COMPLETE METHODS

Animal Preparation and Care

This study was performed on male Sprague-Dawley rats (n = 15, 400±50 g) after approval by the Institutional Animal Care and Use Committee of the University of Pennsylvania (Philadephia, Pennsylvania). Briefly, general anesthesia was induced and maintained with intraperitoneal pentobarbital (40–60 mg/kg initially), the trachea intubated (14-gauge catheter; BD, Franklin Lakes, NJ), and the glottis sealed (DAP Products Inc., Baltimore, MD) to prevent gas leakage.[1] During imaging, a magnetic resonance imaging (MRI)-compatible small animal ventilator capable of mixing and delivering helium and oxygen was used. Paralysis was obtained with pancuronium bromide (1 mg/kg IV; Abbot Labs, North Chicago, IL). Airway pressure was recorded using a fiber-optic sensor (Samba Sensors AB, Vastra Frolunda, Sweden), and heart rate and peripheral oxygen saturation levels were monitored by a veterinary pulse-oximeter (Nonin Medical, Inc. Plymouth, MN) attached to the hind foot. Finally, a rectal probe was used to monitor the animal's body temperature, which was carefully maintained at 37 °C by a flow of warm air through the bore of the scanner. After the last set of measurements, animals were removed from the scanner and euthanized by lethal pentobarbital injection.

Outline of the experiments

Ten animals underwent hyperpolarized gas MRI (HPMRI) in this study in order to measure the apparent diffusion coefficient (ADC) of ^{3}He during ascending and descending positive end expiratory pressure (PEEP) trials. To quantify lung volume hysteresis and recruitment, computerized tomography (CT) was performed in place of MRI on a separate group of five animals undergoing similar ventilator protocols. After completion of the study, all animals were removed from the scanner and euthanized with a lethal pentobarbital injection. The ADC measurements obtained at PEEP 0 cmH_2O in 5 animals of the MRI group have been used as healthy baseline in a previously published experiment.[2]

Mechanical Ventilation Protocol

Animals were maintained supine throughout imaging and were ventilated by an MRI-compatible small animal ventilator developed in the authors' laboratory.[1] Mechanical ventilation was initially performed with a fixed tidal volume (V_T) of 10 mL/kg, respiratory rate of 53 breaths per minute, and an inspiratory to expiratory ratio of 1:2, FiO_2 0.21. With the exception of PEEP and FiO_2, these settings were maintained unchanged throughout the entire imaging session. PEEP was generated by connecting the expiratory gas line to a graduated water column. To standardize lung recruitment history, an alveolar recruitment maneuver was performed 3 min prior to the first image acquisition. This recruitment maneuver consisted of serial increases of PEEP in increments of 3 cmH_2O up to 9 cmH_2O, each maintained for 20 s. At the end of the recruitment maneuver, PEEP was again returned to 0 cmH_2O. After performing the baseline measurements, PEEP was increased from 0 to 9 cmH_2O in 3 cmH_2O increments and returned to baseline in a similar fashion. In HPMRI rats, each PEEP level was maintained for a 90-s period to minimize depolarization of ^{3}He gas, at the end of which an ADC measurement was performed. For the animals that received CT, each PEEP level was maintained for an additional 8 min to account for the longer image acquisition time.

Imaging Protocols

HPMRI: Imaging was performed as previously described [3] with a 50-cm bore 4.7-T MRI scanner (Agilent Technologies, Santa Clara, CA). The MRI scanner was equipped with 12-cm 25 G/cm gradients and a quadrature 8-leg birdcage radiofrequency coil with an internal diameter of 7 cm (Stark Contrast, Erlangen, Germany) that was tuned to the ^{3}He resonance frequency of 152.95 MHz. Using a commercial prototype (IGI.9600.He, GE Healthcare, Durham, NC),[4] ^{3}He gas was hyperpolarized to approximately 30% over 14 h. For all animals, ADC imaging was performed on axial slices using a diffusion-weighted gradient echo imaging pulse sequence with Cartesian centric *k*-space sampling. The parameters used

included: planar resolution = 0.94×0.94 mm^2, field of view = 6 × 6 cm^2, flip angle α = 4–5°, repetition time = 4.5 ms, and echo time = 3.3 ms.[3, 5] The 20-mm axial slice was positioned directly under the heart in order to minimize the cardiac motion artifacts and to maximize the lung parenchyma included in the imaged slab. The inspired gas was switched to a $^4He:O_2$ (4:1) mixture exactly 30 breaths before each image acquisition. By switching the gas at this point, we were able to prevent diffusion measurement errors induced by variability in resident gas concentration present in the functional residual capacity at different PEEP levels. Furthermore, such changes in lung volume can also alter the ADC signal by affecting the alveolar oxygen concentration and ^3He depolarization.[6] The source gas was then switched to hyperpolarized $^3He:O_2$ (4:1) prior to imaging. After five breaths in the presence of $^3He:O_2$, a 4-s inspiratory pause was instituted to perform ADC, following a 500 ms delay to ensure a uniform distribution of the inspired gas. Diffusion weighting was performed along the phase-encoding direction, and corresponding to the left-right direction of the rat body. A diffusion time Δ = 1 ms was used along with a gradient duration δ =200 μs, and b = 0, 5.27, 3.09, 1.41, and 0 s/cm^2, all with a fixed ramp time of τ = 180 μs. The zero value for b was repeated at both the beginning and end of the sequence in order to enhance the accuracy of the α estimate for each voxel.

CT: High resolution whole lung CT scans were acquired at each PEEP level using a commercial microCT scanner (eXplore CT120 system, Gamma Medica, Inc., Northridge, CA). The lungs were easily contained within the default field of view (85 mm transaxial diameter). Settings used for Imaging were: 80 kVp, 32 mA, 16 ms exposure time, 220 projections (half-scan), and 100 μm isotropic resolutions. To avoid blurring due to respiratory motion, imaging was ventilator-gated and performed during 500-ms end-inspiratory breath-holds. Only a single view per breath was acquired during each breath-hold, with the scan time for each image totaling about 8 min.

Image and Data Analysis

HPMRI and CT data analysis was performed using MATLAB (Mathworks, Natick, MA) codes developed in the authors' laboratory. ADC was analyzed on a pixel-by-pixel basis at a planar resolution of approximately 0.94×0.94 mm^2. The acquired signal was corrected for any bias due to background noise.[7]

The signal-to-noise ratio threshold was iteratively varied between 12-20 for each image, and the highest threshold value was selected in order to ensure that the entire lung parenchyma was preserved after masking the low signal-to-noise pixels. The time evolution of valid pixels' signal intensity was then fitted to a standard equation to yield maps of regional ADC values.[3] Mean, standard deviation, and skewness of the ADC distribution were calculated for each imaged slice. To measure the vertical distribution of ADC and investigate whether or not the effects of PEEP and hysteresis are a function of position along the top to bottom direction, maps were subdivided in three horizontal bins of equal thickness: mean ±standard deviation ADC was obtained for each bin.

CT image reconstruction was performed using a proprietary program supplied by the scanner manufacturer (MicroView 2.2, GE Healthcare). For lung gas volume determination, image thresholding[8] was used to obtain images of all relevant three-dimensional regions of interest (ROI), including each lung in its entirety. All pixels with CT density higher than -200 Hounsfield Units (HU) were excluded, thereby ensuring that the image thresholding could produce an adequate delimitation of the aerated lung parenchyma from the surrounding chest wall, and from other nonpulmonary tissue in healthy lungs. End-inspiratory lung gas volume was calculated using CT density analysis methodology, [8-10] following the threshold segmentation, based on the following equation:

$$\text{Gas volume}_{ROI} = \text{Total volume}_{ROI} \times \frac{Mean\ CT_{ROI}}{-1000}$$

Where the Total volume$_{ROI}$ included both gas and tissue volumes, Mean CT$_{ROI}$ denoted the mean of the

lung density in HU within each ROI.

For determination of atelectasis and lung aeration, whole-lung three dimensional ROI were obtained at PEEP 0 cmH_2O (in the ascending and in the descending ramp) and at 9 cmH_2O, by semiautomatic segmentation of CT slices (excluding the heart, the central airways, and the major vessels but including all lung tissue). The total weight of the lungs was then obtained and partitioned between tissue compartments with different aeration. These were defined based on density ranges:[11, 12] [a] nonaerated (-100 to +100 HU); [b] poorly aerated (-500 to -101 HU); [c] normally aerated (-900 to -501 HU); and, [d] hyperinflated (-1,000 to -901 HU). Each compartment was measured as the percentage of the total lung weight. Because the automatic and the semi-automatic methods resulted in pulmonary gas volume values that differed by no more than 5%, end-inspiratory lung gas volume calculated using the automatic thresholding levels was used for analysis of hysteresis.

The airway pressure tracing recorded at the end of each PEEP period was used to obtain the peak inspiratory pressure. Dynamic respiratory system compliance was calculated as $C_{dyn} = V_T/(PIP–PEEP)$.

Statistical Analysis: Statistical analysis was performed using "R" (R Foundation for Statistical Computing; Vienna, Austria[*]) applications developed in the authors' laboratory. Group mean and standard deviation values of all computed quantities were calculated for each PEEP level, in the ascending and descending ramps. The relative changes of measured quantities were compared using repeated measurements two-way ANOVA to examine the main effects of PEEP level (between 0 and 6 cmH_2O) and type of PEEP ramp (ascending *vs*. descending); this was followed by *post-hoc* comparisons to test differences between individual ascending or descending PEEP values, using paired-*t* tests and the Bonferroni correction for multiple comparisons when appropriate. [13] The area under the curve method for serial measurement analysis [14] was used to test for differences in measured variables between ascending and descending PEEP ramps. $P < 0.05$ (for two-tailed hypothesis) was considered statistically significant.

[*] http://www.R-project.org; last accessed July 31, 2013.

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