
Hyperpolarized ^3He MRI in Asthma: Measurements of Regional Ventilation Following Allergic Sensitization and Challenge in Mice—Preliminary Results¹

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Rationale and Objectives. Quantitative regional measurement of physiological parameters of lung may improve both early detection of asthma and its response to treatment by elucidating the characteristics of airway obstruction. Recent emergence of hyperpolarized helium-3 magnetic resonance imaging as a sensitive pulmonary imaging tool has shown great potential in capturing important structural and functional aspects of normal and diseased lungs. The objective of this study was to investigate regional ventilation changes in the mouse lung following allergen sensitization and challenge.

Materials and Methods. A murine model of allergic airway inflammation was created in mice following allergen challenge using *Af* and IgE-mediated asthma. The creation of model was verified using pulmonary function test and histology. Regional fractional ventilation was then measured in the animals using hyperpolarized ^3He MRI on a pixel-by-pixel basis with a planar resolution of 0.24 mm. The sensitized and healthy animals were then compared statistically to assess the potential sensitivity of this technique in detection of such pulmonary abnormalities.

Results. In this work, we have demonstrated for the first time the quantitative measurement of regional ventilation in normal and asthmatic mice. Results of this study show significant changes in regional ventilation in murine model of allergic airway sensitization compared with that in normal control animals.

Conclusion. Further development of this technique can potentially serve as a quantitative marker to investigate the physiology of allergen-induced airway hyperresponsiveness and to assist in disease treatment and prevention.

Key Words. Airway hyperresponsiveness; hyperpolarized ^3He magnetic resonance imaging; regional fractional ventilation; pulmonary functional imaging; asthma.

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Decreased airway obstruction, a major characteristic of asthma, can be elicited by various mechanisms (1). Acute

exposure to allergen, for instance, evokes reversible airway obstruction in part due to airway smooth muscle shortening, inflammatory cell influx, submucosal edema, and release of numerous proinflammatory mediators. Alternatively, chronic asthma is characterized by irreversible remodeling of the airways with basement membrane thickening, collagen deposition, and prominent smooth muscle hyperplasia and hypertrophy. Fixed airway obstruction is also a hallmark of chronic obstructive pulmonary disease, a complex disorder of airway and lung parenchymal changes (2). Although correct diagnosis and assessment of disease severity are crucial in order to provide adequate treatment (3), the traditional tools for

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structural and functional evaluation of the lungs are insufficient.

Spirometric tests to evaluate chronic airflow obstruction are available in clinical settings and suitable for screening of large cohort studies, but spirometry cannot effectively dissect large or small airways obstruction (4). Bronchoscopic mucosal biopsy has been widely used to evaluate histological changes (5); the specimens that can be obtained this way, however, are small and the procedure is invasive, limiting its clinical usefulness as part of a routine diagnostic workup. Computed tomography (CT) has recently been used to assess airway changes, in particular, chronic remodeling (6–10). This technique can provide quantitative assessment for central airway dimensions and for peripheral airway abnormality. However, CT cannot discriminate pathologic details, and there are concerns about exposing patients to ionizing radiation. Thus, a technique for “high resolution/low-risk” visualization of the airways, which could significantly improve diagnosis by providing information on the characteristics of airway obstruction, is greatly needed.

Magnetic resonance imaging (MRI) is a clinically sensitive imaging method that does not expose the patients to ionizing radiation. Due to absence of substantial water and large susceptibility gradients at air–tissue interfaces, conventional MRI has not been very effective in imaging lungs and airways. In the 1960s, it was discovered that the nucleus of ³He and, later, that of ¹²⁹Xe can be polarized with optical pumping. The hyperpolarized state can be maintained for hours. Polarization by optical pumping results in magnetization 10⁵ times greater than the thermal polarizations commonly used in standard MRI magnets. The idea of using hyperpolarized gases in medicine was originated by William Happer and Gordon Cates, physicists at Princeton University, together with Mitchell Albert, now at Harvard University. Due to the highly increased SNR of gaseous states of such hyperpolarized nuclei, hyperpolarized (HP) ³He MRI opened the possibility to visualize ventilated air spaces. In 1994, Albert and colleagues demonstrated imaging using HP ¹²⁹Xe gas in a lung of a dead mouse (11). Similar images were acquired using HP ³He in guinea pigs (12). In vivo HP ³He images were later published in guinea pig (13), and then in healthy (14) and asthmatic human subjects (15). Samee and colleagues (15) showed that MRI of lungs in patients with asthma who have inhaled polarized helium demonstrates ventilation defects that are well correlated with lung function and worsen with challenge of either methacholine (MCh) or exercise. The defects occur heteroge-

neously throughout the lung and do not immediately resolve. These findings established polarized helium imaging as a potentially important imaging technique for asthma.

With the exception of the apparent diffusion coefficient (ADC), which has been widely and successfully used in various species (16–20), most of the HP ³He imaging studies have focused on obtaining gas density or static ventilation images. The real power of HP ³He imaging, however, lies in the capability of quantitative measurement of physiological parameters of lung. Recently, HP ³He imaging has been used to determine regional alveolar partial pressure of oxygen (21,22) and regional fractional ventilation in different animals (23–25). Variations in regional ventilation during inflammatory changes of the airways can be a sensitive quantitative marker with the potential to noninvasively assess the severity of disease and progression of treatment.

The variety of strains known to exhibit a broad range of pulmonary characteristics and diverse disease models have made mice attractive candidates in pulmonary studies, making the challenges associated with HP pulmonary MRI of these species worth the effort. These difficulties mainly depict themselves in the relatively smaller tidal volume (TV) and higher respiration rate (RR) of this species. This study is a preliminary attempt to assess quantitative changes in regional fractional ventilation in the presence of allergic airway inflammation and hyperresponsiveness in a mouse model.

MATERIALS AND METHODS

Murine Model of Allergic Airway Sensitization

Airway inflammation is thought to play an important role in mediating airflow obstruction. The inflammatory changes associated with asthma are usually characterized by an accumulation of eosinophils, T lymphocytes, mast cells, and neutrophils in the airway walls, lung tissue, and airway lumen and are variably associated with changes in airway responsiveness to nonspecific bronchoconstrictors. Acutely or chronically, these changes may be accompanied by airflow limitations (5).

Murine models of allergic airway disease have helped to reveal the underlying mechanisms in development of allergen-induced inflammation and AHR (26,27). Such a model was established to characterize and quantify regional ventilation following allergen challenge. *Aspergillus fumigatus* (Af; Bayer Pharmaceuticals, Elkhart, IN), an airborne

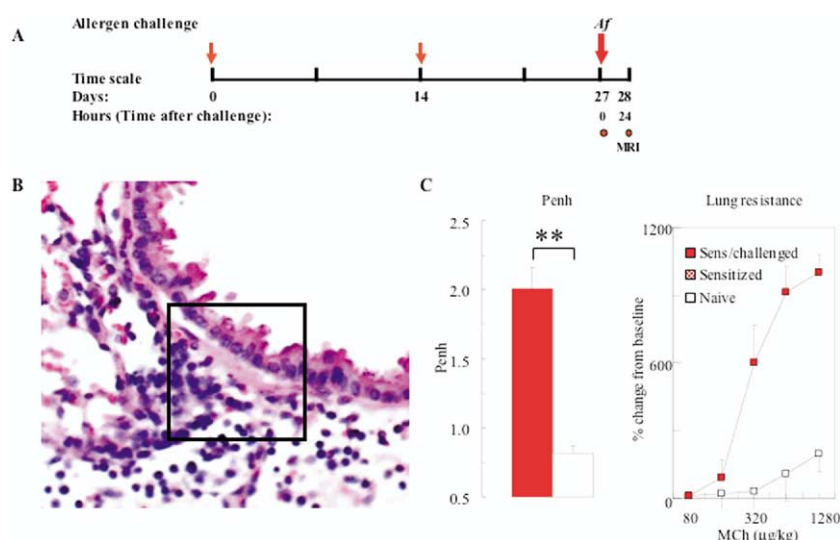


Figure 1. Morphological changes in the asthmatic airways are associated with altered lung physiology. Mice were sensitized with i.p. *Af*/alum and received a single i.n. *Af* challenge (**A**). At 24 hours following allergen challenge of sensitized mice BAL and lung tissue was obtained (**B**) and lung function was measured using the enhanced pause (P_{enh}) and lung resistance changes to MCh (**C**). Using a separate group of mice, MRI was performed. $**P < .01$. Each measurement represents mean $\pm$ SEM of $n = 6$ mice.

fungus responsible for a number of allergic disorders including allergic bronchopulmonary aspergillosis and IgE-mediated asthma (28), was used to create this model. All animal experiments were conducted with approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania. We used 8- to 10-week-old, female normal BALB/c mice (Jackson Laboratories, Bar Harbor, ME) housed under pathogen-free conditions as previously described (29,30). Sensitized mice received two intraperitoneal (i.p.) injections of 20 μ g *Af* together with 20 mg alum (Imject Alum; Pierce, Rockford, IL) in 100 μ l PBS on day 0 and day 14, followed by a single intranasal (i.n.) challenge on day 27 with 25 μ l of *Af* extract in PBS (12.5 μ g in 21% glycerol, PBS) (Fig. 1A). For the i.n. treatment, sensitized mice were anesthetized by isoflurane inhalation, and 25 μ l of *Af* extract or vehicle was applied to the left nares (30). Naïve, nonsensitized mice were treated i.n. with either 25 μ l glycerol or PBS alone.

Analysis of Allergic Inflammatory Changes

Lungs were inflated and fixed in paraformaldehyde (4% with sodium cacodylate 0.1 mol/L, pH 7.3) for histological analysis. Paraffin sections prepared from the lungs of naïve and sensitized mice were stained with hematoxylin and eosin for evaluation of airway inflammation.

Measurement of Lung Function in Mice

Lung function measurements, tissue collection and MRI was performed 24 hours after the i.n. treatment (Fig. 1A). Pulmonary function tests were carried out both using an unrestrained plethysmographic method (Buxco Electronics, Wilmington, NC) and by measuring lung resistance (R_L) in cannulated animals. The noninvasive index of airway hyperresponsiveness, *enhanced pause* (P_{enh})—an empirically derived dimensionless quantity based on the pressure waveform in the plethysmograph box—correlates closely with R_L and can be used as a measure of airway responsiveness to allergen and MCh in a whole-body plethysmograph (30,31). P_{enh} represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and a function of the timing of expiration.

Unrestrained, spontaneously breathing mice were placed inside the main chamber of a whole body plethysmography system, and pressure differences between this chamber and a reference chamber were recorded. The box pressure signal is caused by volume changes and resultant changes in pressure during the respiratory cycle of the animal. From this signal the phases of the respiratory cycle TVs and P_{enh} can be calculated.

R_L measurements were performed on anesthetized mice, cannulated through the trachea and ventilated

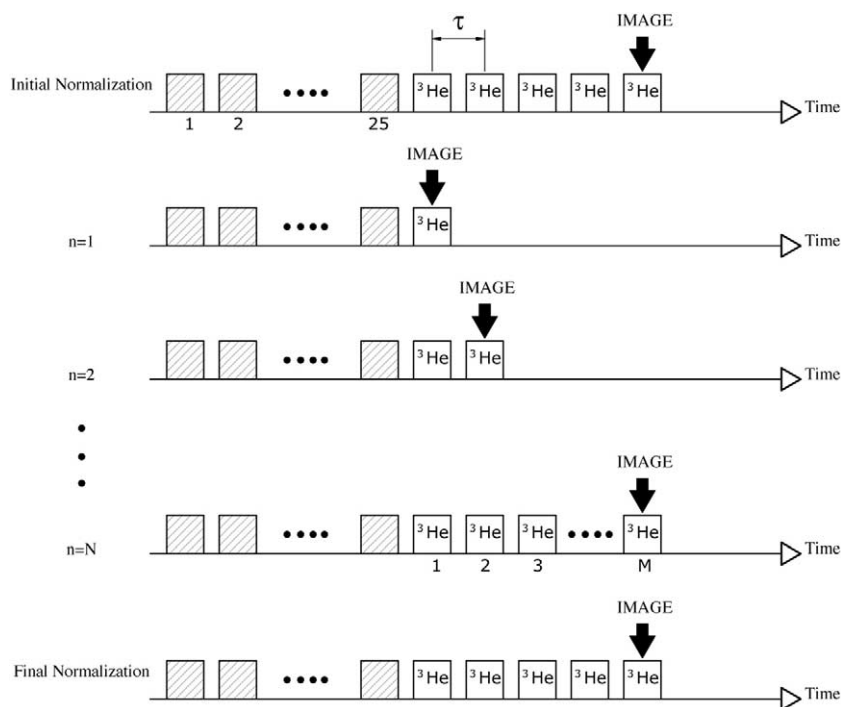


Figure 2. The ventilation sequence used in mice. Hatched blocks represent normal air breaths. Black arrows indicate imaging during breath hold.

(RR = 140 breaths/min, TV = 0.3 ml) following administration of pancuronium bromide (1.0 mg/kg). Transduced alveolar pressure and airflow rate were used to calculate R_L values by a computer (Buxco Electronics, Inc, Wilmington, NC). Baseline values were established and after administration of saline, MCh was given intravenously at concentrations ranging from 80 to 1280 $\mu\text{g/kg}$ in five increments (Fig. 1C, right). The advantage of this technique over noninvasive measurements is the possibility to gain direct information on the function of lower airways by exclusion of the upper airways.

Prior to the MRI experiment, mice were placed in the plethysmography chamber as described earlier, and their breathing frequency and TV were recorded and averaged over a 5-minute period. These parameters were then used to ventilate the animal following anesthesia.

Quantitative Measurement of Regional Fractional Ventilation

Regional fractional ventilation is defined as the amount of gas added to a region of interest (ROI) during inspiration (V_{new}) normalized by the total lung volume of that ROI at the end inspiration (V_{total}):

$$r = \frac{V_{\text{new}}}{V_{\text{total}}} = \frac{V_{\text{new}}}{V_{\text{new}} + V_{\text{old}}} \quad (1)$$

An $r = 0$ indicates no gas replacement and an $r = 1$ indicates complete gas exchange. A volume's gas content is assumed to be divided between r , which is delivered fresh at reservoir polarization level with each polarized gas breath, and $q = 1 - r$, which measures the residual capacity of the studied volume.

The ventilation sequence is based on the original Deninger et al.'s study (25) and consists of a consecutively increasing number of HP ³He gas breaths as shown in Figure 2. The time interval between two consecutive breaths is equal to τ . Before each cycle of polarized gas breaths, a number of normal air breaths is delivered sufficient to wash out all of the helium out of the residual capacity.

Signal intensity in higher-ventilation regions grows at a higher rate than lower-ventilation regions for a given succession of polarized gas breaths. Theoretically, the signal in all regions will converge to a steady state value (S_{∞}) after an infinite number of breaths. In practice, there exists a limited number of breaths large enough to wash

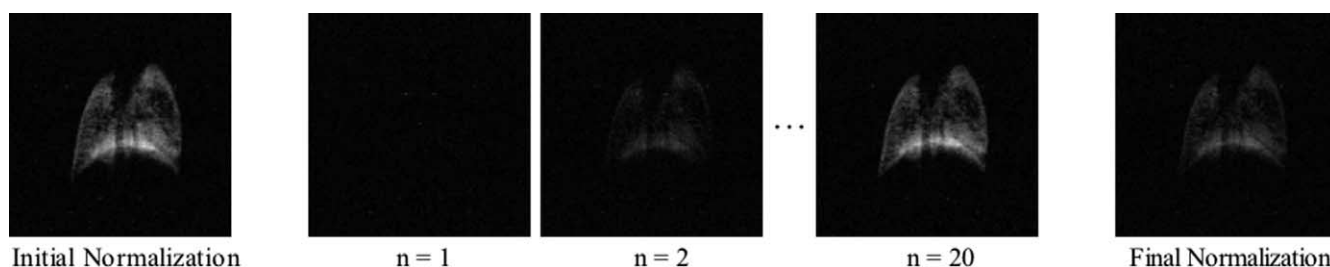


Figure 3. Images in a typical ventilation sequence acquired at 4.7 T. The first and last displayed images in the ventilation sequence are five polarized-breath normalization images for the estimation of $T_{1,\text{ext}}$ of the polarized helium in its reservoir. Sufficient normal breaths are delivered between each image to eliminate any remaining polarized ^3He from the residual capacity and to supply oxygen to the animal. FOV = $3 \times 3 \text{ cm}^2$ and ST = 3 mm.

Table 1
Data Used to Estimate the Average Partial Pressure of Oxygen in the Mouse Lung

Parameter	Value	Description
p_{A,CO_2}	53.3 mbar	Alveolar CO_2 pressure
f_{I,O_2}	0.21 mbar	Fractional concentration of O_2 in dry air
R	0.72 in vivo (34)	Respiratory exchange ratio (ratio of the volume of exhaled CO_2 to the volume of O_2 taken up)
p_B	1013 mbar	Barometric pressure
$p_{\text{H}_2\text{O}}$	62.7 mbar	Water vapor pressure

out almost all of the remaining gas from previous inhalations. Therefore, S_∞ is a specific plateau for each region of the lung and is proportional to the airway volume present in that given region.

Due to the depolarization effect of the applied RF pulse, the lung is ventilated with an adequate number of normal (air) breaths—25 for mice—and refilled with the desired number of fresh hyperpolarized gas breaths in order to guarantee the same initial conditions for each image. The normal breaths also provide the animal with necessary oxygen. The number of steps and the number of polarized gas breaths per step in the ventilation sequence were fine-tuned for the use in mice. Eight steps were used with $n = 1, 2, 3, 4, 5, 8, 15$, and 20 polarized gas breaths, respectively. The maximum number of helium breaths was established empirically on live, anesthetized mice that were continuously monitored for body temperature and electrocardiography. Neither of these parameters indicated morbid alterations during the helium inhalation cycles. In each step, imaging occurred during a 3-second breath hold following the inhalation of the final polarized gas breath. Figure 3 shows a typical sequence of images acquired with this procedure.

The available signal level of ^3He within a given voxel after a certain number of polarized gas breaths is a function of the magnetization of the fraction r of the fresh ^3He and magnetization of the fraction q of ^3He remaining from previous breaths of the same cycle. The first fraction is subject to spin-lattice relaxation in the external reservoir, $T_{1,\text{ext}}$, while the second fraction is affected by oxygen-induced relaxation in the lung, T_{1,O_2} , during the interval τ . The alveolar partial pressure of oxygen, p_{A,O_2} can be estimated as (32):

$$p_{A,\text{O}_2} = p_{I,\text{O}_2} - p_{A,\text{CO}_2} \left(f_{I,\text{O}_2} + \frac{1 - f_{I,\text{O}_2}}{R} \right) \quad (2)$$

for which the parameters are defined Table 1 (33). Using Equation (2) and partial pressure of O_2 in the inhaled air defined as:

$$p_{I,\text{O}_2} = f_{I,\text{O}_2} (p_B - p_{\text{H}_2\text{O}}) \quad (3)$$

p_{A,O_2} can be estimated as $p_{\text{O}} = 130 \text{ mbar}$. This value was assumed to hold for the entire ventilation analysis. Oxygen-induced relaxation of hyperpolarized helium T_{1,O_2} is governed by:

$$T_{1,\text{O}_2} = \xi / p_{A,\text{O}_2} \quad (4)$$

The above estimation of p_{A,O_2} and the proportionality constant $\xi \approx 2.6 \text{ bar.s}$ (35) gives $T_{1,\text{O}_2} \approx 20 \text{ sec}$. Neglecting the uptake of oxygen into the blood (25), the oxygen-induced relaxation time of ^3He will in practice be a variable function of number of breaths $T_{1,\text{O}_2}(j)$ (hence a function of time) where j is the number of breaths in a given step. So $T_{1,\text{O}_2}(j)$ in this approximation is influenced by the wash-out of p_{A,O_2} as:

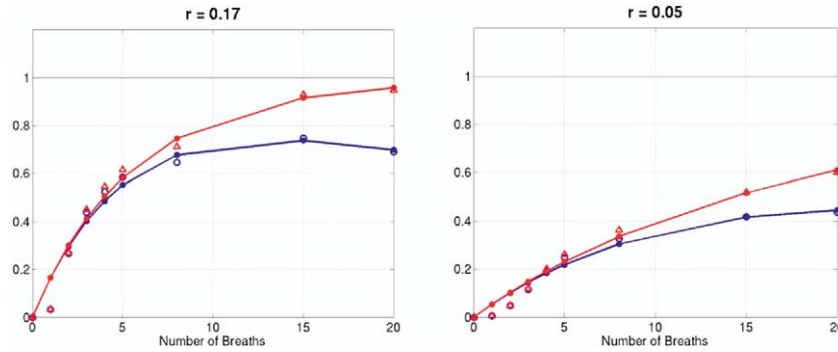


Figure 4. Illustration of curve plots used to determine fractional ventilation for various points. The circular data points are original measurements, and the triangular points are the same data corrected for depolarization in the helium reservoir. Y-axis values are normalized signal intensities with respect to S_{∞} .

$$T_{1,O_2}(j) = \xi/p_{A,O_2}(j) = \xi/(p_0 q^j) \quad (5)$$

In order to estimate the $T_{1,ext}$ decay rate for a given study, two *normalization* images were acquired immediately before and after the actual ventilation sequence (as indicated in Figs. 2 and 3). Assuming a linearly decaying polarization in the reservoir and given the signal level in the normalization images and the elapsed time, the external relaxation time of the external HP ³He reservoir, $T_{1,ext}$, can be estimated. This value was typically in the range of 7–8 minutes for our experimental setup. Having acquired the signal values for $n = N$ steps of the ventilation sequence, and correcting for external relaxation effect of helium, a nonlinear equation of the form (25):

$$S(n) = \psi(n) \cdot r \sum_{k=0}^{n-1} \phi(n, k) \cdot q^k \exp \left[-\frac{p_0 \tau q^{n-k} (1 - q^k)}{\xi(1 - q)} \right] \quad (6)$$

can be solved numerically for r or q on a regional basis, where $\psi = c \cdot \Delta^{(n-1)(N+\frac{n}{2})}$ and $\phi = \Delta^{n-k-1}$, with $\Delta = \exp(-\tau T_{1,ext})$. Figure 4 shows example ventilation curves in a normal control mouse. The signal intensities are plotted as a function of HP ³He breaths for two ROIs. The circular data points represent the raw and the triangular data points represent the corrected data points for the external relaxation. The curve to Equation (6) fits are shown with continuous lines. All corrected curves are normalized with respect to their respective theoretical S_{∞} , hence converging to 1. This normalization also makes it possible to compare lung voxels with different airspace volumes. The relative rapid rise of signal intensity in the higher-ventilation region is evident from the ventilation curve. This

procedure is carried out on a voxel-by-voxel basis in order to generate the regional ventilation map for the given slice.

Animal Preparation

For preliminary evaluation of the sensitivity of regional fractional ventilation to pulmonary characteristics of the murine model of asthma, one animal from each group (normal versus sensitized) was selected. ³He imaging experiments were carried out in anesthetized mice. The mice were cannulated, placed on an MRI-compatible small animal ventilator (GE Healthcare, Durham, NC), and were given an intramuscular injection of pancuronium bromide to halt spontaneous breathing. The ventilator setting was adjusted for each mouse using pulmonary function test measurements performed prior to the anesthesia (TV $\approx$ 0.3 ml, RR = 140 breaths/min). During the entire MRI procedure, the heart rate was monitored and temperature was maintained at 35°C using a flow of warm air inside the bore of magnet.

Hyperpolarized Gas Imaging

The imaging helium gas (Spectra Gases, Branchburg, NJ) has a nominal concentration of 99.25% ³He and 0.75% N₂. The actual concentration of N₂ in the used batch was 0.81%. This mixture was polarized through spin exchange collisions with optically pumped rubidium (Rb) atoms, as previously described (36) using a prototype commercial polarizer (IGI.9600.He; GE Healthcare). The ³He gas was prepared for approximately 14 hours to a polarization level of 30–35%.

Imaging was performed on a small-bore 4.7-T MRI scanner (Varian Inc, Palo Alto, CA) with a homemade

quadrature 12-leg birdcage body coil (1¼-inch inner diameter) tuned to the ^3He resonance frequency of 152.95 MHz. The animal was placed supine in the coil, which was then placed into the bore of the magnet. All imaging was performed using a fast gradient echo pulse sequence with the following parameters: field of view (FOV) = $3 \times 3 \text{ cm}^2$, slice thickness (ST) = 3 mm, flip angle (α) = 10° , matrix size = 128×128 pixels, repetition time (T_R) = 6.8 msec, and echo time (T_E) = 3.4 msec. Coronal slices were selected by performing preliminary scout images to determine position in the three major planes.

Data Analysis

Analysis was performed on 1-pixel $\times$ 1-pixel voxels resulting in a planar resolution of $\sim 0.24 \text{ mm}$. In order to distinguish lung tissue from background, bins with an SNR below the cutoff ratio were excluded from analysis. The cutoff ratio was approximately 10% of the SNR found in the bin of maximum signal intensity but was slightly adjusted for each set of data.

RESULTS AND DISCUSSION

Allergic Airway Sensitization Induced Eosinophilic Inflammatory Changes of the Airways and Hyperresponsiveness to Methacholine

The inflammatory response following allergen challenge is illustrated in Figure 1B. This hematoxylin and eosin-stained section shows that *Af* induces a predominantly peribronchial inflammatory infiltrate that contains mainly mononuclear cells and eosinophils, with the lung parenchyma and the alveolar spaces being relatively preserved. Thus, similar to previously characterized ovalbumin-induced models of allergic AHR (37,38), intraperitoneal sensitization and intranasal challenges with *Af* induced a predominantly Th2-like inflammatory response manifested by an IL-5/eotaxin driven eosinophil accumulation in the lungs (30) closely mimicking the inflammatory profile of human, *Af*-induced asthma.

Figure 1C shows the pulmonary function test results by plethysmography (30) (left panel) and by measuring lung resistance (R_L) (right panel) (39), also reported by others (31). As can be seen, P_{enh} is characteristically increased 24 hours after challenge of sensitized mice. At this time, where the maximum inflammatory changes are assumed to occur (29,30), there was a significant airway hyperresponsiveness to MCh. In addition, regression analysis revealed a significant correlation between the num-

bers of eosinophils and the extent of R_L given at an MCh dose of $320 \mu\text{g/kg}$, confirming a relationship between *Af*-induced allergic airway eosinophilia and airway hyperresponsiveness (39).

Comparison of R_L and P_{enh} measurements in the same mice confirmed the involvement of the lower airways in response to aerosolized MCh. Further, the increases in P_{enh} values were inhibited by pretreatment of the mice with a beta 2-agonist indicating that changes in P_{enh} —at least partly—are mediated by smooth muscle contraction-induced bronchoconstriction.

Regional Ventilation Maps Reveal Functional Differences Associated with Allergic Airway Inflammation

Although murine models are valuable in simulating various pathological and physiological abnormalities in the lung, performing functional HP ^3He MRI in these animals posed technical difficulties primarily caused by their relatively small TV ($\sim 0.3 \text{ ml}$) as opposed to rats ($\sim 3 \text{ ml}$) and rabbits ($\sim 20 \text{ ml}$). The mice's high respiratory rate (120 to 140 breaths/min) also makes administration of such a small volume of gas a mechanically challenging problem, which is probably why such measurements have not been carried out in earlier studies.

Regional assessment of fractional ventilation was performed on a sensitized mouse in comparison with a healthy mouse. For the acquired image slice of both animals, the majority of fractional ventilation values were observed to be between 0.0 and 0.5. The upper limit is primarily a function of slice selection and is based on the specific selected slice. We selected a slice where the high- r -value regions such as trachea and bronchioles were excluded. The contribution of these regions is primarily at the higher end of the spectrum (namely above 0.5, as demonstrated in rats [24] due to the effective gas exchange in these airways). Because the contribution of these high- r -value regions is highly slice dependent and can have a nonuniform effect on the statistics of distribution, it is necessary to exclude them from the data set in order to achieve a fair comparison between different animals.

Figure 5 displays maps of fractional ventilation for both healthy and sensitized mice along with respective frequency distribution histograms. The mean fractional ventilation in the healthy mouse is 0.21, with a standard deviation (SD) of 0.09. The respective values for the asthmatic mouse are 0.13 and 0.06. The ventilation map reveals that regional ventilation in a normal

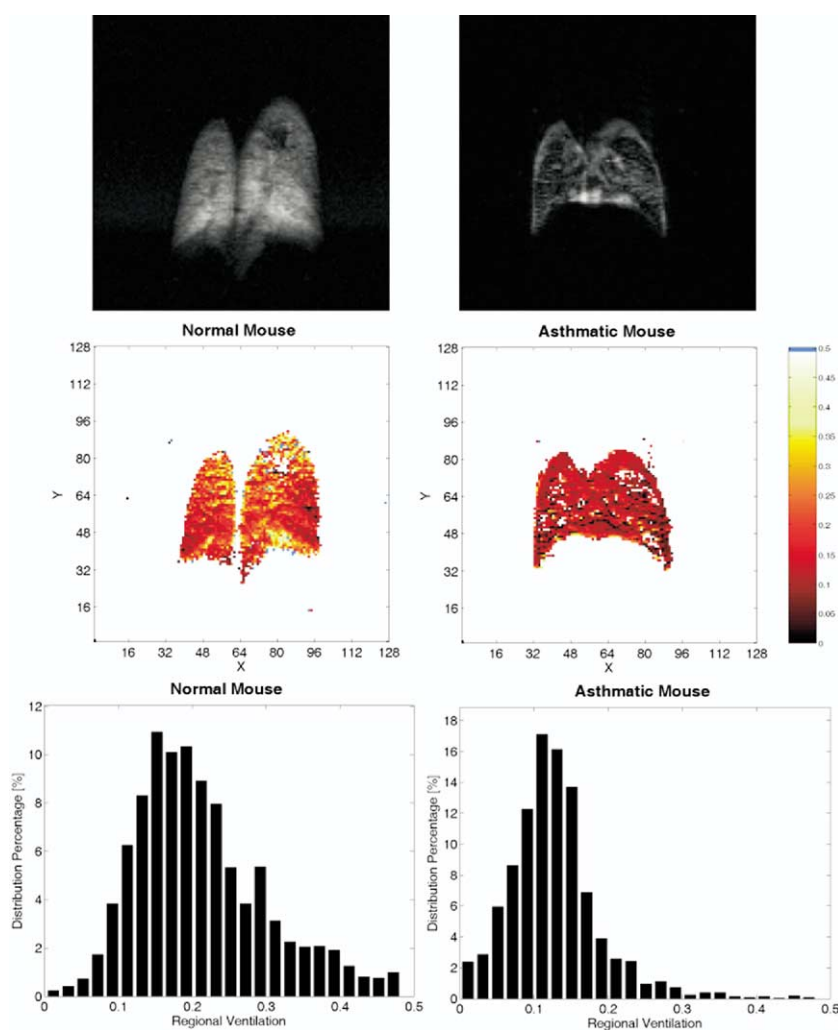


Figure 5. Ventilation images and maps of fractional ventilation in (a) healthy and (b) asthmatic mouse lung. Dark red indicates no gas replacement ($r \approx 0$), while light yellow indicates a gas replacement of $r \approx 0.5$. White areas are regions classified as background (due to low SNR) and are therefore excluded from analysis. Blue areas are regions with $r > 0.5$. Percent frequency distributions for regional fractional ventilation in these two subjects are shown respectively.

mouse changes smoothly from outer to inner regions. In comparison, the asthmatic mouse has a nonuniform distribution with scattered areas of low regional ventilation surrounded by areas of higher ventilation. As can be seen in Figure 5 the average regional ventilation value and the maximum population value for the healthy mouse are almost 50% larger than that of the asthmatic mouse.

The preliminary results suggest that airway changes in a murine model of allergic airway hyperresponsiveness depict themselves in fractional ventilation of mouse lung. Measurement of fractional ventilation at a regional level provides quantitative differences between

the normal and an allergen challenged mouse. These characteristic differences suggest that regional fractional ventilation may be used as a functional tool to assess and monitor the formation and progress of airways restriction. Even though the repeatability and consistency of results has to be assessed on a statistically larger group of subjects, our observations indicate quantitative deviation of both regional and global fractional ventilation as a result of the induced allergic AHR and supports utilizing hyperpolarized ³He MRI as an important functional diagnostic tool in evaluation of symptoms and severity of such pulmonary abnormalities.

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