Chapter 7

Enhanced airway dilation by positive-pressure inflation of the lungs compared with active deep inspiration in patients with asthma

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Abstract

Background: Deep inspiration temporarily reduces induced airways obstruction in healthy subjects. This bronchodilatory effect of deep inspiration is impaired in asthma. Passive machine-assisted lung inflation may augment bronchodilation compared with an active deep inspiration in patients with asthma by either opening closed airways or by reducing fluid flux across the airway wall during deep inspiration, and thereby increasing the tethering forces on the airway wall.

Methods: We recruited 24 patients with asthma [18–46 yr old, forced expiratory volume in 1 second (FEV₁) > 70% predicted; provocative concentration of methacholine inducing a 20% fall in FEV₁ (PC₂₀) < 8 mg/ml], with either an impaired (n = 12) or an intact (n = 12) bronchodilatory response to deep inspiration. Two methacholine challenges were performed on separate days. At a 50% increase in respiratory resistance (forced oscillation technique at 8 Hz), the change in resistance by a positive-pressure inflation (computer-driven syringe) or an active deep inspiration was measured in randomized order.

Results: The reduction in resistance by positive-pressure inflation was significantly greater than by active deep inspiration in the impaired deep inspiration response group (mean change ± SE: −0.6 ± 0.1 vs. −0.03 ± 0.2 cmH₂O·l⁻¹·s, P = 0.002). No significant difference was found between positive-pressure inflation and active deep inspiration in the intact deep inspiration response group (−0.6 ± 0.2 vs. −1.0 ± 0.3 cmH₂O·l⁻¹·s, P = 0.18).

Conclusion: Positive-pressure inflation of the lungs can significantly enhance deep inspiration-induced bronchodilation in patients with asthma.
Introduction

The functional response of the airways to deep inspiration is different between healthy subjects and patients with asthma\textsuperscript{27,41}. In healthy subjects deep inspirations reverse induced bronchoconstriction almost to baseline level\textsuperscript{4,32,39}. In patients with mild asthma, however, dilation of constricted airways by deep inspiration is impaired\textsuperscript{16,38,44}. The impairment of this bronchodilatory effect is related to asthma severity and disease status\textsuperscript{28,40}. Restoring this physiological mechanism that reverses airways obstruction in patients with asthma might reduce the need of current asthma treatment.

During a deep inspiration the transpulmonary pressure is transmitted through the parenchymal tissue and distends all intrapulmonary structures, including the airways\textsuperscript{42}. Airway wall thickening by chronic inflammation in asthma would decrease the strain transmission from the parenchyma to the airway wall, thereby reducing airway distension. Using a mathematical model it has been shown that peribronchial inflammation decreases both the load and the slope of the relationship between peribronchial and pleural pressure\textsuperscript{30}. Furthermore, Burns and Gibson\textsuperscript{12} showed that adding a resistance during deep inspiration, to enhance the subatmospheric intrathoracic pressure, decreased airway conductance (sGaw) in asthmatic patients. They concluded that the large subatmospheric pressures during deep inspiration may lead to extravasation of fluid in the inflamed asthmatic airway wall, thereby enhancing airway wall thickness. However, two studies using fast intravenous infusion of saline, resulting in airway wall thickening by edema and fluid flux\textsuperscript{9,11}, showed no effect on deep inspiration-induced bronchodilation\textsuperscript{37}.

On the other hand, the inflammation-induced remodeling processes in chronic asthma\textsuperscript{13,25} may increase the airway wall stiffness\textsuperscript{5} and make it more resistant to imposed stretch by deep inspiration\textsuperscript{17,33,35,47}. Using high-resolution CT scans, Brown and Mitzner\textsuperscript{8} demonstrated in mechanically ventilated dogs that increased airway wall stiffness by smooth muscle tone resulted in impaired dilation of the airways by positive pressure compared with relaxed airways. Even though it has been observed that the airways of asthmatic patients dilated to the same extent as those of healthy subjects by a deep inspiration, the airways of the patients with asthma constricted following the deep inspiration\textsuperscript{10}. The latter may be a result of altered smooth muscle function in asthma leading to impaired bronchodilatation by deep inspiration\textsuperscript{2}.

It can be postulated that airway wall distension can be improved by manipulation of the intrathoracic pressures by passive lung inflation in patients with asthma. Mechanical inflation of the lungs would induce stretch of the airways without large subatmospheric intrathoracic pressures and could therefore prevent extravasation of fluid in inflamed airways. In addition, the inflated volume may open closed airways, thereby redistributing tethering forces of the parenchyma on the airway wall.

Therefore, we hypothesized that positive-pressure machine-assisted lung inflation will reduce the level of airways obstruction in patients with asthma who do not feature bronchodilation
following active deep inspiration. To that end, we developed a computer-controlled syringe, which can inflate the lungs with a predetermined individual volume and inspiration time for each subject. This was connected to a forced oscillation device, which measured resistance (Rrs) and reactance (Xrs) of the respiratory system continuously during the breathing maneuvers.

The aim of this study was to compare changes in Rrs and Xrs in response to active or positive-pressure (syringe inflated) deep inspiration maneuvers in asthmatic patients who have an intact or an impaired bronchodilatory response to active deep inspiration while provoked with inhaled methacholine.

**Methods**

**Subjects**
For this study we recruited 24 patients with mild to moderate persistent asthma. All patients had a history of wheezing, breathlessness, or cough. They were all atopic, as demonstrated by a positive skin reaction to 1 of 10 common aeroallergen extracts (HAL, Haarlem, The Netherlands) and were hyperresponsive to methacholine [provocative concentration of methacholine inducing > 50% increase in Rrs (PC<sub>50</sub> Rrs) < 8 mg/ml] (7). The subjects were clinically stable and used β<sub>2</sub>-agonists on demand only or in combination with inhaled corticosteroids. Short- and long-acting β<sub>2</sub>-agonists were stopped, respectively, 8 and 24 h before the challenges. None of the participants had a recent upper respiratory tract infection or other relevant diseases. The Medical Ethics Committee of the Leiden University Medical Center approved the study, and the subjects gave their written informed consent before entering the study.

**Study design**
Clinical status, atopy, and hyperresponsiveness to methacholine were assessed during a screening visit. The initial response of the airways to an active deep inspiration was measured at the end of the methacholine challenge of the screening visit. The response determined whether the subject was included in group A (intact response to deep inspiration, the “intact DI response group”) or group B (impaired response to deep inspiration, the “impaired DI response group”) (see also Statistical analysis). On two subsequent randomized visits two additional methacholine challenges were performed to measure the response of the airways to either an active deep inspiration or a positive-pressure inflation performed by the computer-controlled motor-driven syringe (Figure 1).

**Methacholine challenges**
The response of the airways to methacholine was measured using the forced oscillation technique (FOT) at 8 Hz. At baseline, forced expiratory volume in 1 s (FEV<sub>1</sub>) was measured three times following saline inhalation. One minute later, three measurements of Rrs during 30 s of
tidal breathing were performed. The mean of these three measurements was used as baseline Rrs. Serial doubling doses of methacholine bromide (0.03–9.6 mg/ml) in normal saline were aerosolized and inhaled for 2 min by tidal breathing at 5-min intervals. Following each dose Rrs was measured during 30 s of tidal breathing starting at 30 and 90 s after methacholine inhalation. The challenge was stopped when the highest Rrs of these two measurements was increased by more than 50% from baseline. Another measurement was performed to calculate the change in Rrs induced by deep inspiration. Finally spirometry was performed to measure the fall in FEV₁.

**Machine-assisted positive-pressure inflation**

We developed a computer-controlled motorized syringe system and software to perform a passive deep inspiration maneuver [artificial lung function generator (ALFG)]. The ALFG was connected to a forced oscillation device (Figure 2). We used valves to switch between FOT measurements and positive-pressure inflation. Therefore, the measurements taken before and after active deep inspiration were done under the exact same conditions as before and after positive-pressure inflation. The maneuver started with tidal breathing, followed by an expiratory reserve volume (ERV) maneuver. The ERV was calculated from the volume difference between last end-expiratory value and the plateau of the ERV maneuver. Using this ERV, the specific inspiratory capacity (IC) was calculated by subtracting the ERV value from the previously measured baseline vital capacity (VC). As a safety procedure to prevent overinflation, we used 90% of IC as the inspiratory volume to be inflated by the machine. In addition, if ERV following methacholine inhalation was less than baseline ERV, we used baseline ERV. The ERV maneuver was followed by tidal breathing to determine the starting point and duration of the positive-pressure inflation. The function of a cosine during a half period (period of π radians)
was used to simulate the flow-driven passive deep inspiration maneuver, with the inspiration time as signal period time, and the predetermined inspiration capacity as the amplitude of the cosine. $R_{rs}$ and $X_{rs}$ were measured continuously, except during the positive-pressure inflation itself. The active deep inspiration was preceded by the same maneuvers to keep the active and passive deep inspiration measurements comparable.

**Forced oscillation technique**

$R_{rs}$ and $X_{rs}$ were measured continuously during the breathing maneuvers using a forced oscillation device (Woolcock Institute)\textsuperscript{38,44} with an applied oscillation frequency of 8 Hz and an amplitude of $\pm 1\text{ cmH}_2\text{O}$. Flow was measured using a 50-mm-diameter Fleisch pneumotachograph (Vitalograph, Maids Moreton, UK), and differential pressure was measured using a $\pm 2.5\text{ cmH}_2\text{O}$ solid-state transducer (SurSense DCAL4; Honeywell Sensing and Control, Milpitas, CA). Mouth pressure was measured using a similar transducer with a higher range ($\pm 12.5\text{ cmH}_2\text{O}$).

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**Figure 2.** Schematic overview of artificial lung function generator (ALFG) and forced oscillation technique (FOT). The top panel shows the forced oscillation device and the lower panel shows the connected ALFG. During all the measurements the black valve was closed and the white valves were open. In this situation the experimental setting resembles a regular FOT measurement. Just before the positive-pressure inflation the valves were switched (white valves closed and black valve opened), and at the end of the positive-pressure inflation (at total lung capacity) the valves were switched back and FOT measurements started again.
Analog pressure and flow signals were digitized at 400 Hz. The time- and frequency-dependent respiratory impedance $Z_{rs}$ was estimated based on the hypothesis that random errors occur in both pressure and flow. This yields a total least squares (TLS) estimate of respiratory impedance as a function of time and frequency and allows an estimation of confidence intervals in the course of time. This method has been fully described in a previous study.  

**Statistical analysis**

Mean $R_{rs}$ and $X_{rs}$ were calculated from all data points during three tidal inspirations ($R_{rs_{insp}}$ and $X_{rs_{insp}}$) and three tidal expirations ($R_{rs_{exp}}$ and $X_{rs_{exp}}$) separately. The response of the airways was calculated as the difference between $R_{rs}$ and $X_{rs}$ following and preceding the deep inspiration. An impaired response to deep inspiration was defined as a decrease in $R_{rs_{exp}}$ of less than 2 SDs of $R_{rs_{exp}}$ preceding the deep inspiration, whereas an intact response to deep inspiration was defined as a decrease in $R_{rs_{exp}}$ of more than 2 SDs (Figure 3).

The sample size of 12 patients per group was based on our data with regard to $R_{rs}$ measurements, allowing the detection of a 1 cmH$_2$O·L$^{-1}$·s difference within and between the groups.

![Figure 3](image.png)

**Figure 3.** Example of calculation of DI response.  
2 examples of $R_{rs}$ measurements at the end of the methacholine challenge of the screening visit. **A**: the change in resistance of the respiratory system ($R_{rs}$) in a patient with an intact DI response. The top solid line represents the mean of the 3 tidal expirations before DI, the dashed line represents the mean minus 2 SDs, and the bottom solid line represents the mean of 3 tidal expirations following DI. This example clearly shows that the DI decreased $R_{rs}$ by more than 2 SDs. **B**: the change in $R_{rs}$ in a patient with an impaired DI response. In this case the active DI did not decrease $R_{rs}$ by more than 2 SDs.
if α = 0.05, and 1 − β = 0.80. Between-group differences were analyzed using Mann-Whitney U-tests. Within-group differences were explored using Wilcoxon signed-rank tests. Correlations were examined using Spearman’s rank correlation coefficients. We used SPSS version 12.01 for all analyses (SPSS, Chicago, IL). P values < 0.05 were considered statistically significant.

**Results**

All patients performed the measurements without any personal or medical problems. The patient characteristics are given in Table 1. The groups were not significantly different with regard to sex, age, steroid usage, lung volumes (VC, ERV, IC), or lung function (FEV₁, % predicted). A methacholine-induced increase of more than 50% in Rrs could not be reached in all patients. This occurred in 6 of 24 patients: 2 in the intact DI response group, and 4 in the impaired DI response group. The challenge was then stopped if apparent breathlessness or wheezing occurred in combination with a further decrease in Xrs. FEV₁ dropped by more than 20% in all cases (Table 1). We calculated mean resistance and reactance over tidal inspirations (Rrs<sub>insp</sub>, Xrs<sub>insp</sub>) and tidal expirations (Rrs<sub>exp</sub>, Xrs<sub>exp</sub>) separately. However, the parameters showed the same results, indicating that deep inspiration and positive-pressure inflation altered both parameters in the same way. We have therefore only presented the results of Rrs<sub>exp</sub> and Xrs<sub>exp</sub>.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
<th>Intact DI Response Group</th>
<th>Impaired DI Response Group</th>
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<tr>
<td>Sex, M/F</td>
<td>3/9</td>
<td>3/9</td>
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<tr>
<td>Age, yr</td>
<td>28.1±9.1</td>
<td>26.7±9.7</td>
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<tr>
<td>ICS usage, yes/no</td>
<td>5/7</td>
<td>7/5</td>
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<td>FEV₁, %predicted</td>
<td>96.6±15.2</td>
<td>90.6±11.7</td>
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<td>VC, liters</td>
<td>4.6±1.1</td>
<td>4.2±0.8</td>
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<tr>
<td>ERV, liters</td>
<td>1.6±0.5</td>
<td>1.5±0.4</td>
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<tr>
<td>IC, liters</td>
<td>2.9±0.7</td>
<td>2.7±0.7</td>
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<td>PC₅₀ Rrs methacholine, mg/ml</td>
<td>0.73±1.6</td>
<td>0.45±1.5</td>
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<tr>
<td>Fall in FEV₁, %</td>
<td>30.9±10.9</td>
<td>37.4±11.5</td>
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<tr>
<td>IV post-methacholine, %</td>
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<tr>
<td>Active deep inspiration</td>
<td>81.6±12.2</td>
<td>70.5±13.1*</td>
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<tr>
<td>Positive-pressure inflation</td>
<td>82.0±8.7</td>
<td>85.2±5.6</td>
</tr>
</tbody>
</table>

Data are expressed as number [male/female (M/F); inhaled corticosteroid (ICS) usage] or means ± SD. DI, deep inspiration; FEV₁, forced expiratory volume in 1 s; VC, vital capacity; ERV, expiratory reserve volume; IC, inspiratory capacity; Rrs, resistance of the respiratory system; PC₅₀, provocative concentration of methacholine inducing a 50% increase in Rrs. Only the inspiratory volume as a percentage of baseline inspiratory capacity (IV %) for the active deep inspiration was significantly different between the groups (*P = 0.027).
Baseline measurements
Baseline $R_{rs_{exp}}$ and $X_{rs_{exp}}$ were not significantly different between the groups at both visits ($P > 0.1$). Active deep inspiration significantly decreased $R_{rs_{exp}}$ at baseline in the intact DI response group (mean change ± SE: $-0.12 ± 0.06 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$, $P = 0.04$) but not in the impaired DI response group ($+0.05 ± 0.10 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$). Positive-pressure inflation had no effect on baseline $R_{rs_{exp}}$ in both groups ($P = 0.9$). $X_{rs_{exp}}$ was not significantly changed by either deep inspiration or positive-pressure inflation ($P > 0.2$).

Methacholine-induced changes
Methacholine significantly increased $R_{rs_{exp}}$ and decreased $X_{rs_{exp}}$ in both groups ($P < 0.01$; Figure 4A and 4B) at both visits. Both $R_{rs_{exp}}$ and $X_{rs_{exp}}$ were not significantly different between the groups following methacholine inhalation before deep inspiration or positive-pressure inflation ($P > 0.11$). The changes in $R_{rs_{exp}}$ and $X_{rs_{exp}}$ by methacholine and deep inspiration or positive-pressure inflation are summarized in Table 2.

Active deep inspiration
Active deep inspiration significantly decreased $R_{rs_{exp}}$ in the intact DI response group ($P = 0.003$, Figure 4A) but not in the impaired DI response group ($P = 0.9$), which confirmed the findings from the screening visit. Also the change in $R_{rs_{exp}}$ by active deep inspiration was significantly larger in the intact DI response group compared with the impaired DI response group ($P < 0.01$), which resulted in a significantly lower $R_{rs_{exp}}$ during the three tidal expirations following the active deep inspiration in the intact DI response group ($P < 0.01$). Interestingly, $X_{rs_{exp}}$ was significantly increased by active deep inspiration in both groups ($P < 0.02$; Fig. 4B), and the change in $X_{rs_{exp}}$ was not significantly different between the groups ($P = 0.5$). However, it resulted in a significantly higher $X_{rs_{exp}}$ following active deep inspiration in the intact DI response group ($P = 0.02$).

Positive-pressure inflation
$R_{rs_{exp}}$ was significantly decreased by positive-pressure inflation in both groups ($P < 0.02$), and the change induced by the positive-pressure inflation was not significantly different between the groups ($P = 0.8$). Also, $X_{rs_{exp}}$ was significantly increased by positive-pressure inflation in both groups ($P < 0.01$; Figure 4B) with no significant differences between the groups. Notably, the change induced in $R_{rs_{exp}}$ by the positive-pressure inflation was significantly larger than the change induced by active deep inspiration ($P = 0.002$) in the impaired DI response group, but not in the intact DI response group ($P = 0.18$) (Table 3).

Inspiratory volumes
VC, ERV, and IC were measured at baseline to calculate the volume to be inflated by the machine. Also, the actual inspired volume during both the active and positive-pressure inflation
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was measured. Using these values we calculated the percentage inspired volume of baseline inspiratory capacity during the maneuvers following methacholine inhalation. The percentage inspiratory volume of the active deep inspiration following methacholine inhalation was significantly lower in the impaired DI response group (mean ± SD: 71 ± 13%) compared with the intact DI response group (82 ± 12%, \( P = 0.027 \)) and was significantly increased by positive-pressure

Figure 4. Mean resistance of the respiratory system during tidal expiration (Rrs\text{exp}) during the challenges. Mean ± SE for Rrs\text{exp} (A) and reactance of the respiratory system during tidal expiration (Xrs\text{exp}; B) before methacholine inhalation (pre-mch), after methacholine inhalation (post-mch), and following deep inspiration (post-DI) for the intact DI response group (■) and the impaired DI response group (▲) are shown. The data points in the left panels represent the measurements of the active deep inspiration (ADI), and those in the right panels represent the measurements of the positive-pressure inflation (PPI). A: Rrs\text{exp} was significantly increased by methacholine at both visits (*\( P < 0.01 \)) in both groups. In the intact DI response group, Rrs\text{exp} was significantly decreased by both the active deep inspiration and positive-pressure inflation (†\( P < 0.01 \)). In the impaired DI response group Rrs\text{exp} was only significantly reduced by positive-pressure inflation (‡\( P < 0.02 \)). B: Xrs\text{exp} was significantly decreased by methacholine at both visits (*\( P < 0.01 \)) in both groups. In contrast with the Rrs results, Xrs\text{exp} was significantly increased by both the active deep inspiration and positive-pressure inflation in both groups (†‡\( P < 0.02 \)). However, Xrs\text{exp} following active deep inspiration was significantly higher in the intact DI response group (*\( P = 0.02 \)).
Data are expressed as means ± SE. All the Rrs and reactance of the respiratory system (Xrs) data measured at baseline and following methacholine inhalation (Post-methacholine), during active deep inspiration (ADI) and positive-pressure inflation (PPI), are shown for each group separately. Rrs at total lung capacity (TLC) could not be measured during PPI due to valve switching at that time point. Exp, expiration; FRC, functional residual capacity; pre-DI, before deep inspiration; post-DI, after deep inspiration (*P = 0.006). The change in Rrs<sub>exp</sub> by PPI was significantly larger than by ADI in the impaired DI response group †(P = 0.002).

inflation (mean change ± SD: 15 ± 14%; P = 0.011). Notably, although the reduction in Rrs<sub>exp</sub> was not related to the percentage inspiratory volume (P > 0.1; Figure 5), the increase in percentage inspiratory volume by positive-pressure inflation correlated with the increase in reduction of Rrs<sub>exp</sub> by positive-pressure inflation (P < 0.01; Figure 6).
Discussion

This study shows that airways obstruction can be reduced by positive-pressure inflation of the lungs in asthma. Interestingly, this could also be achieved in patients with asthma who were not capable of reducing respiratory resistance by an active deep inspiration. These results suggest that influencing transpulmonary pressures by mechanical inflation of the lung can restore the beneficial bronchodilatory effects of lung inflation in patients with asthma.

To our knowledge this is the first study that used a computer-controlled syringe to inflate the lungs of conscious subjects in the upright position, and at the same time continuously measure the change in airways obstruction by lung inflation by the positive-pressure inflation. The mean
reduction in $R_{rs exp}$ in all patients by the active deep inspiration was $0.7 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$, which is in line with our previous data measured by FOT showing a reduction of $0.6 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$ \cite{44}. Our data further extend the findings by Burns and Gibson \cite{12}, who showed that deep inspiration through an added resistance, compared with a regular deep inspiration, resulted in lower airway conductance in patients with asthma, but not in healthy subjects. They proposed that inflation by positive pressure could prevent the occurrence of edema. And indeed, positive-pressure inflation of the lungs resulted in an improvement of bronchodilation in these patients with asthma.

In our study we included 24 patients with mild to moderate persistent asthma. However, only in 12 patients was $R_{rs exp}$ significantly reduced by active deep inspiration. We questioned whether this difference was due to either limited dilation of the airways or to a difference in the response of the airways to stretch. Therefore, we calculated the $R_{rs}$ at the TLC and FRC level. We found that $R_{rs}$ at TLC was not significantly different between the groups (mean ± SE: impaired DI response group $2.1 \pm 0.2 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$; intact DI response group $1.9 \pm 0.1 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$; $P = 0.6$), but tended to be higher at FRC in the impaired DI response group ($3.8 \pm 0.1$ vs. $3.5 \pm 0.4 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$; $P = 0.09$). Following active deep inspiration $R_{rs}$ was significantly higher in the impaired DI response group (Table 2). This suggests that positive pressure and active deep inspiration dilated the airways to the same extent, but following active deep inspiration airways reconstricted more in the patients from the impaired DI response group.

Our data are in line with the results by Brown et al \cite{10} using high-resolution CT scans. They demonstrated that distension of constricted airways (>3 mm) by deep inspiration is not significantly different between healthy subjects and patients with mild asthma. However, following deep inspiration, the airways of healthy subjects remained dilated, whereas bronchoconstriction

\begin{figure}
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\includegraphics[width=0.5\textwidth]{figure6.png}
\caption{Correlation between the changes in reduction in $R_{rs exp}$ and percentage inspiratory volume by positive-pressure inflation.}
\end{figure}

We calculated the changes in % inspiratory volume (of baseline inspiratory capacity) and bronchodilation (reduction in $R_{rs exp}$) as induced by positive-pressure inflation compared with active deep inspiration. This figure shows the correlation between the changes in the percentage inspiratory volume and the changes in bronchodilation (reduction of $R_{rs exp}$) (Spearman rho = 0.6, $P = 0.04$; $y = -0.029x + 0.151$). Thus an increase in the percentage inspiratory volume by positive-pressure inflation was associated with more bronchodilation.
occurred in the asthmatic patients. These data, together with ours, suggest that the airway-parenchyma interdependence is not the reason for impaired bronchodilation following deep inspiration in patients with mild asthma. Other studies that used the FOT also demonstrated rapid renarrowing of airways following deep inspiration in mild asthma. A reduction in dilation of airways was only demonstrated in severe asthma. Therefore, airway distension during deep inspiration may become impaired with increasing asthma severity, whereas the airway response following deep inspiration is already impaired in mild asthma. This is also observed with the loss of bronchoprotection; therefore these two phenomena could clearly be linked.

We succeeded in safely inflating the airways of conscious sitting patients with a volume and inspiration time specific for each patient, and measured $R_{rs}$ and $X_{rs}$ continuously during these measurements. We used 8 Hz to measure impedance of the respiratory system, because this is close to the resonance frequency and thus would represent airway caliber predominantly. Respiratory resistance is frequency dependent. At lower frequencies (0.1–2 Hz) inhomogeneity and tissue resistance may increasingly affect impedance (constant-phase model). However, these frequencies are too close to the breathing frequency. The changes in $R_{rs\ exp}$ and $X_{rs\ exp}$ by deep inspiration and positive-pressure inflation were different. Possibly, the changes in $X_{rs\ exp}$ represent opening of closed airways or a change in compliance of the airway wall. On the other hand, $X_{rs\ exp}$ may be more sensitive, compared with $R_{rs\ exp}$, to measure changes in airway caliber or airway compliance. We did not measure FRC before the deep inspiration or positive-pressure inflation, and therefore we cannot exclude a difference in hyperinflation between the patients. However, we randomized the order of the inspiratory maneuvers, while both maneuvers were performed in the exact same way. Thus a difference in hyperinflation or calculated IC is unlikely to explain the differences between the groups. Unfortunately we did not include the measurement of transpulmonary pressures as well. Although it is most likely that the pressures within the alveoli and airways were supra-atmospheric during the inflation, we do not have access to these data. In addition, we asked the patients to completely relax and not to assist during the positive-pressure inflation. We have not observed any obvious active inspiration during the positive-pressure inflation, but we cannot exclude that this may have occurred. Nevertheless, the AFLG was able to reduce airways obstruction, as expected, and therefore can be used in future study designs to further investigate the pathophysiological mechanism of airway dilation by lung inflation.

The patients were selected based on their medical history regarding asthma symptoms, atopy, and airway hyperresponsiveness. Interestingly, the number of patients on inhaled steroids was equal among the two groups, confirming that under steroid treatment patients with asthma can exhibit both impaired and intact deep inspiration-induced bronchodilation. The patients were assigned to the impaired or intact DI response group based on the reduction in $R_{rs\ exp}$ following methacholine inhalation on the screening visit. However, the results shown for the reduction in $R_{rs\ exp}$ by active deep inspiration were measured on a randomized subsequent visit. The improvement in bronchodilation following positive-pressure inflation compared with
active deep inspiration was therefore not a result of regression to the mean. The response of the airways to either active deep inspiration or positive-pressure inflation was measured following methacholine inhalation only, since baseline intrinsic airway tone can be highly variable. The dose of methacholine that increased $R_{rs}$ by 50% ($PC_{50}R_{rs}$) was not significantly different between the groups at both visits and was not significantly different between the visits in each group. Therefore, we believe that the level of bronchoconstriction was similar between the groups, and between the two visits. Nevertheless, smooth muscle activity can still be variable even at the same fall in respiratory resistance, which cannot be excluded in this human in vivo study.

We aimed to inflate the lungs by 90% of baseline IC (VC minus ERV) for safety reasons to prevent overinflation. The mean percentage inspiratory volume of baseline IC by positive-pressure inflation was 83.5%. The discordance between the actual inflated percent volume and the calculated volume can be due to leakage of air between the lips and mouth piece, early start of the apparatus, or an increased ERV. Surprisingly, the positive-pressure inflation actually reached a higher percentage of baseline IC than an active deep inspiration in the impaired DI response group. Therefore, we do believe that the inflated volume was enough to dilate the airways adequately.

How could positive-pressure inflation of the lungs induce bronchodilation in patients who cannot achieve this by an active deep inspiration? First, positive-pressure inflation may have opened closed airways that could not be opened by active deep inspiration. Indeed, the improvement in reduction of airways obstruction by positive-pressure inflation over active deep inspiration was related to an increase in the percent inspired volume (Figure 6). However, the reduction in $R_{s}\ exp$ by active deep inspiration was not related to the percentage inspired volume. In addition, both active deep inspiration and positive-pressure inflation led to a significant increase in $X_{rs}\ exp$ in the impaired DI response group. Both observations may therefore not represent a causal relationship, but parallel consequences of the positive-pressure inflation. However, in both cases increased inspired volume by positive-pressure inflation may have led to an increase in tethering of the alveolar attachments, thereby improving the distension of the intraparenchymal airways.

On the other hand, positive-pressure inflation may have led to an increase in stretch of smooth muscle within the airway wall. Using high-resolution CT scans Brown and Mitzner showed in dogs that airways, with increased smooth muscle tone, cannot be dilated to their maximal diameter by transpulmonary pressures of up to 25 cmH$_2$O. This suggests that under physiological conditions it may be impossible to stretch constricted airways to the maximal diameter. This may be augmented by increased stiffness of the airway wall in patients with asthma as a result of chronic inflammation and remodeling. Many in vitro and in vivo animal studies have shown that length oscillations of smooth muscle cells are necessary to reduce stiffness and contractility of the cells. Positive-pressure inflation may have induced greater stretching forces on the airways, and thereby increased stretch of smooth muscle cells and thus reduced airway wall stiffness.
What may be the clinical implication of our study? Although we developed the ALFG in an experimental setting, noninvasive mechanical ventilation is a realistic treatment option in asthma at the emergency department\textsuperscript{15,31,46}. Especially during exacerbations of asthma it has been shown that deep inspirations can induce bronchoconstriction\textsuperscript{28}. Whether this is due to altered smooth muscle function that further constricts on stretch, or to acute inflammatory edema enhanced by large subatmospheric pressures during deep inspiration, remains unclear. However, in both conditions occasional inflation of the lungs (mimicking a deep inspiration) may perturb the ongoing pathophysiological process and act synergistically with pharmaceutical bronchodilators to reduce the airway narrowing\textsuperscript{21}. In the intensive care setting is has been shown that high-volume ventilation recruits closed airways, and prevents closure in combination with positive end-expiratory pressures at the mouth\textsuperscript{1,19,20}. Further studies are required before the use of positive-pressure deep inspirations could be implemented as an actual additional treatment option in the clinical setting.

In conclusion, positive-pressure inflation of the lungs can significantly enhance the reduction in airways obstruction compared with active deep inspiration in patients with asthma. In addition, the inspired volume during the active deep inspiration was significantly lower in patients who were not capable of reducing airways obstruction by deep inspiration compared with patients with an intact bronchodilatory effect of deep inspiration. This suggests that the tethering forces of the parenchyma during active deep inspiration, possibly in relation to the magnitude of the inspired volume, are not strong enough to adequately stretch the airway wall, which may be overcome by positive-pressure inflation.
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