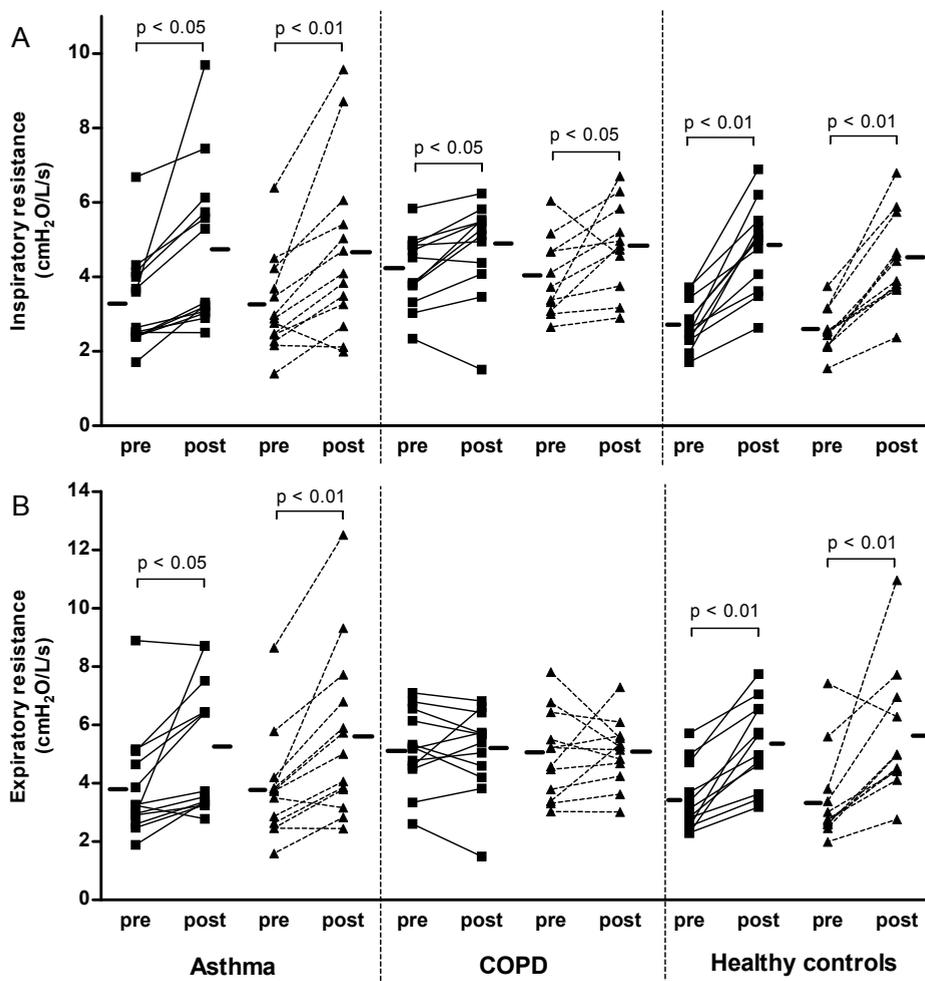


Figure 2. Changes in respiratory resistance by methacholine.

This figure shows the individual data points per group before (pre) and after (post) Mch inhalation for inspiratory (A) and expiratory (B) resistance. Data are expressed in cm H₂O/L/second, and the horizontal lines represent the mean. Squares connected by solid lines represent the data with no deep inspirations taken before Mch inhalation (Figure 1A); triangles connected by dashed lines represent the challenge taken when five deep inspirations were taken before Mch inhalation (Figure 1B). Mch significantly increased inspiratory resistance in all three groups, and expiratory resistance in only the asthma group and healthy control subjects, and not in the COPD group. Five deep inspirations did not protect against the increase in inspiratory and expiratory resistance in any of the three groups.

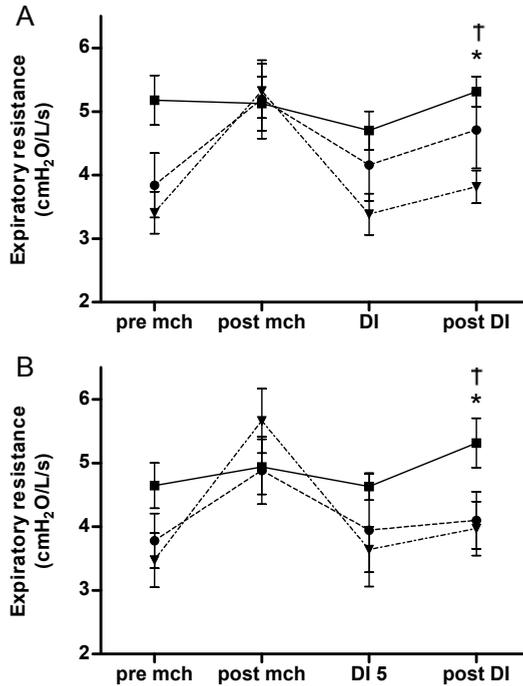


Figure 3. Changes in expiratory resistance by deep breath.

In this figure, the paired data (mean \pm SEM) for patients with asthma (circles, dashed line), those with COPD (squares, solid lines), and healthy control subjects (inverted triangles, dashed-dotted lines) are depicted. The data are expressed as the mean expiratory resistance during three tidal expirations before Mch inhalation (pre mch), three tidal expirations after Mch inhalation (post mch), the passive expiration of the deep inspiration (DI), and three tidal expirations after deep inspiration (post DI). **(A)** Data of the measurement when one deep inspiration was taken after Mch inhalation (**Figure 1A**). **(B)** Data of the measurement when five deep inspirations were taken (**Figure 1C**), where data point "DI 5" represents the mean of the resistance during the five passive expirations of the five deep breaths. The reduction in expiratory resistance during tidal breathing by one and by five deep breaths was significantly larger in healthy subjects as compared with patients with asthma ($^{\dagger}p < 0.05$) and those with COPD ($^{*}p < 0.05$). Furthermore, the reduction in expiratory resistance during tidal breathing by five deep breaths was significantly larger in patients with asthma than in those with COPD ($p < 0.05$).

($p = 0.08$). Tidal volume before and after methacholine inhalation was not significantly different between the groups ($p > 0.7$), nor was the inspiratory volume of either one (mean \pm SD: asthma, 1.6 L \pm 0.5; COPD, 1.6 L \pm 0.5; healthy control subjects, 1.5 L \pm 0.5; $p = 0.9$) or the mean of five deep inspirations (asthma, 2.2 L \pm 0.7; COPD, 2.0 L \pm 0.4; healthy control subjects, 2.1 L \pm 0.6; $p = 0.6$). In the three groups, inspiratory resistance was significantly increased by the single dose of methacholine (**Figure 2A**; mean change \pm SD: asthma, +1.4 \pm 1.5 cm H₂O/L/s; COPD, +0.6 \pm 0.7 cm H₂O/L/s; healthy control subjects, +2.1 \pm 1.0 cm H₂O/L/s). Expiratory resistance was also significantly increased in asthma and healthy control subjects (**Figure 2B**; +1.4 \pm 1.6 and +1.9 \pm 1.1 cm H₂O/L/s, respectively), but not in patients with COPD (-0.05 ± 0.9 cm H₂O/L/s). The increase in resistance by a single dose of methacholine was not significantly reduced when

Table 2. Changes in inspiratory and expiratory resistance by one and five deep inspirations

Change in Resistance (cm H ₂ O/L/s)						
No. of Deep Inspirations	Patients with Asthma		Patients with COPD		Healthy Control Subjects	
	Insp	Exp	Insp	Exp	Insp	Exp
One	-0.6 ± 0.3	-0.5 ± 0.2	-0.2 ± 0.3	0.2 ± 0.3	-1.5 ± 0.3 [†]	-1.5 ± 0.4 ^{††}
Five	-1.1 ± 0.4	-0.9 ± 0.3 [*]	0.0 ± 0.4	0.4 ± 0.3	-1.9 ± 0.4	-2.0 ± 0.4 ^{††}

Data are expressed as mean ± SEM. Analysis of variance, *post hoc* Tukey's honestly significant difference test. **p* < 0.05 Patients with asthma vs. those with COPD. [†]*p* < 0.05 Healthy control subjects vs. patients with COPD. ^{††}*p* < 0.05 Healthy control subjects vs. patients with asthma.

five deep inspirations were taken before methacholine (bronchoprotection) in any of the three groups (**Figure 2**).

In patients with asthma and healthy subjects, after inhalation of methacholine, both one and five deep inspirations significantly reduced inspiratory and expiratory resistance (**Table 2; Figures 3A and 3B** for one and five deep inspirations, respectively). In COPD, no significant reduction in expiratory resistance was observed by either one or five deep inspirations, and only inspiratory resistance was significantly reduced by five deep inspirations. The reduction in expiratory resistance induced by both one and five deep breaths was significantly larger in healthy control subjects than in patients with asthma and COPD (Table 2; *p* < 0.05 and 0.01, respectively). Furthermore, the reduction in expiratory resistance during tidal breathing by five deep breaths was significantly larger in asthma than in COPD (**Table 2**; *p* < 0.05). The absolute change induced in expiratory resistance by five deep inspirations is a mean (± SD) percent reduction of 67(±4.4)% in healthy control subjects and 22(±2.3)% in patients with asthma, and a percent increase of 13(±3.3)% in patients with COPD.

Bronchial inflammation

The numbers of inflammatory cells in the lamina propria of bronchial biopsies per cell type are shown in **Table 3**. Patients with asthma had significantly more eosinophils (EG2+ cells) in the

Table 3. Inflammatory cell counts in bronchial biopsies

Cell Type	Asthma	COPD
CD3 ⁺ cells	53.3 (14.0–134.0)	25.5 (3.0–160.5)
CD4 ⁺ cells	24.8 (9.5–86.0)	12.0 (1.5–83.0)
CD8 ⁺ cells	25.8 (7.0–62.0)	15.0 (5.0–93.0)
CD4 ⁺ /CD8 ⁺ cells	1.7 (0.6–4.4)	0.6 (0.1–4.4)
EG2 ⁺ cells	1.5 (0.0–8.0)*	0.3 (0.0–3.0)
AA1 ⁺ cells	10.0 (1.0–24.0)	16.0 (2.0–56.0)
AA1 ⁺ cells in airway smooth muscle bundles	2 (0.0–7.0)	1.5 (1.0–3.0)
CD68 ⁺ cells	19.0 (8.0–53.0)	9.3 (3.0–100.0)
NE ⁺ cells	1.8 (0.0–14.0)	2.0 (0.0–41.0)

The numbers of cells are expressed as median (range) per 0.1 mm². **p* < 0.05 between groups.

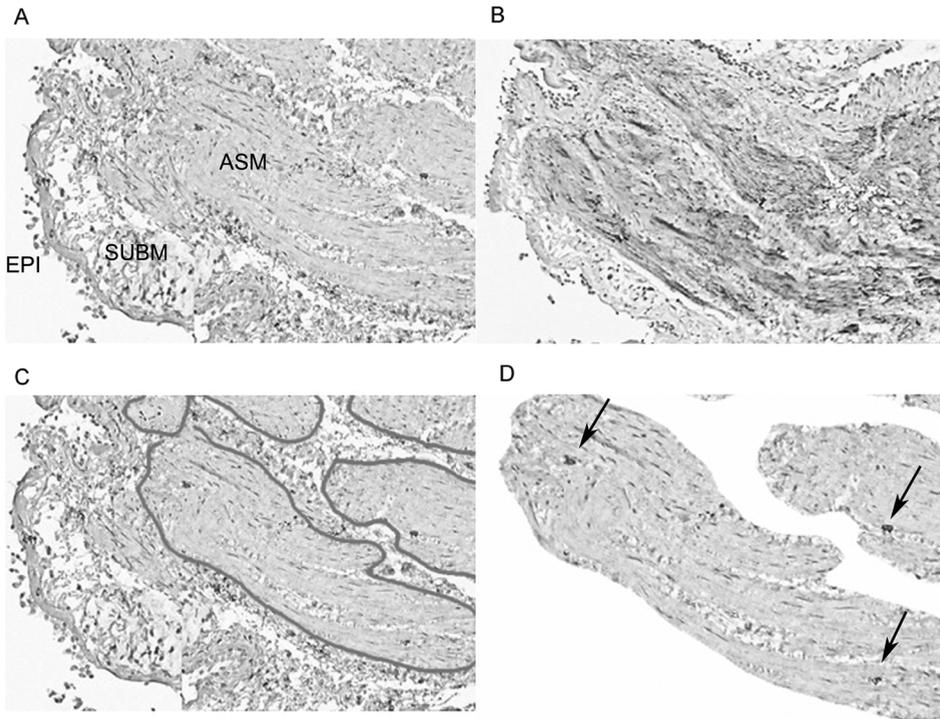


Figure 4. Photomicrographs of mast cell and myosin staining.

Example of a bronchial biopsy section immunohistochemically stained for **(A)** tryptase-positive mast cells (ASM = airway smooth muscle; EPI = epithelium; SUBM = submucosa), and **(B)** a serial section of the same biopsy stained for myosin. The smooth muscle area was manually selected **(C)** in the mast cell staining by myosin staining. In the selected area **(D)**, mast cells (arrows) were automatically counted. Original magnification: x200.

lamina propria as compared with patients with COPD. Also, the number of CD4+ lymphocytes and the CD4+/CD8+ lymphocyte ratio tended to be higher in asthma than in COPD, but this did not reach significance ($p = 0.09$ and 0.06 , respectively). Among the inflammatory cell types analyzed, predominantly mast cells were observed in the airway smooth muscle bundles (**Figure 4**). In asthma, 74%, and in COPD, 76% of the biopsies contained sufficient ($> 0.1 \text{ mm}^2$) airway smooth muscle area. The mean area analyzed in asthma was $0.24 (\pm 0.11) \text{ mm}^2$ and, in COPD, $0.36 (\pm 0.20) \text{ mm}^2$ ($p = 0.11$).

In asthma, the reduction in resistance by one deep breath was positively associated with the number of CD4+ cells per 0.1 mm^2 ($r = 0.61$; $p = 0.04$; **Figure 5A**). In addition, the number of mast cells in the airway smooth muscle bundles correlated positively with the reduction in resistance by five deep breaths ($r = 0.72$; $p = 0.03$, **Figure 5B**). In COPD, there were no significant correlations between the changes in resistance by deep inspirations and inflammatory cell counts within the lamina propria, or the number of mast cells in the airway smooth muscle bundles.

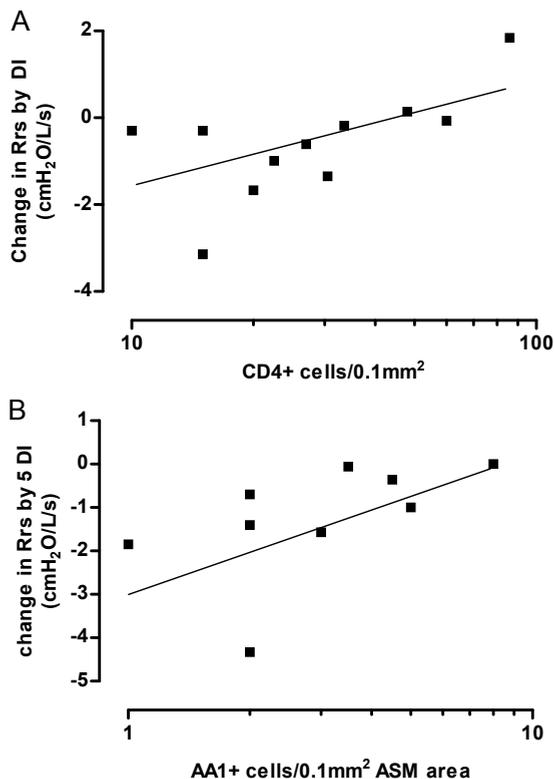


Figure 5. Relationship between inflammatory cell counts and deep inspiration–induced bronchodilation in asthma.

This figure shows the relationship between the change in inspiratory resistance (Rrs) by (A) one deep inspiration (DI) and the number of CD4+ lymphocytes/0.1 mm² in the lamina propria ($r = 0.61$; $p = 0.04$), and by (B) five deep inspirations and the number of tryptase-positive (AA1+) mast cells/0.1 mm² in the airway smooth muscle (ASM) bundles ($r = 0.73$; $p = 0.03$).

Discussion

The results of this study demonstrate that the bronchodilatory effect of deep inspiration is impaired in intermittent and mild persistent asthma as compared with that in healthy subjects, and even more markedly impaired in patients with mild to moderate COPD. Interestingly, in asthma, the reduced bronchodilatory effect of a deep inspiration was associated with increased numbers of mast cells within the airway smooth muscle bundles and increased CD4+ lymphocyte counts in the bronchial lamina propria. These findings suggest that the impairment of deep inspiration–induced bronchodilation in asthma is a result of inflammatory mechanisms within the airway smooth muscle area and bronchial wall, possibly resulting in altered airway mechanics by influencing airway smooth muscle characteristics or increasing airway wall thickness. To our knowledge, this is the first study showing a relationship of inflammatory

cell counts in the airway smooth muscle area and lamina propria of bronchial biopsies with airway responses to deep inspiration in asthma. In general, our physiologic results are in line with previous studies showing reduced bronchodilation after deep inspiration in asthma and COPD as compared with that in healthy control subjects^{23,33}. Although, we did find a significant reduction in respiratory resistance by both one and five deep inspirations in the patients with asthma, this was significantly less than in the healthy subjects. This partly preserved deep inspiration-induced bronchodilation in asthma differs from other studies showing almost no reduction in airways obstruction by deep inspiration in patients with asthma³⁴. This might be explained by differences in disease severity and the level of airway hyperresponsiveness of the participating subjects. The patients with asthma in our study had intermittent or mild persistent asthma, needing no other medication than bronchodilators on demand. Furthermore, the method of measuring airway responses to deep inspiration differs among studies, and may influence the outcome parameters as well³⁵.

Notably, we did not find a bronchoprotective effect of deep inspirations in the healthy control group, whereas this has been shown by several studies in the past^{3,5}. This seems to be explained by the methods used to assess airways obstruction. Bronchoprotection by deep inspiration has predominantly been observed by using measurements implicitly including a deep breath, such as FEV₁, whereas it could not be established by parameters without a deep breath during the measurement³⁶. We purposely chose the latter to examine the unaffected protective effect of deep inspirations against the dynamics of airway narrowing and, therefore, may have missed bronchoprotection as reported when using FEV₁. Taken together, these findings suggest that deep inspirations taken before methacholine inhalations improve subsequent bronchodilatory effects of deep breaths in healthy subjects, and thus prevent a fall in FEV₁, but may not necessarily prevent the obstruction itself.

We aimed to look at relationships between bronchial inflammation and deep-breath effects within a group of patients with asthma and those with COPD, and therefore selected the patients that matched the key features of these two distinct disease groups. As expected, this resulted in significant differences between the groups with regard to age and lung function. However, neither in COPD nor in healthy control subjects was a relationship found between deep breath-induced reduction in respiratory resistance and age or lung function ($r < 0.4$; $p > 0.2$). Therefore, the differences between COPD and healthy control subjects are most likely a result of pathophysiologic changes in COPD.

We used a modified single-dose methacholine challenge to induce a given level of airways obstruction in all subjects to measure both the bronchoprotective and the bronchodilatory effect of deep inspirations. During the first challenge, we established the dose that induced a reduction in FEV₁ of at least 20%, and used that dose for the other two challenges. We could not determine whether the subsequent single-dose challenges induced the same fall in FEV₁ in absence of performing spirometry. However, because there was no significant difference within the groups between the three challenges with regard to respiratory resistance

after methacholine inhalation, we presume that the level of obstruction was approximately the same as in the dose-finding challenge. Interestingly, in the patients with COPD, the fall in FEV₁ induced by methacholine was not accompanied by a significant increase in respiratory resistance, a finding that we cannot fully explain. This may have limited the possibility of reducing respiratory resistance by deep inspiration in this group. However, there was no direct relationship between the increase in respiratory resistance by methacholine and the reduction in respiratory resistance by deep inspirations, suggesting that the absence of the bronchodilatory effect of deep inspirations in COPD was not necessarily dependent on the absence of an increase in respiratory resistance. Hence, this finding may provide new information on the functionally relevant pathophysiology of the airways in patients with mild to moderate COPD, which requires further investigation. In this study protocol, we have used the forced oscillation technique to measure airway responses to deep inspiration. The limitation of this method is that the results represent resistance of the complete respiratory system, including the upper airways, and thus the site of the obstruction or deep inspiration-induced bronchodilation is difficult to determine. However, this technique enabled us to monitor respiratory resistance continuously, and, therefore, we were able to measure the effect of deep breaths on the dynamics of airway obstruction during both the deep breaths and tidal breathing. How can we interpret these results? During a deep inspiration, the airways are dilated, as shown on computed tomographic scan³⁷, both in healthy adults and those with asthma, presumably as a result of the airway–parenchymal coupling.

However, it appeared that, in asthma, deep breaths could not reduce respiratory resistance to the same extent as in healthy subjects. We found that increased numbers of CD4+ lymphocytes in the lamina propria of bronchial biopsies were associated with impaired bronchodilation after a deep breath in asthma. It is likely that these cells indirectly reflect the inflammatory changes within the bronchial wall that prevent adequate stretch of the airways and airway smooth muscle layer. CD4+ lymphocytes are involved in eosinophilic inflammation, and are associated with vasodilation and microvascular leakage³⁸. These inflammatory changes may narrow the internal airway diameter, and, at the same time, increase the outer wall perimeter, thereby decreasing the force applied to the airways by the parenchyma during deep inspiration^{17,39}. In addition, a similar relationship with CD4+ cells was not found at slightly larger changes in resistance induced by five deep inspirations. This may indicate that inflammation, as reflected by CD4+ lymphocytes within the lamina propria, indeed decreases deep inspiration-induced stretch of the airways, but does not fully prevent it, which may be overcome by multiple stretching maneuvers. Another hypothesis regarding the role of inflammation in the impairment of the bronchodilatory effect of deep inspiration is the reduction in the stretch-induced release of inhibiting factors, such as nitric oxide. The CD4+ lymphocytes within the bronchial wall may counteract these active bronchodilating mechanisms. Most strikingly, we found a correlation between the number of mast cells within the smooth muscle bundles and deep inspiration-induced bronchodilation in asthma. Mast cells can promote airway smooth

muscle contraction by releasing histamine, prostaglandin D₂, and tumor necrosis factor- α ^{29,40}. We speculate that the localization of the mast cells within the smooth muscle cells could result in a physiologically altered intrinsic contractile function, leading to an increased formation of actin and myosin cross bridges, more difficult to disrupt by deep inspiration-induced stretch of the airways, which has been referred to as the latch state⁴¹. These data further extend the results obtained by Brightling and colleagues²⁹, showing increased numbers of mast cells in the airway smooth muscle bundles in bronchial biopsies of patients with asthma as compared with healthy control subjects or patients with eosinophilic bronchitis, which was related to airway hyperresponsiveness. Interestingly, in COPD, there was no significant reduction in respiratory resistance by deep breaths. An absolute loss of alveolar attachments might explain this observation, as this would result in uncoupling of the airway–parenchyma interdependence, leading to less strain imposed on the airways by the parenchyma during deep inspiration⁴². Indeed, it has been shown that the loss of alveolar attachments was related to less bronchodilation by deep breaths in patients with mild to moderate COPD¹³. Because we did not find a direct relationship between inflammatory cells within the bronchial wall and deep breath-induced bronchodilation in COPD, we speculate that the marked loss in the ability to reduce respiratory resistance by deep inspiration is predominantly due to structural damage of the airways or lung parenchyma in this disease.

What could be the clinical implication of our study? The correlation of inflammatory cells within the submucosa and airway smooth muscle bundles with the bronchodilatory effect of a deep inspiration in asthma indicates that the impaired airway mechanics may, at least partially, be restored by treatment. Indeed, it has been shown that airway responses to deep inspirations can be improved by treatment with (inhaled) corticosteroids^{18–20}. Furthermore, because deep inspirations are likely to play a role in airway hyperresponsiveness³, perceived symptoms⁴³, and exacerbations⁹ in asthma, measurement of airway responses to deep inspiration may give additional information on current disease status. In COPD, our findings indicate that airway inflammation plays a less prominent role in the pathophysiologic mechanism of deep breath-induced bronchodilation, which limits the options for intervention. However, deep-breath responses may be a sensitive parameter for finding early lung damage caused by smoking.

We conclude that deep inspiration-induced bronchodilation is reduced in patients with intermittent and mild persistent asthma as compared with healthy subjects, and absent in patients with mild to moderate COPD. In asthma, the bronchodilatory effect of deep inspirations is related to inflammatory cell counts within airway smooth muscle bundles and bronchial wall, whereas in moderate COPD, this relationship could not be found. These results indicate that the physiologic protection against airway narrowing by deep inspiration is impaired in both asthma and COPD, but this may be due to different pathophysiologic mechanisms.

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