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(Article begins on next page)
Ventilation Defect Formation in Healthy and Asthma Subjects Is Determined by Lung Inflation

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Abstract

Background: Imaging studies have demonstrated that ventilation during bronchoconstriction in subjects with asthma is patchy with large ventilation defective areas (Vdefs). Based on a theoretical model, we postulated that during bronchoconstriction, as smooth muscle force activation increases, a patchy distribution of ventilation should emerge, even in the presence of minimal heterogeneity the lung. We therefore theorized that in normal lungs, Vdefs should also emerge in regions of the lung with reduced expansion.

Objective: We studied 12 healthy subjects to evaluate whether Vdefs formed during bronchoconstriction, and compared their Vdefs with those observed in 9 subjects with mild asthma.

Methods: Spirometry, low frequency (0.15 Hz) lung elastance and resistance, and regional ventilation by intravenous 13NN-saline positron emission tomography were measured before and after a challenge with nebulized methacholine. Vdefs were defined as regions with elevated residual 13NN after a period of washout. The average location, ventilation, volume, and fractional gas content of the Vdefs, relative to those of the rest of the lung, were calculated for both groups.

Results: Consistent with the predictions of the theoretical model, both healthy subjects and those with asthma developed Vdefs. These Vdefs tended to form in regions that, at baseline, had a lower degree of lung inflation and, in healthy subjects, tended to occur in more dependent locations than in subjects with asthma.

Conclusion: The formation of Vdefs is determined by the state of inflation prior to bronchoconstriction.

Introduction

Using different imaging techniques, ventilation defective regions (Vdefs) have been demonstrated in asymptomatic asthma subjects [1] and after inhalation of methacholine [2–5] or after exercise [5,6]. Positron-emission tomography (PET) dynamic imaging studies of intravenously injected 13NN-saline showed the development of Vdefs during acute bronchoconstriction consistent with severely hyperventilating units clustered in relatively large, contiguous regions of the lung [3,4,7]. In a previous study of asthma subjects [3], we found that the ventilation defects tended to be located towards the dependent regions of the lung regardless of whether the same subject was imaged supine or prone, and attributed that to a vertical gradient of lung inflation. In addition, we found that in the prone position, where the subject’s lungs had a greater state of inflation, Vdefs tended to be smaller than in the supine position [3]. Those results were consistent with a theoretical model of bronchoconstriction that includes dynamic inter-dependence among parenchymal forces, gas pressures and airways in a tree structure [7]. The model also predicts that, for a given smooth muscle activation, a reduction in forces on the airway wall, due to less expanded parenchyma, should result in the emergence of Vdefs, and these should increase in size with further reductions in lung inflation [8].

Despite the fact that Vdefs tended to form in dependent regions of the lung, they did not form exclusively there [4]. This, it was speculated, could be because of other factors in the airways of asthma subjects such as inflammation, edema, and local smooth muscle hyperresponsiveness may predispose specific regions to become Vdefs. These considerations led us to question whether in subjects without asthma, Vdefs could form under bronchoconstrictive challenge and, if so, to wonder where the Vdefs would be located. Although Vdefs have been demonstrated widely in asthma subjects, they have not been previously shown to occur in healthy subjects challenged with methacholine. In this study, we sought to test whether Vdefs are formed in healthy subjects and compare their location with those seen in asthma subjects. Specifically, since healthy subject’s airways would be less likely to have airway inflammation, edema or remodeling and thus have only the regional influence of lung inflation in Vdef formation, we tested whether in healthy subjects after a bronchoconstrictive stimulus,
$V_{def}$ should be even more likely to form in more dependent zones compared to asthma subjects.

**Methods**

**Ethics statement**

The protocol, procedures, consent form and consent process were reviewed and approved by the Human Research Committee of the Massachusetts General Hospital. Before participation, written informed consent was obtained from all subjects.

**Subject characteristics**

Nine subjects with mild asthma and twelve healthy subjects were studied. Baseline characteristics are given in Table 1. Nine subjects with mild asthma and twelve healthy subjects were studied. The subjects were recruited by advertisements posted in the hospital and through general e-mail announcements within the Partners Healthcare System. Subjects with mild asthma were considered eligible if they had been diagnosed with asthma, were over age 18, had not had an upper respiratory infection in the last month prior to screening, had not been smoking for the 3 months prior to screening and had less than a 10 pack-year smoking history. Subjects with asthma were questioned to determine if their asthma met the National Asthma Education and Prevention Program’s definition for mild to moderate asthma [9]. Healthy subjects were considered eligible if they were over 18, had not been smoking for the 3 months prior to screening and had less than a 10 pack-year smoking history, had normal PFT results, had a PC20 dose >8 mg/ml, and had not had an upper respiratory infection in the last month prior to screening. Both subjects with asthma and healthy subjects were excluded if they were a member of the study staff, had other lung diseases or heart disease, were pregnant, or had been exposed to more than half of the expected radiation dose for the protocol in the past year (3.75 mSv). Subjects with asthma were also excluded if they had a history of being unresponsive to alllurterol, had taken oral steroids in the past year for subjects with asthma, or had an absolute contraindication for methacholine challenge testing (FEV$_1$<50% predicted or <1 L, heart attack or stroke in the last 3 months, uncontrolled hypertension, or known aortic aneurysm).

**Study protocol**

The study protocol consisted of one screening and one imaging session. If the subject had not had a standard methacholine (MCh) challenge test within the past year, one was conducted during the screening session in the seated position at least one week before the imaging session. If subjects were taking asthma medications, these were stopped prior to MCh challenge testing and imaging according to MCh challenge guidelines. The provocative concentration (Provocochline, Methapharm, Coral Springs, FL, USA) that caused a 20% fall in FEV$_1$ (PC20) was determined for all subjects up to a maximum dose of 25 mg/mL. Subjects with a PC20 dose >8 mg/mL and a past medical history of asthma were considered subjects with asthma while those with PC20 dose >8 mg/mL and a past medical history of asthma were considered healthy subjects. Subjects who did not meet these criteria were excluded. On the study day, before any study conditions was repeated starting five minutes after administration of the methacholine. Post-bronchoconstriction measurements were finished within 20 minutes to ensure being done between the peak and end of plateau phase of MCh action [13].

**Data analysis**

For baseline and during bronchoconstriction, images displaying the topographic distribution of $^{13}$NN tracer retention were generated from the activity remaining in the lung at the end of the 3-minute washout period. Using the tracer retention image taken during bronchoconstriction, a $V_{def}$ region of interest (ROI) was defined by selecting a set of voxels containing more than 20% of the highest tracer concentration on that image. The threshold value was a trade-off between obtaining a region large enough to reduce the effect of noise and small enough to include only areas of significant tracer retention. Little change in the size of the $V_{def}$ was seen when the value for thresholding was varied in the vicinity of 20%. Once the $V_{def}$ ROI was defined, all voxels outside of this region, but within the lung mask, defined better ventilated areas outside of $V_{def}$ (Out). The fraction of lung occupied by $V_{def}$ was calculated by dividing the number of voxels within the $V_{def}$ ROI by the total number of voxels in the lung mask ($F_{V_{def}}$).

The specific ventilation (alveolar ventilation per unit volume, $sV_A$) within $V_{def}$ was calculated from the washout kinetics of the average ROI $^{13}$NN concentration and expressed as a ratio of the $sV_A$ of the rest of the imaged lung ($V_{def}$/Out). The heterogeneity of the voxel-by-voxel $sV_A$ distribution for the imaged lung was characterized by the mean-normalized variance of the tracer washout rate [$cov^2(sV_A = (SD/mean)^2)$]. Regional fractional gas content (Fgas), defined as the volume fraction of gas contained in a lung region, was calculated for each ROI from the transmission scans [4]. Average Fgas for the entire imaged lung, the vertical gradient in Fgas, and the relative Fgas (Fgas/0/ROI) of $V_{def}$ regions in relation to that of the rest of the lung were also calculated. The
average location of the Vdefs ROI, relative to the imaged lung, was calculated as the distance between the geometric center of the lung mask \(G_{\text{CLung}}\) and that of the Vdefs \(G_{\text{CVdefs}}\) in the left-to-right and dorso-ventral directions (Figure 1). The deviations between the \(G_{\text{CLung}}\) and \(G_{\text{CVdefs}}\) were normalized by the corresponding width (right-left) and height (ventral-dorsal) with the positive sign assigned to the right and ventral directions.

Because of the limited sample size of the study, the non-parametric Wilcoxon two sample rank sum test was used to assess significance comparing before and after methacholine at a level of \(p < 0.05\) and analysis was performed using STATISTICA (StatSoft, Inc.; Tulsa, OK). Data are expressed as mean \(\pm SD\). Linear regression was performed on plots of \(F_{\text{gas}}\) versus height using MATLAB (The Mathworks, Natick, MA).

### Results

In the upright position, healthy subjects and subjects with mild asthma had normal pulmonary function test results measured at baseline (Table 2). In that position, the methacholine dose to the asthmatic subjects was selected such that FEV\(_1\), was reduced by 20% (PC\(_{20} = 1.23 \pm 1.24 \text{ mg/mL}\)). Also, in the erect position, by protocol, the maximum methacholine dose given to the normal subjects (25 mg/mL) reduced FEV\(_1\) by less than 20%. These results contrast with those measured in the supine position the day of the study: FEV\(_1\) decreased by almost 40% in asthma subjects versus 25% in healthy subjects \((p < 0.05\) both much greater than the reductions seen in the erect position (Table 2). Consistent with the greater degree of FEV\(_1\) drop during the methacholine challenge, \(R_{\text{low}}\) increased significantly more in asthma subjects than in healthy subjects. \(E_{\text{low}}\) after methacholine was reduced in both groups but there was no difference in the percent drop between the two groups in (Table 2).

#### Formation of ventilation defects

After the methacholine challenge Vdefs formed in both groups, with similar degrees of hypoventilation relative to the rest of the lung.

The specific ventilation of Vdefs was less than half of that of the rest of the lung (Table 2), and this was not different between both groups. Similarly, the fraction if the imaged volume of lung occupied by Vdefs for both subject groups was not different (Table 2).

The global heterogeneity in ventilation (measured as the coefficient of variation of specific ventilation) increased in both groups during the methacholine challenge but there was no

### Table 1. Subject Characteristics.

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<th>Height (in)</th>
<th>Weight (lb)</th>
<th>PC20 (mg/mL)</th>
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<th>Weight (lb)</th>
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<td>M</td>
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<td>51.1</td>
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doi:10.1371/journal.pone.0053216.t001
significant difference in heterogeneity between the two groups at baseline or during bronchoconstriction.

Before the challenge, the regions that later became Vdefs had an average specific ventilation that was not different to that of the rest of the lung. In other words, the Vdefs and the rest of the lung had similar specific ventilation at baseline. In both healthy subjects and subjects with asthma, Vdefs were formed generally, but not always, in the most dependent (dorsal) regions of the lung (Figure 1). In average, the geometric center of the Vdefs was vertically more dependent than the geometric center of the imaged lung, (p<0.05) in both of the groups. However, the healthy subjects had, on average, a more dependent deviation of the Vdefs (p<0.05, Figure 1 and Figure 2). In the horizontal (right to left) direction, in average there were not systematic deviations between the geometric centers of the Vdefs and the lung.

### Mean and regional lung inflation

Both groups experienced a similar average increase in Fgas of 7% during bronchoconstriction with the magnitude of the increase negatively related with the baseline mean Fgas (Figure 3 and Table 2). Before bronchoconstriction, the ratio in Fgas within areas that became ventilation defects over that of the rest of the lung (FgasVdef/Out) was in average less than unity (p<0.05) in both healthy subjects and subjects with asthma (Figure 4). This means that at baseline the areas that after challenge became Vdefs were less expanded than the rest of the lung. In addition, mean FgasVdef/Out at baseline was significantly lower (p<0.05) in healthy subjects compared to that in subjects with asthma. Thus, compared with the rest of the lung, parenchymal expansion at baseline of areas that became Vdefs after challenge was lower in the healthy than in asthma subjects (Table 2 and Figure 4). During bronchoconstriction there was no difference in mean FgasVdef/Out between groups (Table 2 and Figure 4), indicating that once the Vdefs were formed, the contrast in lung expansion between Vdefs and is the rest of the lung was similar in asthma and healthy subjects.

### Vertical gradient

At baseline, there were systematic vertical gradients in Fgas such that Fgas increased with the height above the lower surface of the imaged lung (gradient of Fgas >0, p<0.05). Also, there was no

---

**Table 2. Results.**

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Asthma</th>
<th>% Change</th>
<th>Baseline</th>
<th>BC</th>
<th>% Change</th>
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</thead>
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<td>FEV1 (L)</td>
<td>2.74±0.89</td>
<td>2.05±0.80</td>
<td>−25.1±14.6</td>
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<td>1.62±0.36</td>
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<tr>
<td>FVC (L)</td>
<td>3.18±1.00</td>
<td>2.51±0.95</td>
<td>−21.2±12.8</td>
<td>3.02±0.58</td>
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<td>Rlow</td>
<td>5.43±1.00</td>
<td>92.0±54.7</td>
<td>1730±1250</td>
<td>5.87±2.58</td>
<td>177±103</td>
<td>3300±2090*</td>
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<tr>
<td>Fgas</td>
<td>6.27±1.90</td>
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<td>82.3±74.0</td>
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<td>Mean Lung Fgas</td>
<td>0.65±0.06</td>
<td>0.69±0.04</td>
<td>7.79±5.38</td>
<td>0.64±0.04</td>
<td>0.69±0.04</td>
<td>7.33±5.24</td>
</tr>
<tr>
<td>FgasVdef/Out</td>
<td>0.94±0.04*†</td>
<td>1.00±0.02</td>
<td>6.94±4.98</td>
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<tr>
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<td>0.34±0.14</td>
<td></td>
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</table>

Values are means ± SD. Rlow, low-frequency resistance; Elow, low-frequency elastance; Fgas, fractional gas content; Vdef, ventilation defect; VV/Vdef/Out, specific ventilation inside Vdefs vs. outside; cov2 x Vdef, coefficient of variation of specific ventilation; F Vdef, fraction of total imaged volume occupied by Vdefs. % change refers to the change during bronchoconstriction compared to baseline. *P<0.05 compared to Healthy.

P<0.05 compared to ratio of 1.

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difference in mean $F_{\text{gas}}$ gradient between both groups (Table 2 and Figure 5). During bronchoconstriction, both groups demonstrated a reduction in $F_{\text{gas}}$ gradient, which was reduced from 0.0069±0.003 cm$^{-2}$ to 0.0042±0.002 cm$^{-2}$ in healthy subjects, and from 0.0068±0.0034 cm$^{-2}$ to 0.0004±0.0039 cm$^{-2}$ in subjects with asthma. Thus, during bronchoconstriction the average $F_{\text{gas}}$ gradient of both groups was no longer greater than zero. It follows that the 0.07 increase in mean $F_{\text{gas}}$ following methacholine challenge, and the loss of the vertical gradient were largely driven by a regional increase in $F_{\text{gas}}$ in the most dependent lung (Figure 6).

Discussion

This study demonstrated that, as in asthma subjects, large ventilation defects do form in healthy subjects after inhalation of methacholine. Although by protocol, the dose of the agonist given to healthy subjects reduced FEV$_1$ less than 20% when tested in erect position; when measured during scanning in the supine position the reduction of FEV$_1$ was in average 25%. Both in healthy and asthma subjects the $V_{\text{defs}}$ tended to develop in dependent and less expanded areas (lower $F_{\text{gas}}$) of the lung. Healthy subjects do not differ from asthma subjects in this regard, except that $V_{\text{defs}}$ of healthy subjects were more dependent than those in asthma subjects. Given that both asthma and healthy subjects demonstrated vertical gradients in lung inflation (Figure 5 and Figure 6), taken together these results imply that for healthy subjects, the $V_{\text{defs}}$ are favored to form in regions of reduced lung inflation prior to agonist inhalation, whereas in asthma subjects, the formation of $V_{\text{defs}}$ may be affected by other factors that are not necessarily localized in dependent regions of the lung.

Before discussing the significance of these results, it is important to note some limitations of this study. The general experimental limitations of the PET imaging technique have been discussed in previous reports [3,4,14–16]. Among others they include the limited spatial resolution of PET, the fact that the tracer is delivered by perfusion into distal regions, and the need to study the subject in the recumbent positions. The relatively low spatial resolution prevents the visualization and direct quantification of ventilation and $F_{\text{gas}}$ heterogeneities within regions $<\sim1$ cm$^3$. This limits the volume at which $V_{\text{defs}}$ can be detected and reduced the contrast in specific ventilation between $V_{\text{defs}}$ and the rest of the lung. However, given the inherent patchiness of bronchoconstriction [7] observed both in animals [17,18] and humans [4], the limited spatial resolution should not invalidate the main result of this paper: that $V_{\text{defs}}$ also form in healthy supine subjects and that these tend to develop in the dependent and less distended regions of the lung.

The delivery of the $^{13}$NN tracer intravenously implies that the intrapulmonary distribution of the tracer before the washout is proportional to by perfusion. Thus, if the $V_{\text{defs}}$ received less perfusion than the rest of the lung, as we have previously observed [4], regions of severe vasoconstriction could have been excluded from $V_{\text{defs}}$. However, in spite of the reduced initial tracer concentration in $V_{\text{defs}}$, at the end of the washout period the activity remaining was much higher that that in the rest of the lung.
lung. In fact, because the tracer delivered intravenously bypasses the elevated resistance of peripheral airways, the $^{13}$NN method can be more sensitive detecting regions of severe hypoventilation than methods delivering a tracer by inhalation.

Finally, it is unclear whether $V_{\text{defs}}$ would form in healthy erect subjects following methacholine inhalation. For obvious regions this study was limited to imaging subjects in the recumbent position. This has two consequences: 1) the reduction in functional residual capacity of the supine compared with the erect position was probably responsible for the exaggerated obstruction by methacholine inhalation and additional reduction in $FEV_1$ [19], and 2) in addition to the effect of gravity on the lung parenchyma and mediastinal structures, the position and shape of the diaphragm [20] was likely responsible for the reduced lung inflation of the dependent regions that potentiated the formation of $V_{\text{defs}}$.

We limited the study to subjects with mild asthma, in which other factors such as airway remodeling, inflammation and mucus are likely to be small or absent. These factors may have greater importance in affecting the formation of $V_{\text{defs}}$ as the severity of

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**Figure 5. Fractional gas content ($F_{\text{gas}}$) gradients in healthy controls and subjects with asthma at baseline and during bronchoconstriction.** The grayscale of the squares in the plots corresponds to the number of data points with that value such that the darker the square, the greater the number of points with that value. The linear regression line (solid black) is shown and the number at the top of each line is the gradient ($x10^{-2}/\text{cm}$) for each plot.
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asthma worsens. Another limitation of the protocol was the difference in methacholine dose given to subjects. The asthma subjects received their respective PC20 doses, but healthy subjects were only challenged with the maximum recommended dose for methacholine challenge tests (25 mg/mL). This means that although healthy subjects actually received the highest doses of methacholine, based on the functional (FEV1) change, they were less constricted than the subjects with asthma. Thus from this study we cannot rule out that if the bronchoconstrictive stimulus was matched in both groups the difference in vertical dependence of Vdefs would disappear. However, we found that plotting the Vdef location versus change in FEV1 did not show any correlation between strength of constriction and vertical location of Vdefs for

Figure 6. Height above the lung versus gas fraction (Fgas) at baseline (solid line) and during bronchoconstriction (dotted line). Except for h056 and h058 in the healthy control group and h032 and h034 in the asthma group, the observed increase in mean lung Fgas was mostly due to an increase in Fgas in the most dependent lung.

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subjects with asthma. In addition, at baseline deviation of could have been distributed more homogeneously. Methacholine being given in the prone position, where the aerosol present study (subjects studied in the supine position was slightly less than in the healthy subjects (Table 2 and Figure 2). Also, subjects with asthma having a tendency to form in dependent zones, had less vertical dependent zones. A small difference of parenchymal distending forces in the supine together, these findings support the notion that in healthy subjects decreasing in the dorsal to ventral direction (Figure 5). Taken asthma and healthy controls is likely not due to any difference in the dose of methacholine. doi:10.1371/journal.pone.0053216.g007

healthy subjects, even for those subjects with FEV1 changes similar to those of the asthma subjects (Figure 7).

Ventilation defective regions have been demonstrated in asthma subjects after methacholine using PET [3,4,7] or MRI [1,2,21], after exercise [5] and in stable patients with asthma [22] using hyperpolarized gas magnetic resonance imaging (MRI). Our group previously reported a nearly equal vertical dependence of Vdefs formation in asthma subjects regardless of position, either prone or supine [3]. The ventral deviation of Vdefs in those asthma subjects studied in the supine position was slightly less than in the present study (−0.12 versus −0.17), which may be a result of the methacholine being given in the prone position, where the aerosol could have been distributed more homogeneously.

Although Vdefs formed preferentially in dependent regions of the lung for both groups (Figure 2), in healthy subjects, the vertical deviation of Vdefs was more pronounced compared with that of subjects with asthma. In addition, at baseline FgasVdef/out was less than unity in both groups but significantly lower in healthy subjects compared to that of subjects with asthma. The observation of a baseline FgasVdef/out lower than unity is consistent with a previous study where in 9 out of 11 subjects with mild asthma had a baseline FgasVdef/out<1 [4]. Furthermore, healthy subjects demonstrate clear vertical gradients in Fgas, with Fgas decreasing in the dorsal to ventral direction (Figure 5). Taken together, these findings support the notion that in healthy subjects a small difference of parenchymal distending forces in the supine position was enough to drive the formation of ventilation defects in dependent zones.

Ventilation defective regions in subjects with asthma, while still having a tendency to form in dependent zones, had less vertical deviation toward dependent regions when compared to that of healthy subjects (Table 2 and Figure 2). Also, subjects with asthma had slightly greater FgasVdef/out compared to that of healthy subjects but yet had similar baseline and bronchoconstriction Fgas gradients as those of healthy subjects. These results suggest that the reduced vertical dependence of Vdefs formation in subjects compared to that of healthy subjects was not due to differences in local lung inflation but may have been related to other factors that could precipitate the formation of Vdefs. Examples of such factors may include differences in airway wall thickness (smooth muscle hyperplasia, edema and/or inflammation), smooth muscle contractile strength (hyperresponsiveness), airway mucus, or uncoupling between airway wall and parenchyma reducing local parenchymal tethering forces [3]. Another factor potentially affecting the location of Vdefs in the asthma subjects could have been a heterogeneous deposition of the methacholine aerosol due to heterogeneous ventilation, which would have been affected by the factors previously described.

We found an increase in lung mean Fgas and of imaged lung field volume as a result of the methacholine challenge for healthy and asthma subjects. These increases are similar to those measured for supine subjects in previous studies [3,4]. What is notable in this study is that, despite a greater degree of global obstruction caused by the methacholine challenge in the asthma subjects (higher Rlow and lower FEV1), both groups had similar increases in lung volume and similar sized Vdefs, which are consistent with the lack of difference of the change in Elow between the two groups. In addition, a relative increase in regional expansion of the ventilation defective regions (FgasVdef/out increased in all regions) was detected during bronchoconstriction. As in previously studies [3,4], the relatively greater increase in Fgas observed in Vdefs compared with the rest of the lung may be caused by either a relative reduction in local blood volume or to dynamic hyperinflation of these regions.

There are several potential clinical implications of these findings. It is known that the supine position results in greater bronchial hyperresponsiveness compared with the erect position [19] and a many patients with asthma often exhibit increased symptoms at night [23]. We have shown previously [3] that reduced lung volume in the supine position compared with prone during bronchoconstriction results in larger Vdefs for the same dose of MCh. Indeed, studies using nocturnal continuous positive airway pressure in asthma have shown improved nocturnal asthma symptoms [24], quality of life [25] and airway hyperresponsiveness [26].

In conclusion, in healthy subjects and very mild asthma, the formation of Vdefs depends on the state of regional lung inflation prior to inhalation of agonist. What is remarkable is how such a small difference in parenchymal distension at baseline can predispose the formation of Vdefs. This finding, however, is consistent with the theoretical predictions of model of the lung that included short and long distance dynamic interactions between airway pressure, parenchymal forces and the airway smooth muscle. In that model, a reduction in lung volume decreases the critical level of smooth muscle constriction needed to trigger Vdef formation [7]. Thus, in the presence of elevated smooth muscle activity, even a small local reduction in lung volume can be sufficient to trigger the emergence of a Vdef. In more severe asthma, it can be expected that other factors may be more important in triggering the formation of Vdefs, such as airway inflammation, smooth muscle hypertrophy, or airway edema.

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Author Contributions

Conceived and designed the experiments: RSH JGV. Performed the experiments: RSH TW GM MFVM JGV. Analyzed the data: RSH TW GM MFVM JGV. Wrote the paper: RSH HFR TW GM MFVM JGV.
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