

³He MRI in Mouse Models of Asthma

Bastiaan Driehuys,^{1*} Julia Walker,² Jim Pollaro,¹ Gary P. Cofer,¹ Nilesh Mistry,^{1,3} David Schwartz,⁴ and G. Allan Johnson¹

In the study of asthma, a vital role is played by mouse models, because knockout or transgenic methods can be used to alter disease pathways and identify therapeutic targets that affect lung function. Assessment of lung function in rodents by available methods is insensitive because these techniques lack regional specificity. A more sensitive method for evaluating lung function in human asthma patients uses hyperpolarized (HP) ³He MRI before and after bronchoconstriction induced by methacholine (MCh). We now report the ability to perform such ³He imaging of MCh response in mice, where voxels must be ~3000 times smaller than in humans and ³He diffusion becomes an impediment to resolving the airways. We show three-dimensional (3D) images that reveal airway structure down to the fifth branching and visualize ventilation at a resolution of 125 × 125 × 1000 μm³. Images of ovalbumin (OVA)-sensitized mice acquired after MCh show both airway closure and ventilation loss. To also observe the MCh response in naive mice, we developed a non-slice-selective 2D protocol with 187 × 187 μm² resolution that was fast enough to record the MCh response and recovery with 12-s temporal resolution. The extension of ³He MRI to mouse models should make it a valuable translational tool in asthma research. Magn Reson Med 58: 893–900, 2007. © 2007 Wiley-Liss, Inc.

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In asthma research, efforts to understand the disease pathways and identify new therapeutic targets are increasingly reliant on mouse models. The value of the mouse is driven by several factors. First, the murine genome has been mapped in detail. Second, the mouse immune system is well characterized, providing a detailed understanding of how recurrent antigen presentation relates to inflammatory response. Third, mice can be genetically engineered to overexpress or suppress targeted genes. These powerful capabilities to create and modify disease generate an equally strong demand for methods to evaluate the associated physiological phenotype. Common methods to assess lung function in small animals rely on inducing bron-

choconstriction with methacholine (MCh) and measuring the resultant changes in airway pressure or resistance (1,2). Unfortunately, such metrics, which provide only global lung function information, cannot answer many of the most critical questions. What is the degree of small vs. large airway involvement? Is the disease focal or diffuse? Are the sites of airway hyperresponsiveness (AHR) reproducible? Are airways closing or merely narrowing? How well do mouse models reproduce human disease? These long-standing questions could be addressed by measuring regional ventilation and airway caliber changes through imaging.

Ventilation imaging in human asthma patients has gained prominence since Altes et al. (3) first demonstrated that subtle defects could be observed in the lungs of even asymptomatic asthmatics by hyperpolarized (HP) ³He MRI. Images acquired before and after MCh challenge allowed patients to act as their own control and thereby shed new light on asthmatic response by isolating the contribution to airway resistance of specific airways and lung regions (4). Such regional characterization of AHR by ³He MRI offers a more sensitive and specific way to study asthma.

It is similarly desirable to use ³He MRI to study AHR in mouse models of asthma. However, ³He imaging in mice introduces several additional hurdles. First, the ³He distribution before and after MCh challenge must be imaged with sufficient spatial resolution to resolve the branches of the airways. Second, ³He diffusion can completely eliminate the airway signal if not properly managed. Third, the image should be acquired sufficiently rapidly to capture the response before the airways relax back to their open state. These issues are all exacerbated in small animal MRI, where airway relaxation to baseline is likely to be faster than in humans (5) and voxels must be ~3000 times smaller. High spatial resolution is typically achieved in small animals by acquiring images over multiple breaths (6,7). However, such averaging also lengthens the scan time, which conflicts with the transient nature of the MCh response. Thus, as in many MR experiments, temporal and spatial resolution are in perpetual conflict for imaging airways hyperresponsiveness, and this problem is particularly acute in rodents.

AHR imaging in rodents was significantly advanced by Chen and Johnson (8), who showed that MCh-induced changes in ventilation and airway caliber could be observed in nonsensitized Fisher rats by using ³He MRI. Radial ³He images of a single 4-mm slice were acquired at four time points of the inspiration with a spatial resolution of 200 × 200 μm². The images, which took 2.5 min to acquire, demonstrated a heterogeneous and relatively long-lasting (~10 min in some animals) alteration of regional ventilation and airway caliber. Although these results were obtained after a large injected MCh dose

¹Center for In Vivo Microscopy, Duke University Medical Center, Durham, North Carolina, USA.

²Pulmonary Medicine, Duke University Medical Center, Durham, North Carolina, USA.

³Department of Biomedical Engineering, Duke University, Durham, North Carolina, USA.

⁴National Institute for Environmental Health Sciences, Research Triangle Park, North Carolina, USA.

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*Correspondence to: Bastiaan Driehuys, Ph.D., Center for In Vivo Microscopy, Box 3302, Duke University Medical Center, Durham, NC 27710. E-mail: driehuys@orion.duhs.duke.edu

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($\sim 1200 \mu\text{g/kg}$), they provided evidence that ^3He MR could play a role in studying AHR in small animal models of asthma. The next step was to transition the imaging method from rats to mice.

^3He MRI in mice has been difficult because exquisite resolution is needed to resolve their airways and ^3He administration is made difficult by their small tidal breathing volumes. Compared to the $\sim 2\text{-ml}$ tidal volume of the rat, its $\sim 200\text{-}\mu\text{l}$ tidal volume makes the mouse challenging to ventilate and image with HP ^3He . The first HP ^3He MRI of a mouse was demonstrated by Dugas et al. (9), who used an asymmetric readout gradient recalled echo (GRE) multi-slice sequence with 1.2 ms echo time to achieve a resolution of $0.25 \times 0.25 \times 3\text{ mm}^3$. ^3He imaging of mice was subsequently demonstrated by Haczku et al. (10), who employed a conventional single-slice GRE sequence with $\text{TE} = 3.4\text{ ms}$ to achieve similar ($0.24 \times 0.24 \times 3\text{ mm}^3$) resolution images in a mouse model of asthma. In a mouse sensitized by and challenged with *Aspergillus fumigatus* (Af), Haczku (10) demonstrated reduced regional ventilation (11) compared to a healthy control. However, neither of these imaging studies were able to visualize the mouse airways. The nonvisibility of the airways is significant impediment in the study of asthma where their role is the central question at hand. In this work we will show that absence of airway signal is a limitation of conventional Cartesian GRE ^3He image acquisition. Although it has been a mainstay sequence of clinical and small animal ^3He MRI, this sequence is severely impacted by diffusion attenuation of the MR signal. Attenuation is exacerbated in ^3He mouse imaging (as noted by Dugas et al. (9)) due to the high resolution required. We will show that diffusion attenuation can be nearly completely avoided by employing radial encoding sequences.

The successful use of radial sequences for ^3He imaging in mice was illustrated in a recent study by Chen et al. (12) whose $70 \times 70 \times 800\text{ }\mu\text{m}^3$ resolution images readily depict several airway branches. While succeeding in the goal of airway imaging, this imaging protocol required a large quantity of ^3He (~ 1.2 liters) and image acquisition was too slow (25 min) to have been practical for imaging MCh response. In this work, we now report the ability to produce 2D and 3D images of ^3He distribution in the lungs of mice sufficiently quickly to capture their heterogeneous response to MCh challenge. These images, which depict airway response down to the fifth branching, are enabled by radial encoding. The significant advantage of radial vs. Cartesian encoding in mouse ^3He MRI is also explained.

THE ^3He DIFFUSION ATTENUATION PROBLEM

Diffusion-induced attenuation of ^3He signal is a profound problem when imaging airway structures on the small scale of a mouse. The diffusion problem has begun to receive attention in clinical ^3He imaging, where its careful consideration can yield measurable improvements in signal-to-noise ratio (SNR) (13–15). Here we show that for small animal imaging, the problem can be largely eliminated by starting the image acquisition at the center of Fourier space. This simple switch from Cartesian sampling, which starts at the edge of k -space, has a remarkable impact on small animal ^3He image quality. An additional

problem in small animal ^3He MRI is diffusion attenuation caused by slice selection. This problem can be overcome by avoiding slice selection and using 3D encoding. Because both problems are so critical to small animal ^3He MRI, and neither has received significant attention in the literature, we present calculations of the scale of the effect. Such calculations lead us to conclude that high-resolution ^3He MRI of mouse airways should be performed using non-slice-selective 2D and 3D radial imaging sequences.

We first consider signal attenuation in a Cartesian GRE acquisition caused by the readout gradient. This problem has been addressed by Mugler (16), but results are not available in the published literature. A readout gradient G , of duration T attenuates the transverse magnetization of a spin population with gyromagnetic ratio γ and diffusion coefficient D according to

$$S = S_0 e^{(-\gamma^2 G^2 D T^3/3)}, \quad [1]$$

where S_0 is the unattenuated signal (17,18). This attenuation expression can be expressed as a function of resolution Δx , by calculating the readout gradient strength G for a given field of view (FOV) X and sampling bandwidth Δf . To sample the image without aliasing, Δf must exceed by a factor of 2 the frequency-dispersion that G creates across X

$$\Delta f = \frac{1}{\Delta t} = \frac{\gamma}{\pi} G X. \quad [2]$$

Here Δt is the sampling time per point. Using this expression and equating the spatial and temporal sampling intervals by $T/\Delta t = X/\Delta x$, we solve for G .

$$G = \frac{\pi}{\gamma T \Delta x}. \quad [3]$$

Substituting G into Eq. [1] yields the attenuation function in terms of resolution

$$S = S_0 e^{-1/3 \left(\frac{\pi}{\Delta x} \right)^2 D T}. \quad [4]$$

The relationship in Eq. [4] represents the SNR reduction of a Cartesian vs. radially-acquired GRE image. This is because image SNR is dominated by the signal at the center of k -space. In a Cartesian acquisition, $k = 0$ is not sampled until after the prephasing gradient has taken the magnetization to the edge of k -space and the first half of the read-gradient has returned it to center. This excursion creates a period of diffusion-induced attenuation that under the idealized assumption of equal prephasing and readout gradient amplitudes, lasts for time T , the data sampling period. This period does not exist for a radial acquisition because it starts at the center of k -space and thus no attenuation is accumulated. Of course, both radial and Cartesian acquisition will be attenuated on the remaining k -space trajectory, thus reducing the amplitudes of the higher spatial frequencies. The associated effects of such filtering on HP gas MR microscopy will be the subject of an upcoming paper.

The practical consequences of diffusion attenuation in a Cartesian GRE image are illustrated in Fig. 1a, which, assuming a 1-ms data-sampling period, shows the dramatic signal drop at increased resolution. Attenuation is calculated in two scenarios—restricted ^3He diffusion in the parenchyma of the lung ($D = 0.2 \text{ cm}^2/\text{s}$) and nearly free ^3He diffusion in the airways ($D = 2 \text{ cm}^2/\text{s}$). Under conditions of restricted diffusion in the parenchyma, the signal is completely extinguished at a resolution below $\sim 200 \text{ }\mu\text{m}$. For free diffusion in the airways, the signal is extinguished below $\sim 500 \text{ }\mu\text{m}$. By contrast, radially acquired images shown in this work depict the airways down to the fifth order branching, where the diameter is $\sim 350 \text{ }\mu\text{m}$ (19). These calculations show that, in addition to motion insensitivity and reduced susceptibility problems touted since the early development of HP gas MRI (20–22), the single most important advantage of radial encoding may be “diffusion-proofing.”

Yet another pathway for diffusion attenuation of signal is presented by the slice-select gradient. The issue has been addressed in some detail for clinical ^3He imaging by Wild et al. (15) who note a marked advantage of 3D vs. multislice 2D imaging as slice thickness drops below 10 mm. For our goal of 1-mm-thick slices, diffusion attenuation makes slice selection impossible.

To calculate the attenuating effects of slice selection using Eq. [1], we must calculate the amplitude and duration of the slice select gradient G_{slice} . The slice gradient is set such that across a slice of thickness Δz it causes a frequency dispersion of Δf , which matches the bandwidth of the RF pulse used for excitation.

$$2\pi\Delta f = \gamma\Delta z G_{\text{slice}}. \quad [5]$$

The excitation bandwidth of a sinc-shaped RF pulse, for example, is the inverse of the time t_0 from its peak to its first zero-crossing. However, the excitation frequency profile is only a perfectly boxcar shaped if the RF pulse is infinitely long and possesses infinite zero crossings. An acceptable profile is generally attained by truncating the pulse with 4–10 zero crossings. Denoting these as N_0 and

then substituting $\Delta f = N_0/t_{\text{pulse}}$ into Eq. [5] gives the slice gradient strength

$$G_{\text{slice}} = \frac{2\pi N_0}{\gamma\Delta z t_{\text{pulse}}}. \quad [6]$$

A final step is to calculate the time period over which the slice gradient and the rephasing gradient contribute to the attenuation. The effective period of attenuation from the slice-select gradient lasts from the center of the RF pulse to its end or $t_{\text{pulse}}/2$ (15). Then, the rephrasing lobe, if we assume it to be of equal amplitude to the slice-select gradient, must also last for $t_{\text{pulse}}/2$. Thus, the combined attenuation period becomes t_{pulse} , which, when combined with Eqs. [6] and [1], gives the attenuation due to slice selection

$$S = S_0 e^{-4/3 \left(\frac{N_0 \pi}{\Delta z} \right)^2 D t_{\text{pulse}}}. \quad [7]$$

This expression is exactly analogous to Eq. [4] derived for the readout gradient, except that the exponent is amplified by an additional factor of $4N_0^2$. This exacerbation results from the need to create a frequency profile that is as close to perfectly square as possible, thus requiring the pulse to have more zero-crossings. The additional zero crossings shorten t_0 and thereby increase the bandwidth of the pulse. To match this pulse bandwidth with equal frequency dispersion across the desired slice, the gradient must be commensurately increased. It is thus because well-defined slices require very large gradients, that diffusion attenuation by the slice selection is particularly problematic.

The practical consequences of ^3He signal attenuation as a function of slice thickness are illustrated in Fig. 1b. Assuming a slice selected by a 1-ms sinc pulse with $N_0 = 4$ zero crossings, all signal will vanish in the parenchyma ($D = 0.2 \text{ cm}^2/\text{s}$) for a thickness below 1.5 mm and in the airways ($2 \text{ cm}^2/\text{s}$) for a thickness below 3 mm. Such calculations illustrate that for high-resolution imaging of the mouse lung, slice selection is nonviable. Combined with the attenuation calculations for the readout gradient discussed in the previous paragraphs, these results show that

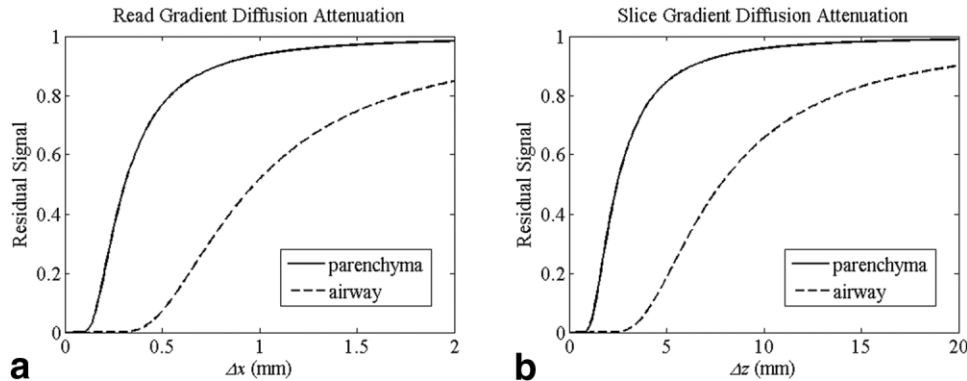


FIG. 1. Impact of resolution and slice thickness on diffusion-induced signal attenuation. **a:** Signal attenuation at the center of k -space versus resolution of an idealized single-echo Cartesian GRE acquisition with 1 ms readout period. This shows the nonviability of Cartesian GRE acquisition for high-resolution ($<500 \text{ }\mu\text{m}$) imaging airways by ^3He . **b:** Signal attenuation as a function of slice thickness assuming a sinc pulse of 1 ms duration with four zero crossings. This shows that slice selection is nonviable for ^3He imaging of airways below a slice thickness of $\sim 4 \text{ mm}$. For both calculations we have assumed $D = 0.2 \text{ cm}^2/\text{s}$ in the parenchyma, and $D = 2.0 \text{ cm}^2/\text{s}$ in the airway.

for ^3He MRI of mouse airways, non-slice-selective radial encoding should be used.

MATERIALS AND METHODS

Overview

HP ^3He imaging before and after MCh administration was performed in five BALB/c mice (two control and three ovalbumin [OVA]-sensitized) and four nonsensitized (naïve) C57BL/6 mice. These mouse strains were chosen because BALB/c mice are most commonly used in allergic models of asthma, and because C57BL/6 is the common platform for many genetically modified animals. The BALB/c mice were imaged using a 3D radial acquisition taking 5.8 min to complete and consuming 96 ml of HP ^3He to yield a resolution of $125 \times 125 \times 1000 \mu\text{m}^3$. The C57BL/6 mice underwent nonselective 2D radial imaging taking 12 s to complete and consuming 3 ml of ^3He to yield a resolution of $187 \times 187 \mu\text{m}^2$.

Animal Preparation—Sensitization

Airway hyperresponsiveness in mice is commonly achieved by sensitization and recurrent challenge with OVA (23). Under a protocol approved by the Duke Institutional Animal Care and Use Committee, 10 μg of grade V OVA (Sigma, St. Louis, MO, USA) adsorbed to 2 mg of alum adjuvant (Pierce Biotechnology Inc., Rockford, Illinois, USA) diluted in saline was administered via intraperitoneal (i.p.) injection on days 0 and 14 to BALB/c mice (Jackson Labs, Bar Harbor, ME, USA). The mice were subsequently challenged for one hour with 1% OVA aerosol on days 21, 22, and 23 (23), and imaged on day 24.

^3He Polarization

^3He was polarized by spin exchange optical pumping (SEOP) in a prototype commercial polarizer (IGI.9600.He; Magnetic Imaging Technologies, Durham, NC, USA). This system uses 60 W of 795-nm laser light to optically pump Rb (24) and typically produces 1.2 liters of ^3He (Spectra Gases, Alpha, NJ, USA) overnight with a time constant of 4–5 h and $P \approx 30\%$. Once the ^3He polarization was saturated, the optical cell was cooled to room temperature and the ^3He was dispensed into a 1-liter Tedlar bag (Jensen Inert Products, Coral Springs, FL, USA) housed in a Plexiglas cylinder. The cylinder was then detached from the polarizer and attached to a HP gas-compatible ventilator. The cylinder sits about 1 m from the opening to the 2T MR scanner, where gradient-induced longitudinal ^3He relaxation (25) is about 20 min, allowing imaging to be performed with acceptable SNR for approximately that duration.

Animal Preparation—Imaging

Mice were anesthetized using an i.p. injection of 75 mg/kg Nembutal (Abbott Labs, Chicago, IL, USA) and maintained with intravenous (i.v.) maintenance doses of 20 mg/kg every 30 min. Each mouse received a 2F-gauge catheter (Sherwood Medical, Tullamore, Ireland) in their jugular vein for delivery of MCh. The MCh delivery line was

extended with a 100-cm-long prefilled PE50 (Becton Dickinson, Sparks, MD, USA) tube connected to a syringe accessible from outside of the magnet. The mouse received a tracheostomy to allow placement of a 18-G tracheal tube, which was secured by sutures to end 2–3 mm above the carina. The tracheal tube was connected to a low-dead-volume ($\sim 40 \mu\text{l}$) Y-connector separating the inhalation and exhalation lines of an MR-compatible constant volume ventilator (12). The mouse was ventilated in the supine position at 100 breaths per minute with 0.2-ml tidal volume (26). A single breath was characterized by a 260-ms inhalation, 100-ms breathhold, and a 240-ms passive exhalation, resulting in a typical peak airway pressure of 8 cm H_2O prior to MCh challenge. To minimize the mouse's potential respiratory efforts against the ventilator, Doxacurium chloride (0.25 mg/kg) (Abbott Labs) paralytic was administered roughly 20 min prior to imaging. The animal was inserted into an 85.1/64.8 MHz dual-tuned birdcage coil ($L = 5.5 \text{ cm}$, $\phi = 3.5 \text{ cm}$) and the entire assembly was inserted into the magnet. The animal's airway pressure, ECG, and body temperature were continuously monitored throughout the study. Body temperature was maintained via feedback from a rectal temperature probe that controlled warm air circulating through the magnet bore.

MRI Hardware

Images were acquired on a 2T horizontal 30-cm clear bore magnet (Oxford Instruments, Oxford, UK) with shielded gradients (18 G/cm), controlled by a GE EXCITE 11.0 console (GE Healthcare, Milwaukee, WI, USA). The scanner was interfaced to the dual-tuned coil using a separate integrated transmit/receive switch with 31-dB gain preamplifier (Nova Medical, Wilmington, MA, USA) for the ^1H or ^3He frequency. These frequencies were created by modifying the intrinsic 63.86-MHz frequency of the scanner to 85.1 or 64.8 MHz using an up-down converter (Cummins Electronics Labs, North Andover, MA, USA).

^3He MR Imaging Sequences

High-resolution 3D images were acquired with an FOV of 3.2 cm in the coronal plane and 1.6 cm in the sagittal direction using 3D radial encoding (27). Images were acquired using a 132- μs hard excitation pulse, $\text{TR} = 5 \text{ ms}$, $\text{TE} = 132 \mu\text{s}$, bandwidth (BW) = 62.5 kHz, with 20 radial k -space views per breath. Data acquisition continued over 5.8 min until 11,520 radial k -space views had been accumulated. Radial data was then regridded on a Cartesian matrix of $256 \times 256 \times 16$ and Fourier-transformed to give images with a Nyquist-limited resolution of $125 \times 125 \times 1000 \mu\text{m}^3$. Although these images were acquired over multiple breaths, they were given the characteristics of a single breath imaging by using a variable flip angle (VFA) to excite subsequent views in a breath (28). From the first to last view, the flip angle was incremented from $\alpha_1 = 13^\circ$ to $\alpha_{20} = 90^\circ$ to both maintain constant magnetization from view to view and to prevent residual magnetization from contributing signal to the next breath. In addition to optimal use of the available ^3He magnetization, this method has the further beneficial effect of highlighting the airways over the parenchyma.

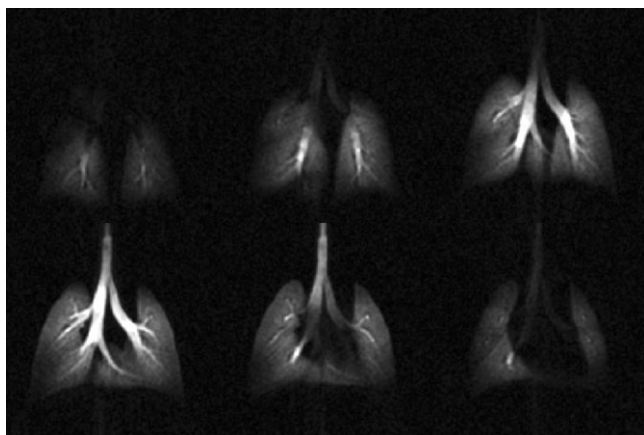


FIG. 2. Representative ^3He image partitions from a 3D dataset acquired in a naive BALB/c mouse (#1 in Table 1). The image has a resolution of $125 \times 125 \times 1000 \mu\text{m}^3$, consumed 96 ml of HP ^3He , and was acquired in 5.8 min.

Non-slice-selective coronal 2D images were similarly acquired with FOV = 2.4 cm, using 2D radial encoding. Images employed a 132- μs hard excitation pulse, TR = 5 ms, TE = 132 μs , BW = 31.25 kHz, with 20 radial k -space views per breath. Data acquisition continued for 12 s until 400 radial k -space views had been accumulated. Radial data was regridded on a Cartesian matrix of 128×128 and Fourier-transformed to yield images with a Nyquist-limited resolution of $187 \times 187 \mu\text{m}^2$. The images were acquired employing the same VFA scheme outlined for 3D imaging in order to highlight the airways relative to the parenchyma.

Experimental Protocol

The protocol consisted of the following steps. The lungs of the mouse were centered in the FOV by using a coarse ^1H localizer. Then, HP ^3He was dispensed and ^3He imaging was immediately commenced by switching the ventilator breathing gas from air to a mixture of 75% HP ^3He mixed with 25% O_2 . Gas mixing occurred at a junction just before the tracheal tube to minimize the depolarizing effects of oxygen on ^3He (29). Roughly 10 breaths were sufficient to establish stable ^3He magnetization in the lungs and image acquisition was started. Image data was collected during the 100-ms breathhold by using the ventilator to trigger the MR acquisition at end inspiration. The first ^3He image acquired in this manner was the baseline image. Immediately upon its completion, MCh was administered (250 $\mu\text{g}/\text{kg}$) in 0.1 ml of saline in a rapid bolus (~ 1 s). Before MCh reaches the lungs, it acts on the cholinergic receptors of the heart and causes a transient slowing of the heart rate (30). As soon as this heartrate reduction was noted (1–2 s postinjection), image acquisition was started. Images were acquired one before and one after MCh for our 3D protocol. For our 2D protocol, 1 image was acquired before MCh and subsequent images were continuously acquired every 12 s for 3–5 min following MCh.

Image Analysis

Both 3D and 2D image data were evaluated to quantify the degree of ventilation alteration induced by MCh. The ven-

tilated lung was first roughly cropped slice-by slice to prevent any background artifacts from being counted. Then a region outside the cropped lung was selected to measure the noise mean and SD. These values were used to retain only voxels with signal 3SD above the background noise mean and these voxels represented the ventilated lung. The ventilated voxels were counted in the pre- and post-MCh images and their ratio was computed to provide a global measure of change in ventilation.

One series of 2D temporal image series was analyzed to demonstrate the post-MCh temporal evolution of both regional ventilation and airway caliber. Regional ventilation was evaluated by selecting three regions of interest (ROIs) representing parts of the lung that appeared to evolve differently in time. Ventilation in the ROIs was calculated for each image according to the method above. For airway analysis, we selected six bronchi that could be visualized in all 23 image frames. These were the left and right main stem bronchus (lSt, and rSt), the right accessory lobe bronchus (V), and the first three lateral bronchi of the left lung (1L1, 1L2, and 1L3). The nomenclature used to label these airways is that set forth by Wallau et al. (31). Airway diameters were measured by importing the image stack into ImageJ (NIH, Bethesda, MD, USA), drawing a transverse line across the bronchi of interest and using the “plot profile” function to identify the increased signal intensity of the airway. The edges of the signal increase were determined by finding the point on either side of the profile where the signal dropped to half of the maximum-baseline intensity and this was taken to be the airway edge.

RESULTS

A typical ^3He dataset is shown in Fig. 2. The images cover the entire lung from front to back with six 1-mm coronal partitions and have an SNR of 50 in the major airways and

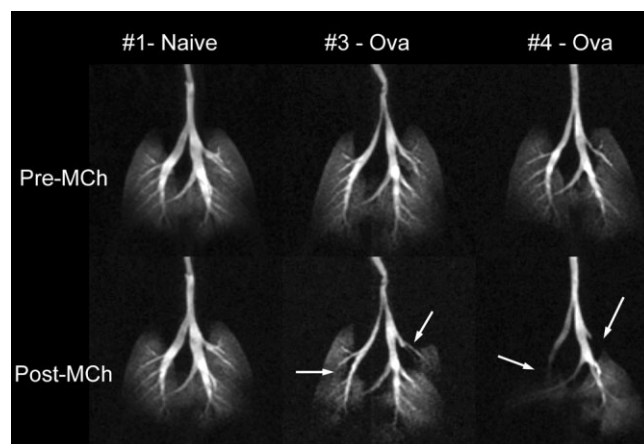


FIG. 3. MIPs generated from 3D ^3He images of 3 BALB/c mice before (top row) and after (bottom row) 250 $\mu\text{g}/\text{kg}$ MCh challenge. From left to right in the figure are a naive (#1), Ova-sensitized (#3), and Ova-sensitized (#4) mouse. The numbering relates these mice to Table 1. In the Ova-sensitized mice, the MCh effect is readily observed (arrows), but it is not readily apparent in the naive mouse. Presumably, in this nonsensitized animal the airway constriction is less severe and does not last for the full 5.8 min of the image acquisition.

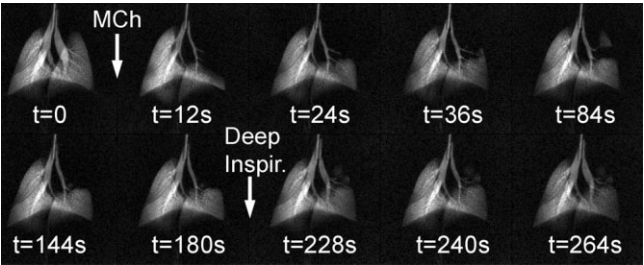


FIG. 4. Temporal response to MCh challenge in an unsensitized C57BL/6 mouse (#7) showing 10 out of 23 frames acquired every 12 s. The mouse exhibits dramatic collapse of the left main stem bronchus and the airway feeding the right accessory lobe. After the mouse receives a deep inspiration (before the $t = 228$ s frame), its airway caliber and ventilation recover nearly fully to their baseline state.

19 in the parenchyma. These SNR values were typical of images acquired immediately after dispensing ^3He . Subsequent images exhibited reduced SNR due to ^3He polarization decay, with a T_1 of roughly 20 min. All images exhibited higher SNR in the airways than parenchyma. This effect is largely attributable to the use of a VFA acquisition, particularly the final 90° flip, which consumes all remaining magnetization in each breath. This highlights the airways because with each breath they replenish 60% to 90% of their ^3He whereas the parenchyma replenishes only 5% to 30% of its ^3He (11,32). The VFA acquisition with terminal 90° pulse thus effectively presents a single breath “snapshot” of ventilation despite having been acquired over multiple breaths. Images acquired in this manner could resolve the airways to approximately the fifth generation where they measure $\sim 350\text{ }\mu\text{m}$ (3 pixels) across.

To readily identify the most responsive airways in the 3D images, we display them as maximum intensity projections (MIPs). Such representations are shown in Fig. 3 depicting images before and after MCh challenge from three mice—a naive control, and two OVA-sensitized mice. In the sensitized mice, the images show focal bronchoconstriction that is sufficient to fully close several large bronchi for at least the 5.8 min required to acquire the 3D scan. Accompanying the constriction is a reduction of ^3He signal intensity in the periphery of the lung served by these airways. By contrast, images of the naive mouse do not show any readily observable reduction in airway caliber or ventilation. However, reduced ventilation was measurable by the pixel-counting method.

Table 1 summarizes our findings on ventilation and airway caliber in all nine mice studied with MCh challenge. All the mice exhibited a reduction in the number of

ventilated pixels after MCh challenge. Of the mice that underwent 3D imaging, two OVA sensitized mice showed a large ventilation reduction (43% and 51%) compared to two naive mice (21% and 26%). One OVA-sensitized mouse showed a ventilation reduction comparable to that

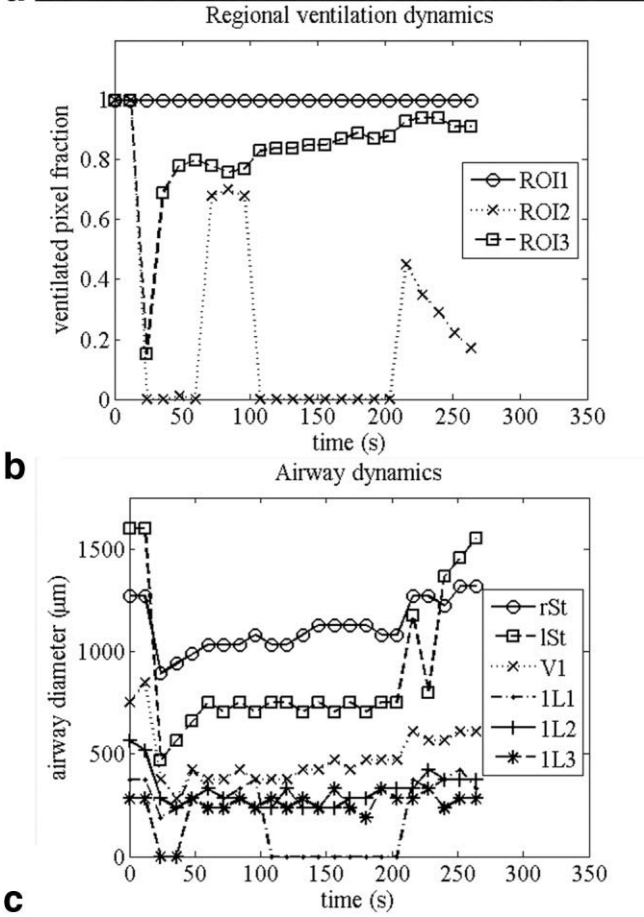
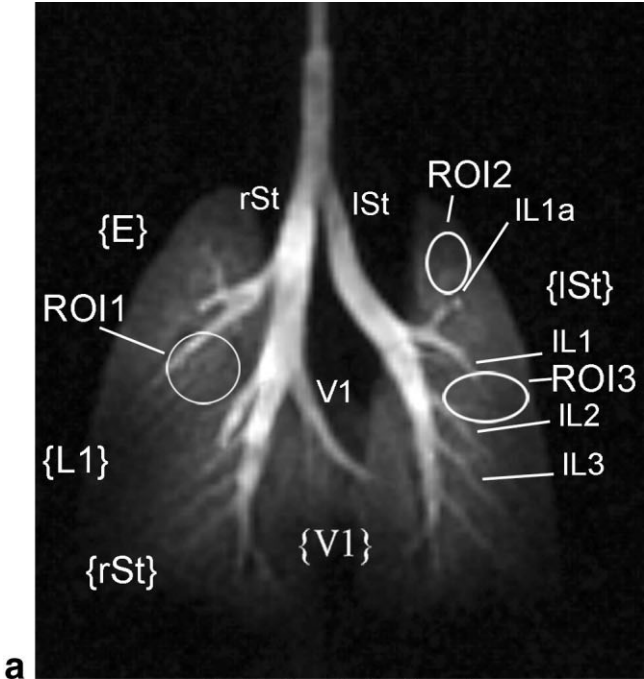


FIG. 5. Quantitative measurements of the regional ventilation and airway dynamics after MCh challenge. **a**: Labeling of airways and lung lobes according to Wallau et al. (31). In addition to the ROIs, we tracked the caliber of the left and right main stem bronchus (ISt, and rSt), the right accessory lobe bronchus (V), and the first three lateral bronchi of the left lung (1L1, 1L2, and 1L3). **b**: Recovery of ventilation in three ROIs exhibiting distinct temporal dynamics. **c**: Recovery of airway caliber in selected bronchi showing similar temporal dynamics, but different initial response.

Table 1
Summary of Imaging Results*

ID	Type	Species	2D/3D	Response (%)	Narrowing
1	Naive	BALB/cJ	3D	21	No
2	Naive	BALB/cJ	3D	26	No
3	OVA	BALB/cJ	3D	43	Yes
4	OVA	BALB/cJ	3D	51	Yes
5	OVA	BALB/cJ	3D	24	Yes
6	Naive	C57BL/6	2D	38	Yes
7	Naive	C57BL/7	2D	42	Yes
8	Naive	C57BL/6	2D	21	Yes
9	Naive	C57BL/6	2D	20	Yes

*Ventilation was reduced after MCh challenge in all animals, both naive and ovalbumin-sensitized. Two out of three sensitized animals showed a measurably larger MCh response than naive controls. Airway narrowing after MCh was seen in all images, except the 3D images of naive animals.

of naive mice (24%). This mouse may not have received the full 250- $\mu\text{g}/\text{kg}$ dose of MCh due to a blockage in the catheter. All mice that underwent 2D imaging showed measurably reduced ventilation after MCh even though they were not specifically sensitized to be hyperresponsive. These mice also showed measurable narrowing of several branches of airways.

For mapping the temporal dynamics of regional ventilation and airway caliber following MCh challenge, the more rapid 2D imaging was most fruitful. Images from such a time-course study are depicted in Fig. 4, which shows a nonsensitized C57BL/6 mouse that nevertheless exhibited a very strong MCh response. In this animal, the left main stem bronchus is severely constricted and the left apical lung shows dramatic reduction of ventilation, while its right main stem bronchus is less constricted and ventilation appears largely unaltered on the right side. The only portion of the right lung that experiences severe constriction is the bronchus feeding the right accessory lobe. After the initial MCh response, all afflicted lung regions and bronchi show a gradual recovery over time. Then, a deep inspiration, applied by stacking three breaths without exhalation at $t = 228$ s, causes nearly complete recovery of ventilation and airway caliber. When this same mouse was given a subsequent MCh challenge 10 min later, it showed the identical pattern of response and recovery. This observation seems to indicate that the lung response in this mouse on a short time-scale is reproducible.

The temporal evolution of airway caliber and ventilation can be readily quantified. Ventilation reduction and recovery shown in Fig. 5b reveals very different temporal dynamics in the selected ROIs. ROI1, in the right lung, has unaltered ventilation for the entire time course. ROI2, in the apex of the left lung, is severely impacted, dropping to zero ventilation, making a slight recovery, dropping to zero, and finally making a slight recovery. ROI3 is in an intermediate zone, where it is initially severely impacted before making a gradual but nearly complete recovery. By contrast, the recovery dynamics of the airways shown in Fig. 5c all follow a fairly similar time course. However, in these airways the initial magnitude of response was different. The observation that ventilation recovers differently

from airway caliber may suggest that airways smaller than those we can observe are significantly involved in the response. The ability to measure both airway caliber and ventilation changes by ^3He MRI provides a means to assess the impact of both resolved and of nonresolved airways.

DISCUSSION

We have illustrated that ^3He MRI of the mouse lung can be performed with sufficient temporal and spatial resolution to capture its transient response to a challenge with MCh. These images reveal both changes in ventilation and airway caliber. The ability to resolve the airways of small animals by ^3He MRI is particularly important to the study of airways hyperreactivity and has historically been accomplished only by groups employing radial sampling, including Duke, Wisconsin (33), and Lyon (34,35). In this work, we have provided simple calculations to explain how using radial imaging and avoiding slice selection mitigates the effects of ^3He diffusion, and makes airway imaging in mice possible.

While we have demonstrated both 3D and 2D ^3He imaging of airways hyperreactivity, we currently find the additional temporal information provided by 2D imaging to be most valuable. Although the 2D images lack resolution in the ventral–dorsal direction, the loss of information is alleviated by the relatively short extent of the mouse lung (~ 1 cm) in this direction, and use of the VFA technique to highlight the airways against the parenchyma. Because the 2D images can be acquired rapidly, additional information about how specific regions recover ventilation and how specific airways return to their fully open state could provide further insights into a variety of asthma models.

These encouraging preliminary imaging results open up the way for considerable continued technical and biological investigations of airways hyperresponsiveness. Future technical milestones will include developing 3D imaging sequences that can be acquired nearly as quickly as the present 2D images. Examples of more efficient 3D sequences include 3D PRIZE (projections inside Z encoding) or ZIPR (Z encoding inside projections), which phase-encode the z-direction while employing radial encoding within the slices (36). Such approaches could cover k -space with about one-fourth of the views of our present fully-radial 3D sequence and thus permit a shorter scan. Furthermore, scan times could be cut four-fold by using newly emerging approaches for radial image reconstruction that employ nonuniform fast Fourier transform (NUFFT) without regridding (37). Thus, the very real potential exists to use these methods to obtain 3D ^3He images of the resolution demonstrated here in less than 1 min of scan time. Though tradeoffs between scan time and spatial resolution will continue to be a challenge, we are optimistic that the outlined approaches will generate considerable improvements.

CONCLUSION

The ability to image alterations in mouse lung function after MCh administration with high spatial and temporal resolution opens up exciting possibilities for quantitative evaluation of asthma phenotypes. These phenotypes are currently only assessed by methods that, though in ubiq-

uitous use in the airway biology community, lack spatial information and are limited in information content. By instead assessing murine asthma models via ^3He imaging, we have an opportunity to open up new avenues of investigation. Imaging will be particularly powerful if, as our initial results suggest, AHR is a heterogeneous phenomenon. The ability of imaging to isolate those regions of the lung that contribute most significantly to its response should provide increased sensitivity. The ability to now image AHR from mouse to human (38), should make HP ^3He MRI a truly translational tool in asthma research.

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