

# Ventilation Defect Formation in Healthy and Asthma Subjects Is Determined by Lung Inflation

R. Scott Harris<sup>1\*</sup>, Hanae Fujii-Rios<sup>1</sup>, Tilo Winkler<sup>2</sup>, Guido Musch<sup>2</sup>, Marcos F. Vidal Melo<sup>2</sup>, José G. Venegas<sup>2</sup>

**1** Department of Medicine (Pulmonary and Critical Care Unit), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States of America, **2** Anesthesia and Critical Care, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States of America

## 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 <sup>13</sup>NN-saline positron emission tomography were measured before and after a challenge with nebulized methacholine. *Vdefs* were defined as regions with elevated residual <sup>13</sup>NN 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.

**Citation:** Harris RS, Fujii-Rios H, Winkler T, Musch G, Vidal Melo MF, et al. (2012) Ventilation Defect Formation in Healthy and Asthma Subjects Is Determined by Lung Inflation. PLoS ONE 7(12): e53216. doi:10.1371/journal.pone.0053216

**Editor:** Marco Idzko, University Hospital Freiburg, Germany

**Received:** August 23, 2012; **Accepted:** November 27, 2012; **Published:** December 28, 2012

**Copyright:** © 2012 Harris et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was supported by grants from the National Institutes of Health: HL-68011 to JGV and HL-04501 to RSH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: rsharris@mgh.harvard.edu

## 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 <sup>13</sup>NN-saline showed the development of *Vdefs* during acute bronchoconstriction consistent with severely hypoventilating 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,

$V_{def}$ s 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 albuterol, 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.<sup>2</sup> The provocative concentration (Provocholine, 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  $\leq 8$  mg/mL and a past medical history of asthma were considered subjects with asthma while those with PC20 dose  $>8$  mg/mL without any past medical history of asthma were considered as healthy subjects. Subjects who did not meet these criteria were excluded. On the study day, before any study procedures all subjects had spirometry, plethysmographic lung volumes and diffusing capacity measured in the upright position to verify that they did not have obstruction or restriction and to confirm that the DLCO was in the normal range. Subjects were then positioned supine in the PET scanner, with their arms outside the scanner resting on armrests, and remained in that position for the rest of the study, including during MCh inhalation. The

imaging field was selected to include the bases of the lung above the diaphragm to maximize the volume of lung within the 10 cm long field of view of the PET scanner. Lung volume changes were monitored continuously by impedance plethysmography (SomnoStar PT, SensorMedics Corp., Yorba Linda, CA, USA) and the signal continuously displayed to the subject on a computer screen. Oscillatory mechanics were measured as previously described [10–12] and the low frequency (0.15 Hz), resistance ( $R_{low}$ ) and elastance ( $E_{low}$ ) were derived. After acquiring a 10-minute transmission scan and baseline oscillatory mechanics, the subject was instructed to take two deep breaths. During the exhalation phase of the second breath, the subject was instructed to stop breathing at lung volume equal to the mean lung volume previously estimated from the impedance plethysmographic signal during steady state breathing. At the start of apnea, a bolus of  $^{13}N$ -saline ( $\sim 30$  ml) was injected intravenously (5 ml/s) and dynamic acquisition of sequential emission scans was initiated [3]. After 30 to 40 s of apnea the subject was instructed to resume breathing while coached to match his/her previous rate and tidal volume as displayed on the computer screen. At the end of a 3-minute washout period, spirometry was performed in the same position using a hand-held portable spirometer (Satellite Spirometer, Jones Medical Instrument Co., Oak Brook, IL, USA). While supine with the head turned to the side, five breaths of methacholine were then administered to the subject at his/her previously determined PC20 dose or up to a maximum of 25 mg/mL for healthy controls via a DeVilbiss nebulizer (model 646, DeVilbiss Healthcare Company, Somerset, PA, USA). An identical imaging sequence and data collection to that acquired in baseline 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  $^{13}N$  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  $V_{def}$ s 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  $V_{def}$ s (Out). The fraction of lung occupied by  $V_{def}$ s 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,  $s\dot{V}_A$ ) within  $V_{def}$ s was calculated from the washout kinetics of the average ROI  $^{13}N$  concentration and expressed as a ratio of the  $s\dot{V}_A$  of the rest of the imaged lung ( $\dot{V}_{V_{def}/Out}$ ). The heterogeneity of the voxel-by-voxel  $s\dot{V}_A$  distribution for the imaged lung was characterized by the mean-normalized variance of the tracer washout rate [ $cov^2 s\dot{V}_A = (SD/mean)^2$ ]. Regional fractional gas content ( $F_{gas}$ ), defined as the volume fraction of gas contained in a lung region, was calculated for each ROI from the transmission scans [4]. Average  $F_{gas}$  for the entire imaged lung, the vertical gradient in  $F_{gas}$ , and the relative  $F_{gas}$  ( $F_{gas_{V_{def}/Out}}$ ) of  $V_{def}$ s regions in relation to that of the rest of the lung were also calculated. The

**Table 1.** Subject Characteristics.

| Subjects       | Age (yr) | Gender | BMI (kg/m <sup>2</sup> ) | Height (in) | Weight (lb) | PC20 (mg/mL) |
|----------------|----------|--------|--------------------------|-------------|-------------|--------------|
| <b>Healthy</b> |          |        |                          |             |             |              |
| h041           | 42       | M      | 23.5                     | 73          | 178         | >25          |
| h044           | 55       | F      | 29.2                     | 63          | 165         | >25          |
| h045           | 20       | M      | 26.6                     | 69          | 180         | >25          |
| h046           | 64       | F      | 29.2                     | 64          | 170         | >25          |
| h047           | 55       | F      | 28.9                     | 63          | 163         | >25          |
| h050           | 34       | F      | 24.5                     | 64          | 143         | >25          |
| h052           | 39       | M      | 24.3                     | 73          | 184         | >25          |
| h053           | 23       | F      | 33.5                     | 64          | 195         | >25          |
| h056           | 24       | F      | 22.7                     | 62.5        | 126         | >25          |
| h057           | 56       | M      | 27.7                     | 70          | 193         | >25          |
| h058           | 49       | F      | 19.9                     | 63.75       | 115         | >25          |
| h064           | 24       | F      | 22.7                     | 62          | 124         | >25          |
| <b>average</b> | 40.4     |        | 26.1                     | 66.0        | 161.3       |              |
| <b>sd</b>      | 15.4     |        | 3.8                      | 4.2         | 27.8        |              |
| Subjects       | Age (yr) | Gender | BMI (kg/m <sup>2</sup> ) | Height (in) | Weight (lb) | PC20 (mg/mL) |
| <b>Asthma</b>  |          |        |                          |             |             |              |
| h029           | 43       | M      | 30                       | 75          | 240         | 2.50         |
| h031           | 56       | F      | 36.9                     | 68          | 243         | 3.46         |
| h032           | 22       | F      | 26.3                     | 62.5        | 146         | 0.38         |
| h034           | 27       | F      | 17.1                     | 67          | 109         | 2.40         |
| h035           | 31       | F      | 23.6                     | 61.5        | 127         | 0.24         |
| h036           | 24       | M      | 24.2                     | 65          | 145         | 1.25         |
| h038           | 39       | M      | 30.3                     | 70          | 211         | 0.25         |
| h043           | 50       | F      | 23.9                     | 66          | 148         | 0.34         |
| h054           | 40       | M      | 30.8                     | 70          | 215         | 0.25         |
| <b>average</b> | 36.9     |        | 27.1                     | 67.2        | 176.0       | 1.23         |
| <b>sd</b>      | 11.8     |        | 5.7                      | 4.2         | 51.1        | 1.24         |

doi:10.1371/journal.pone.0053216.t001

average location of the  $V_{def}$  ROI, relative to the imaged lung, was calculated as the distance between the geometric center of the lung mask ( $GC_{Lung}$ ) and that of the  $V_{def}$  ( $GC_{V_{def}}$ ) in the left-to-right and dorso-ventral directions (Figure 1). The deviations between the  $GC_{Lung}$  and  $GC_{V_{def}}$  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  $F_{gas}$  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% ( $PC_{20} = 1.23 \pm 1.24$  mg/mL). Also, in the erect position, by protocol, the maximum methacholine dose given to the normal

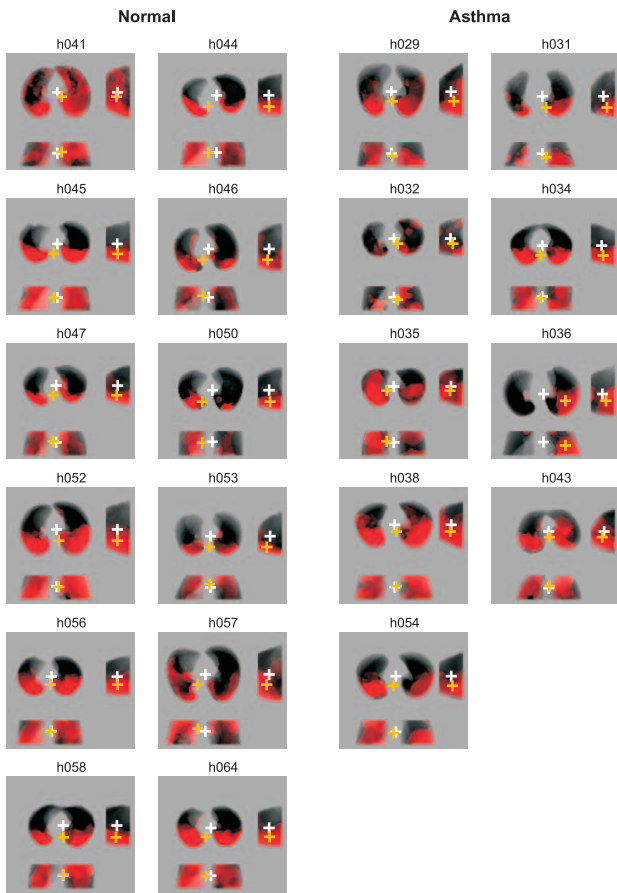
subjects (25 mg/mL), reduced  $FEV_1$  by less than 20%. These results contrast with those measured in the supine position the day of the study:  $FEV_1$  decreased by almost 40% in asthma subjects versus 25% in healthy subjects ( $p < 0.05$ ) both much greater than the reductions seen in the erect position (Table 2). Consistent with the greater degree of  $FEV_1$  drop during the methacholine challenge,  $R_{low}$  increased significantly more in asthma subjects than in healthy subjects.  $E_{low}$  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  $V_{def}$ s formed in both groups, and with similar degrees of hypoventilation relative to the rest of the lung

The specific ventilation of  $V_{def}$ s 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 of the imaged volume of lung occupied by  $V_{def}$ s 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



**Figure 1. Analysis of geometric center of the lungs (GCLung) and ventilation defects (GCVdef).** The left column shows projections of Vdefs (in red) for healthy subjects and the right column for subject with mild asthma. The white cross represents the GCLung and the orange cross is the GCVdef. There are three views of the lung in each image (clockwise from top left): transverse viewed caudo-cranially, sagittal, and coronal. In these images, Vdefs that deviate toward the bottom of the page result in negative distances between GCLung and GCVdef in the transverse, sagittal and coronal planes.  
doi:10.1371/journal.pone.0053216.g001

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 ( $F_{gas\ Vdef/Out}$ ) 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  $F_{gas\ Vdef/Out}$  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  $F_{gas\ Vdef/Out}$  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

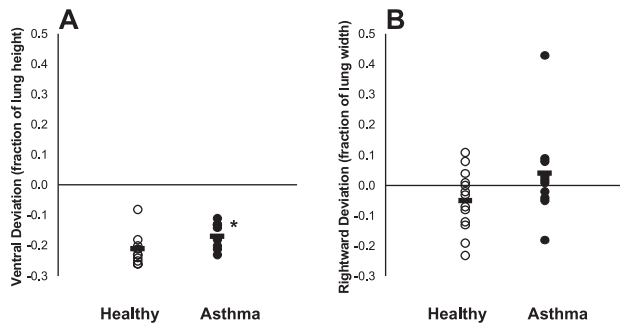
**Table 2. Results.**

|                                | Healthy                |                        |            | Asthma                  |                        |             |
|--------------------------------|------------------------|------------------------|------------|-------------------------|------------------------|-------------|
|                                | Baseline               | BC                     | % Change   | Baseline                | BC                     | % Change    |
| FEV <sub>1</sub> (L)           | 2.74±0.89              | 2.05±0.80              | -25.1±14.6 | 2.64±0.45               | 1.62±0.36              | -38.7±7.23* |
| FVC (L)                        | 3.18±1.00              | 2.51±0.95              | -21.2±12.8 | 3.02±0.58               | 2.06±0.60              | -33.1±8.91* |
| R <sub>low</sub>               | 5.43±1.00              | 92.0±54.7              | 1730±1250  | 5.87±2.58               | 177±103                | 3300±2090*  |
| E <sub>low</sub>               | 6.27±1.90              | 10.1±4.00              | 61.6±37.3  | 8.85±3.00               | 17.2±11.8              | 82.3±74.0   |
| Mean Lung F <sub>gas</sub>     | 0.65±0.06              | 0.69±0.04              | 7.79±5.38  | 0.64±0.04               | 0.69±0.04              | 7.33±5.24   |
| F <sub>gas Vdef/Out</sub>      | 0.94±0.04 <sup>†</sup> | 1.00±0.02              | 6.94±4.98  | 0.98±0.03* <sup>†</sup> | 1.03±0.05              | 5.87±4.86   |
| F <sub>gas gradient</sub>      | 0.0069±0.0033          | 0.0004±0.0020          | -90.0±38.0 | 0.0068±0.0033           | 0.0004±0.0039          | -105±51.8   |
| $\dot{V}_{Vdef/Out}$           | 1.14±0.14 <sup>†</sup> | 0.44±0.15 <sup>†</sup> | -61.0±16.3 | 1.04±0.14               | 0.54±0.16 <sup>†</sup> | -46.0±25.0  |
| cov <sup>2</sup> s $\dot{V}_A$ | 0.57±0.08              | 0.94±0.25              | 65.2±200   | 0.61±0.19               | 1.05±0.24              | 71.2±24.7   |
| F <sub>Vdef</sub>              |                        | 0.34±0.11              |            |                         | 0.34±0.14              |             |

Values are means ± SD. R<sub>low</sub>, low-frequency resistance; E<sub>low</sub>, low-frequency elastance; F<sub>gas</sub>, fractional gas content; Vdef, ventilation defect;  $\dot{V}_{Vdef/Out}$ , specific ventilation inside Vdefs vs. outside; cov<sup>2</sup> s $\dot{V}_A$ , coefficient of variation of specific ventilation; F<sub>Vdef</sub>, fraction of total imaged volume occupied by Vdefs. % change refers to the change during bronchoconstriction compared to baseline. \*P<0.05 compared to Healthy.

<sup>†</sup>P<0.05 compared to ratio of 1.

doi:10.1371/journal.pone.0053216.t002

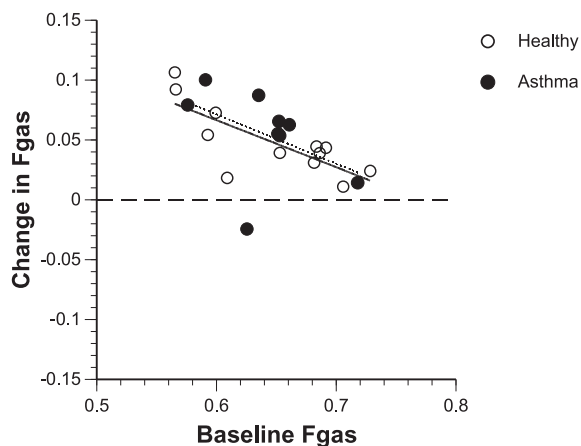


**Figure 2. Plots of Geometric Centers in subjects with asthma vs. healthy subjects.** A. The ventral deviation of calculated geometric center of ventilation defects ( $GCV_{Vdef}$ ) from the geometric center of the lungs ( $GCLung$ ). Both healthy subjects and subjects with asthma demonstrated a vertical dependence in  $Vdefs$ . However healthy subjects have a significantly ( $P = .028$ ) lower ventral deviation compared to that of subjects with asthma. B. The horizontal (x-axis) deviation of calculated geometric center of ventilation defects ( $GCV_{Vdef}$ ) from the geometric center of the lungs ( $GCLung$ ). ( $p > 0.05$ ). doi:10.1371/journal.pone.0053216.g002

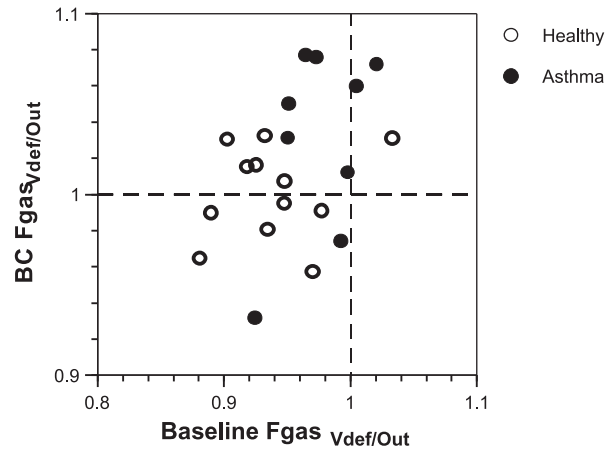
difference in mean  $F_{gas}$  gradient between both groups (Table 2 and Figure 5). During bronchoconstriction, both groups demonstrated a reduction in  $F_{gas}$  gradient, which was reduced from  $0.0069 \pm 0.003 \text{ cm}^{-1}$  to  $0.0004 \pm 0.002 \text{ cm}^{-1}$  in healthy subjects, and from  $0.0068 \pm 0.0034 \text{ cm}^{-1}$  to  $0.0004 \pm 0.0039 \text{ cm}^{-1}$  in subjects with asthma. Thus, during bronchoconstriction the average  $F_{gas}$  gradient of both groups was no longer greater than zero. It follows that the 0.07 increase in mean  $F_{gas}$  following methacholine challenge, and the loss of the vertical gradient were largely driven by a regional increase in  $F_{gas}$  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



**Figure 3. Plot of change in fractional gas content ( $F_{gas}$ ) in healthy subjects and subjects after bronchoconstriction versus baseline  $F_{gas}$ .** Mean Lung  $F_{gas}$  increased after bronchoconstriction for both healthy subjects and subjects with asthma. Subjects that initially had a lower baseline mean lung  $F_{gas}$  had a larger increase in mean lung  $F_{gas}$  during bronchoconstriction when compared to those that started off with a higher baseline mean lung  $F_{gas}$ . doi:10.1371/journal.pone.0053216.g003



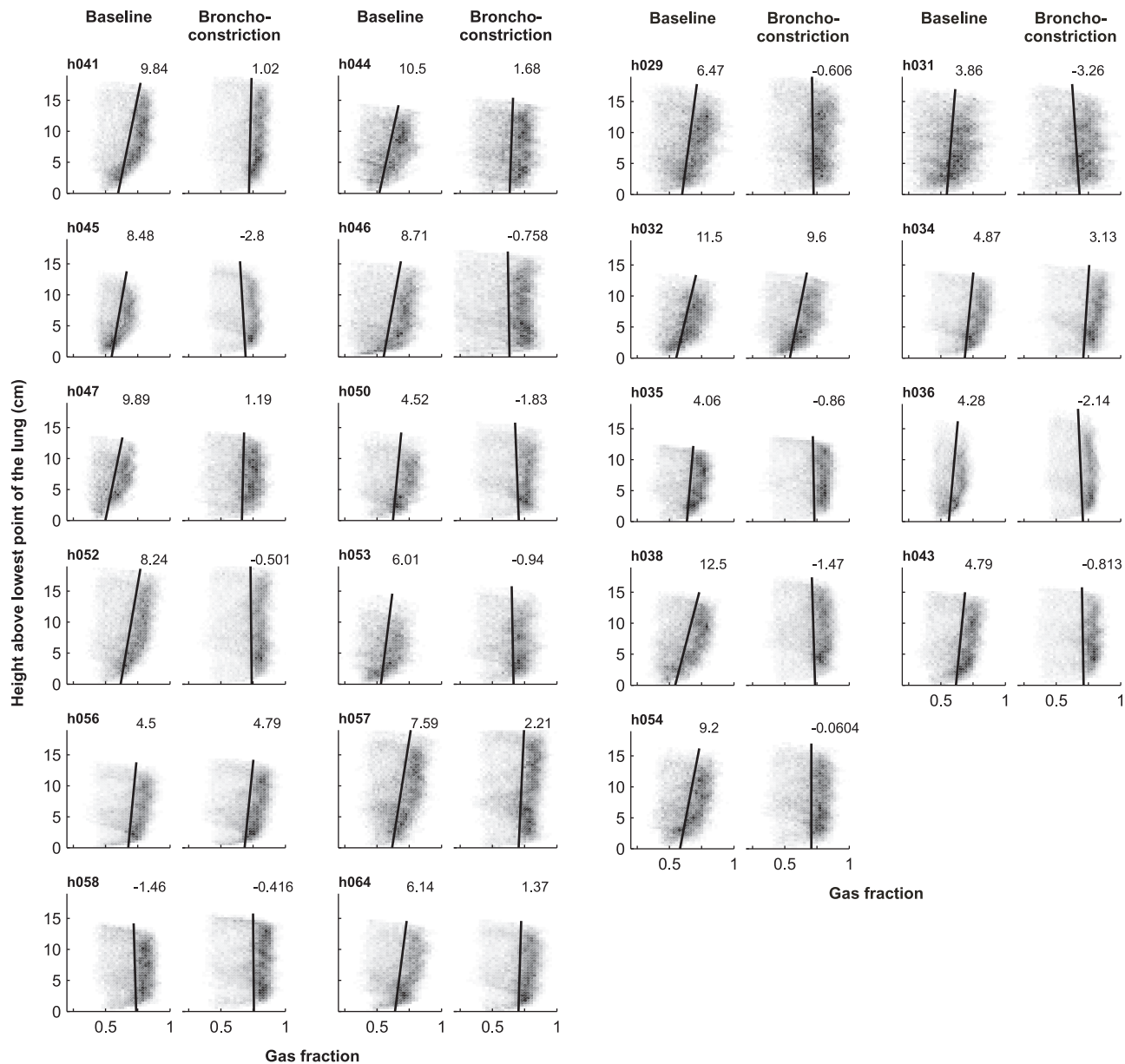
**Figure 4.  $F_{gas}_{Vdef/Out}$  in baseline and bronchoconstriction (BC) conditions for healthy subjects and subjects with asthma.** At baseline,  $F_{gas}$  values are below 1 for most healthy subjects and subjects with asthma. After bronchoconstriction, the  $F_{gas}$  was much more heterogeneous, with values both above and below 1. doi:10.1371/journal.pone.0053216.g004

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  $Vdefs$  tended to develop in dependent and less expanded areas (lower  $F_{gas}$ ) of the lung. Healthy subjects do not differ from asthma subjects in this regard, except that  $Vdefs$  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  $Vdefs$  are favored to form in regions of reduced lung inflation prior to agonist inhalation, whereas in asthma subjects, the formation of  $Vdefs$  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  $F_{gas}$  heterogeneities within regions  $< \sim 1 \text{ cm}^3$ . This limits the volume at which  $Vdefs$  can be detected and reduced the contrast in specific ventilation between  $Vdefs$  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  $Vdefs$  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  $^{13}\text{N}$  tracer intravenously implies that the intrapulmonary distribution of the tracer before the washout is proportional to by perfusion. Thus, if the  $Vdefs$  received less perfusion than the rest of the lung, as we have previously observed [4], regions of severe vasoconstriction could have been excluded from  $Vdefs$ . However, in spite of the reduced initial tracer concentration in  $Vdefs$ , at the end of the washout period the activity remaining was much higher than that in the rest of the

# Healthy Asthma



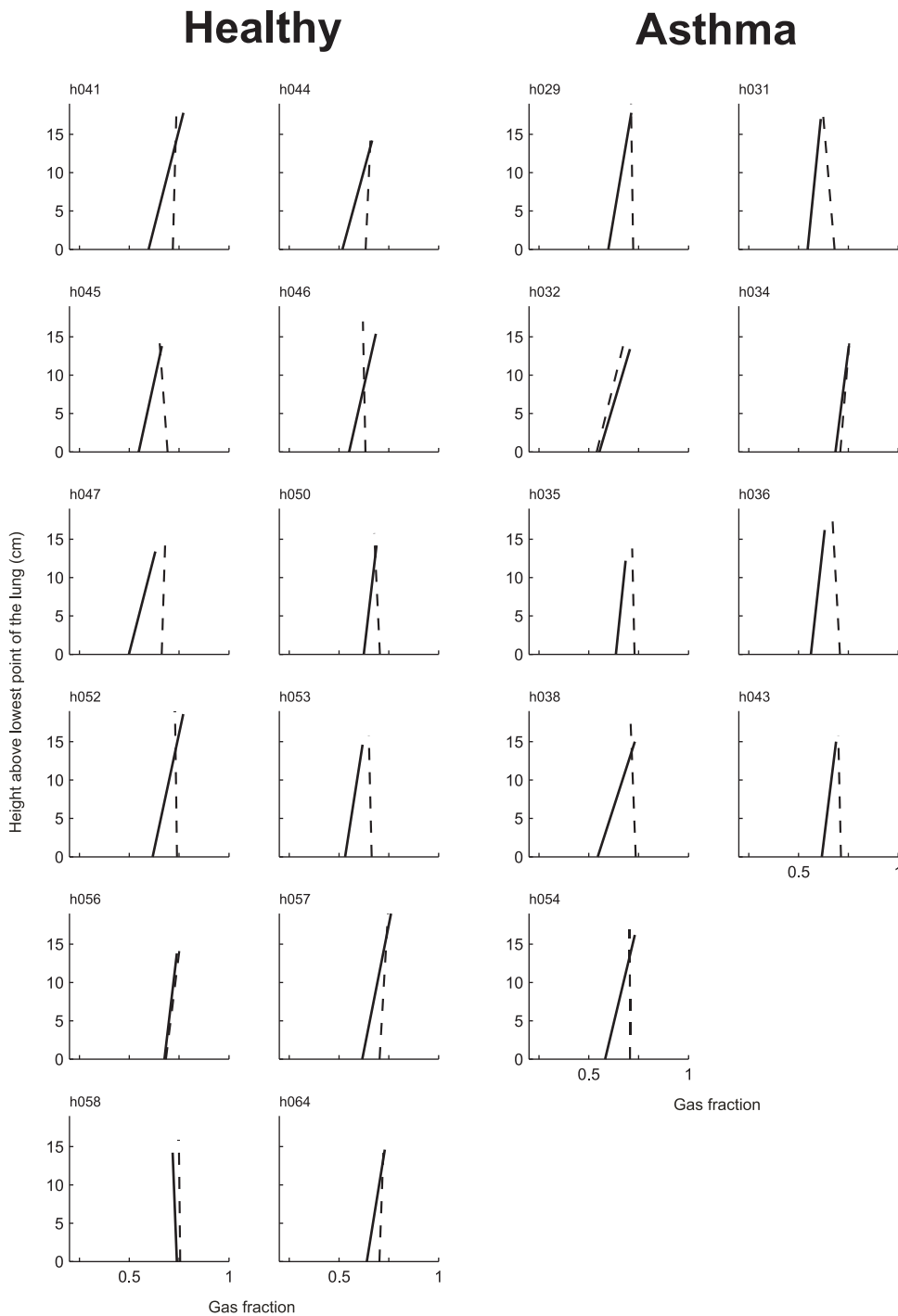
**Figure 5. Fractional gas content ( $F_{gas}$ ) gradients in healthy controls and subjects with asthma at baseline and during bronchoconstriction.** The grayscale of the squares in the plots corresponds to the number of data points with that value such that the darker the square, the greater the number 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 ( $\times 10^{-3}/\text{cm}$ ) for each plot.  
doi:10.1371/journal.pone.0053216.g005

lung. In fact, because the tracer delivered intravenously bypasses the elevated resistance of peripheral airways, the  $^{13}\text{N}$  method can be more sensitive detecting regions of severe hypoventilation than methods delivering a tracer by inhalation.

Finally, it is unclear whether  $V_{def}$ s would form in healthy erect subjects following methacholine inhalation. For obvious reasons 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  $\text{FEV}_1$  [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  $V_{def}$ s.

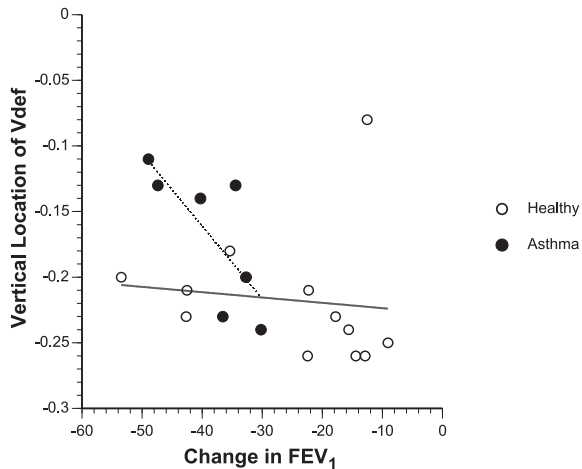
We limited the study to subjects with mild asthma, in which other factors such as airway remodeling, inflammation and mucus are likely to be small or absent. These factors may have greater importance in affecting the formation of  $V_{def}$ s as the severity of



**Figure 6. Height above the lung versus gas fraction ( $F_{gas}$ ) 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  $F_{gas}$  was mostly due to an increase in  $F_{gas}$  in the most dependent lung.  
doi:10.1371/journal.pone.0053216.g006

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 ( $FEV_1$ ) 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  $V_{def}$ s would disappear. However, we found that plotting the  $V_{def}$  location versus change in  $FEV_1$  did not show any correlation between strength of constriction and vertical location of  $V_{def}$ s for



**Figure 7. Vertical location of  $V_{def}$ s versus  $FEV_1$ .** Note that although some asthma subjects had dependent locations of  $V_{def}$ s similar to healthy controls, the healthy controls always had dependent  $V_{def}$ s regardless of the strength of constriction. This suggests that the significant difference in vertical location of  $V_{def}$ s between subjects with 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  $FEV_1$  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  $V_{def}$  formation in asthma subjects regardless of position, either prone or supine [3]. The ventral deviation of  $V_{def}$ s 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  $V_{def}$ s formed preferentially in dependent regions of the lung for both groups (Figure 2), in healthy subjects, the vertical deviation of  $V_{def}$ s was more pronounced compared with that of subjects with asthma. In addition, at baseline  $F_{gasV_{def}/Out}$  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  $F_{gasV_{def}/Out}$  lower than unity is consistent with a previous study where in 9 out of 11 subjects with mild asthma had a baseline  $F_{gasV_{def}/Out} < 1$  [4]. Furthermore, healthy subjects demonstrate clear vertical gradients in  $F_{gas}$ , with  $F_{gas}$  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  $F_{gasV_{def}/Out}$  compared to that of healthy subjects but yet had similar baseline and bronchoconstriction  $F_{gas}$  gradients as those of healthy subjects. These results suggest that the reduced vertical dependence of  $V_{def}$  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  $V_{def}$ s. 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  $V_{def}$ s 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  $F_{gas}$  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  $R_{low}$  and lower  $FEV_1$ ), both groups had similar increases in lung volume and similar sized  $V_{def}$ s, which are consistent with the lack of difference of the change in  $E_{low}$  between the two groups. In addition, a relative increase in regional expansion of the ventilation defective regions ( $F_{gasV_{def}/out}$  increased in all regions) was detected during bronchoconstriction. As in previously studies [3,4], the relatively greater increase in  $F_{gas}$  observed in  $V_{def}$ s 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  $V_{def}$ s 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  $V_{def}$ s 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  $V_{def}$ s. 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  $V_{def}$  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  $V_{def}$ . In more severe asthma, it can be expected that other factors may be more important in triggering the formation of  $V_{def}$ s, such as airway inflammation, smooth muscle hypertrophy, or airway edema.

## Acknowledgments

The authors thank S. A. Barrow and S. B. Weise for technical assistance with image acquisition and processing; R. J. Callahan, Ph.D. and A. Bruce for radiation safety and quality assurance testing of the radioisotope; and J. A. Correia, Ph.D., W. M. Bucelewicz and D. F. Lee for preparation of the radioisotope.

## 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.



## References

- Altes TA, Powers PL, Knight-Scott J, Rakes G, Platts-Mills TA, et al. (2001) Hyperpolarized 3He MR lung ventilation imaging in asthmatics: preliminary findings. *J Magn Reson Imaging* 13: 378–384.
- de Lange EE, Altes TA, Patrie JT, Parmar J, Brookeman JR, et al. (2007) The variability of regional airflow obstruction within the lungs of patients with asthma: assessment with hyperpolarized helium-3 magnetic resonance imaging. *J Allergy Clin Immunol* 119: 1072–1078.
- Harris RS, Winkler T, Musch G, Vidal Melo MF, Schroeder T, et al. (2009) The prone position results in smaller ventilation defects during bronchoconstriction in asthma. *J Appl Physiol* 107: 266–274.
- Harris RS, Winkler T, Tgavalekos N, Musch G, Melo MF, et al. (2006) Regional pulmonary perfusion, inflation, and ventilation defects in bronchoconstricted patients with asthma. *Am J Respir Crit Care Med* 174: 245–253.
- Samee S, Altes T, Powers P, de Lange EE, Knight-Scott J, et al. (2003) Imaging the lungs in asthmatic patients by using hyperpolarized helium-3 magnetic resonance: assessment of response to methacholine and exercise challenge. *J Allergy Clin Immunol* 111: 1205–1211.
- Munkner T, Bundgaard A (1986) Regional V/Q changes in asthmatics after exercise. *Eur J Respir Dis Suppl* 143: 62–66.
- Venegas JG, Winkler T, Musch G, Vidal Melo MF, Layfield D, et al. (2005) Self-organized patchiness in asthma as a prelude to catastrophic shifts. *Nature* 434: 777–782.
- Winkler T, Venegas JG (2007) Complex airway behavior and paradoxical responses to bronchoprovocation. *J Appl Physiol* 103: 655–663.
- National Asthma Education and Prevention Program (2007) Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma—Summary Report 2007. *J Allergy Clin Immunol* 120: S94–138.
- Tgavalekos NT, Musch G, Harris RS, Vidal Melo MF, Winkler T, et al. (2007) Relationship between airway narrowing, patchy ventilation and lung mechanics in asthmatics. *Eur Respir J* 29: 1174–1181.
- Tgavalekos NT, Tawhai M, Harris RS, Musch G, Vidal-Melo M, et al. (2005) Identifying airways responsible for heterogeneous ventilation and mechanical dysfunction in asthma: an image functional modeling approach. *J Appl Physiol* 99: 2388–2397.
- Tgavalekos NT, Venegas JG, Suki B, Lutchen KR (2003) Relation between structure, function, and imaging in a three-dimensional model of the lung. *Ann Biomed Eng* 31: 363–373.
- Cartier A, Malo JL, Begin P, Sestier M, Martin RR (1983) Time course of the bronchoconstriction induced by inhaled histamine and methacholine. *J Appl Physiol* 54: 821–826.
- Melo MF, Harris RS, Layfield JD, Venegas JG (2005) Topographic Basis of Bimodal Ventilation-Perfusion Distributions during Bronchoconstriction in Sheep. *Am J Respir Crit Care Med* 171: 714–721.
- Musch G, Layfield JD, Harris RS, Melo MF, Winkler T, et al. (2002) Topographical distribution of pulmonary perfusion and ventilation, assessed by PET in supine and prone humans. *J Appl Physiol* 93: 1841–1851.
- Vidal Melo MF, Layfield D, Harris RS, O'Neill K, Musch G, et al. (2003) Quantification of regional ventilation-perfusion ratios with PET. *J Nucl Med* 44: 1982–1991.
- Bayat S, Porra L, Suhonen H, Suortti P, Sovijarvi AR (2009) Paradoxical conducting airway responses and heterogeneous regional ventilation after histamine inhalation in rabbit studied by synchrotron radiation CT. *J Appl Physiol* 106: 1949–1958.
- Venegas JG, Schroeder T, Harris S, Winkler RT, Melo MF (2005) The distribution of ventilation during bronchoconstriction is patchy and bimodal: a PET imaging study. *Respir Physiol Neurobiol* 148: 57–64.
- Shardonofsky FR, Martin JG, Eidelman DH (1992) Effect of body posture on concentration-response curves to inhaled methacholine. *Am Rev Respir Dis* 145: 750–755.
- Froese AB (2006) Gravity, the belly, and the diaphragm: you can't ignore physics. *Anesthesiology* 104: 193–196.
- Tzeng YS, Lutchen K, Albert M (2009) The difference in ventilation heterogeneity between asthmatic and healthy subjects quantified using hyperpolarized 3He MRI. *J Appl Physiol* 106: 813–822.
- de Lange EE, Altes TA, Patrie JT, Gaare JD, Knake JJ, et al. (2006) Evaluation of asthma with hyperpolarized helium-3 MRI: correlation with clinical severity and spirometry. *Chest* 130: 1055–1062.
- Kraft M, Pak J, Martin RJ, Kaminsky D, Irvin CG (2001) Distal lung dysfunction at night in nocturnal asthma. *Am J Respir Crit Care Med* 163: 1551–1556.
- Ciftci TU, Ciftci B, Guven SF, Kokturk O, Turktas H (2005) Effect of nasal continuous positive airway pressure in uncontrolled nocturnal asthmatic patients with obstructive sleep apnea syndrome. *Respir Med* 99: 529–534.
- Lafond C, Series F, Lemiere C (2007) Impact of CPAP on asthmatic patients with obstructive sleep apnoea. *Eur Respir J* 29: 307–311.
- Busk M, Busk N, Puntennney P, Hutchins J, Yu Z, et al. (2012) Use of continuous positive airway pressure reduces airway reactivity in adults with asthma. *Eur Respir J*.