Regional correlation of emphysematous changes in lung function and structure: a comparison between pulmonary function testing and hyperpolarized MRI metrics

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Emami K, Chia E, Kadlecsek S, MacDuffie-Woodburn JP, Zhu J, Pickup S, Blum A, Ishii M, Rizi RR. Regional correlation of emphysematous changes in lung function and structure: a comparison between pulmonary function testing and hyperpolarized MRI metrics. J Appl Physiol 110: 225–235, 2011. First published September 30, 2010; doi:10.1152/japplphysiol.00269.2010.—Regional and global relationships of lung function and structure were studied using hyperpolarized 3He MRI in a rat elastase-induced model of emphysema (n = 4) and healthy controls (n = 5). Fractional ventilation (r) and apparent diffusion coefficient (ADC) of 3He were measured at a submillimeter planar resolution in ventral, middle, and dorsal slices 6 mo after model induction. Pulmonary function testing (PFT) was performed before MRI to yield forced expiratory volume in 50 ms (FEV50), airway resistance (R5), and dynamic compliance (Cdyn). Cutoff threshold values of ventilation and diffusion, r* and ADC*, were computed corresponding to 80% population of pixels falling above or below each threshold value, respectively. For correlation analysis, r* was compared with FEV50/functional residual capacity (FRC), R5 and Cdyn, whereas ADC* was compared with FEV50/FRC, total lung capacity (TLC), and Cdyn. Regional correlation of r and ADC was evaluated by dividing each of the three lung slices into four quadrants. Cdyn was significantly larger in elastase rats (0.92 ± 0.16 vs. 0.61 ± 0.12 ml/cmH2O). The difference of R5 and FEV50 was insignificant between the two groups. The r* of healthy rats was significantly larger than the elastase group (0.42 ± 0.03 vs. 0.28 ± 0.06), whereas ADC* was significantly smaller in healthy animals (0.27 ± 0.04 vs. 0.36 ± 0.01 cm/s). No systematic difference in these quantities was observed between the three lung slices. A significant 33% increase in ADC* and a significant 31% decline in r* for elastase rats was observed compared with a significant 51% increase in Cdyn and a nonsignificant 26% decline in FEV50/FRC. Correlation of imaging and PFT metrics revealed that r and ADC divide the rats into two separate clusters in the sample space.

lungs physiology; relationship of lung function and structure; regional fractional ventilation; apparent diffusion coefficient; hyperpolarized gas MRI

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is the fourth leading cause of death in the United States and is now the most common form of chronic lung disease (3). Emphysema, a primary subcategory of COPD, is characterized by abnormal enlargement of lung components distal to the terminal bronchioles (2) and is accompanied by alveolar wall destruction and tissue density loss (28). This disease results principally from inhaled toxic substances, usually chemicals in tobacco smoke, which activate epithelial cells to produce inflammatory mediators that trigger chronic inflammation (3) and progressively deteriorate pulmonary function (23). Emphysema symptomatology can be arrested with early diagnosis, proper bronchodilator treatment, and smoking cessation (6). Conventional methods to diagnose and evaluate emphysema include pulmonary function tests (PFTs), chest radiography, and computed tomography (CT). PFT markers, such as the decline in forced expiratory volume in 1 s (FEV1) (8) and its ratio to forced vital capacity (FVC), are the most common clinical pulmonary markers used to assess the presence and progression of emphysema. PFTs measure the increased time required to force the air out of the lungs due to the effects of increased resistance of small airways (36) and increased lung compliance due to diminished lung elastic recoil (21). These global markers, along with conventional chest radiology, only provide a gross assessment of the state of emphysema, thereby limiting their application to quantitative and localized diagnosis. Furthermore, global pulmonary markers are generally insensitive to early, mild changes in lung function due to emphysema (31), and it is hypothesized that up to 30% of functional lung capacity can be lost despite minimal respiratory symptoms (29).

Computed tomography (CT), by comparison, is more sensitive to early emphysematous changes including regional variations of lung morphometry (15), and good correlation with pathology specimens has been shown (7). Emphysema-induced tissue destruction, however, results in lower x-ray attenuation per unit volume compared with healthy lungs, thereby limiting the achievable contrast in the images. Other disease processes, such as reduced perfusion and air trapping (32), can also reduce attenuation, and these effects may not be directly separable from emphysematous changes. Finally, CT’s high radiation dose limits its use in longitudinal studies and in cases where continuous clinical follow-up is required (5).

A strong link between lung structure and function has been demonstrated by various researchers (4, 16). In addition to structural deterioration, emphysema also directly affects lung function through irreversible airflow limitation (23), a fact that underscores the value of simultaneous measurement of structural and functional changes in the lung during the disease’s progression. Hyperpolarized helium-3 magnetic resonance imaging (HP 3He MRI) techniques have been evolving over the past decade to address the shortcomings of PFT and CT techniques. Since HP 3He MRI allows for the direct visualiz-
tion of the distribution of gas atoms within the airways and alveolar space, it presents a significant potential in identifying changes in both lung structure (25, 35) and function (14, 20), especially in the early stages of pulmonary diseases. Its attractive safety profile makes this sensitive, nonionizing, and noninvasive modality a useful tool for evaluating emphysema progression after therapeutic intervention. However, despite the recent advances in HP 3He MRI technology, the regional relationships between lung structure and function have not been explored. Gas density 3He MR images have been previously reported in other studies as a qualitative tool to assess the ventilation deficiency in both emphysematous (27) and asthmatic subjects (1). More recently, stepwise ventilation techniques have been proposed to provide quantitative regional ventilation information (9, 11), as well as dynamic techniques for qualitative imaging of gas flow in the lungs with a high temporal resolution (18). A few notable works have explored the correlation between functional (19) and structural (26) HP 3He MRI metrics and PFT measurements. The most commonly adopted HP 3He MRI metric to study emphysematous changes in the lung, however, is the 3He apparent diffusion coefficient (ADC) (12, 30), which is fundamentally a structural marker.

In this study, we examined correlations between changes of lung function and structure in a rat model of emphysema by comparing quantitative regional HP 3He MRI markers of alveolar size and fractional ventilation to a number of standard PFT metrics including airway resistance, dynamic compliance, as well as characteristic and forced expiratory lung volumes. The primary aim of this study was to compare the sensitivity of each set of markers to diseased-induced changes in the lungs. In addition, simultaneous regional changes of lung function and structure in healthy and emphysematous animals were examined through measurements of lung ventilation and self-diffusion of gas atoms as the two main mechanisms of gas transport in the lungs. Therefore, the secondary aim of this research was to attain a better understanding of the coupled behavior of function and structure in this disease model and to augment the current understanding of emphysematous changes in the lung.

METHODS

Emphysema model induction. All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania. Two groups of five male Sprague-Dawley rats were used to perform the study. All rats were age-matched and maintained under similar environments and dietary conditions for a period of 6 mo. Elastase was administered via the trachea at 20 U per 100 g body weight. One unit, U, is defined as the amount of elastase that will solubilize 1 mg of elastin in 20 min at 37°C and pH 8.8. Under an anesthesia. It was expected that all animals would exhibit a fairly uniform emphysematous response over this time period. However, since the elastase instillation was carried out through the trachea, the delivery rate to each lobe was not directly controlled. Therefore, it was possible that each lobe in the rat lung experienced a different level of tissue destruction.

Animal preparation and pulmonary function testing. PFTs were performed right before the MRI session on the same day. Animals were sedated with 0.1 g/kg intraperitoneal ketamine and 10 mg/kg xylazine. The dose was repeated every 90 min or as necessary. The rats were intubated with a 2-in.-long, 14-gauge angiocatheter (BD, Franklin Lakes, NJ). A small quantity of soft sealant (UHU Tac adhesive putty; Saunders Mfg., Readfield, ME) was applied to the outside of the tracheal tube to create a tight seal around the endotracheal tube right after the vocal folds to enable a breath hold of up to 25 cmH2O for 5 s with negligible leakage. The rats were then connected to a rodent-specific forced maneuver system (AUT6110, Buxco Research Systems, Wilmington, NC). The PFT system consisted of a restrained whole body plethysmography chamber (PLY3115, Buxco Research Systems, Wilmington, NC) equipped with mouth pressure and body flow transducers for real-time measurements of chest pressure and displacement. A series of programmed forced ventilation maneuvers were used to measure functional residual capacity (FRC), total lung capacity (TLC), FEV50, airway resistance (R), and dynamic compliance (Cdyn), all in less than 10 min. Each measurement was performed a minimum number of three times to ensure reasonable repeatability.

Immediately after PFT measurements, the rats were temporarily paralyzed with 1 mg/kg iv pancuronium bromide (Abbott Labs, North Chicago, IL) and immediately connected to a custom-built MRI-compatible ventilator. Mechanical ventilation was maintained with normal air at a respiratory rate (RR) of 60 breaths/min with a tidal volume (TV) equal to 15% TLC to normalize the forced ventilation effort among different animals. Temperature was monitored using a rectal probe and was maintained at 37°C by a flow of warm air through the bore of the magnet. Heart rate and blood oxygen saturation level were monitored using a veterinary pulse-oximeter (8600V, Nonin Medical, Plymouth, MN) with the optical probe attached to the rat’s hind foot.

Regional measurement of fractional ventilation. Fractional ventilation is defined as the ratio of the amount of fresh gas added to a region of interest (ROI) in the lung during inspiration, noted as Vf, to the total gas space of that ROI at the end of inspiration, Vi (which comprises both Vt and the residual volume, Vr); r = Vf/Vi = Vp/(Vt + Vf). A voxel’s gas content at end inspiration under breath-hold pressure is assumed to be divided between r, which consists of the delivered fresh gas, and q = 1 − r, which represents the residual capacity of the ROI. A measurement of r = 0 indicates no gas replacement, whereas r = 1 indicates complete gas exchange with each breath.

Fractional ventilation imaging was performed using the technique described earlier (11). Briefly, a series of 10 HP gas breaths (3He:O2 = 4:1) was delivered to the rat at the designated tidal volume, and one image was acquired after each breath during a 350-ms breath hold. The polarization of 3He in a given ROI at the jth breath is a function of the arriving fresh gas from the reservoir, Sj, and the residual polarization from the previous breaths, S(j−1), subject to decay mechanisms during the breathing cycle. The HP 3He signal buildup in the rat lung can therefore be expressed as a recursive model of the form:

\[ S(j) = r \cdot S_0 + (1 - r) \cdot S(j - 1) \times \exp(\text{DRF}) \]

which can in turn be solved for r on a pixel-by-pixel basis to yield the fractional ventilation map of the imaged slice. The oxygen-induced depolarization of 3He during each breath is governed by D3He = −60/(RR-T13He), with the time constant T13He = δ/PO2 as a function of the partial pressure of oxygen (PO2) present in the airways, and with
\[ \xi = 2.6 \text{ bar-s} \text{ at normal body temperature} \] (24). The radio frequency (RF) depolarization effect \( D_{RF} = N_{PE} \text{ln}(\cos(\alpha)) \) represents the cumulative effect of repeated RF excitations on HP \(^3\text{He} \), where \( \alpha \) represents the RF pulse flip angle, and \( N_{PE} \) is the number of pulses triggered per image (i.e., the number of phase encode lines). From a practical standpoint, the oxygen decay effect has a negligible effect on signal buildup (11), and therefore, a nominal value of \( P_{O_2} = 140 \) mbar was assumed to hold throughout the lung. In contrast, the RF pulse effect has a substantial effect on signal dynamics and was separately estimated on a regional basis by acquiring a series of five back-to-back ventilation images during a 2-s breath hold at the end of the fractional ventilation maneuver.

Regional measurement of apparent diffusion coefficient. Apparent diffusion coefficient maps of \(^3\text{He} \) were acquired using a double-acquisition ADC imaging technique previously described in (10). The double-acquisition technique has been shown to minimize the effect of cross terms between diffusion gradients and imaging gradients (22), as well as that of background field gradients (including susceptibility gradients induced by air-tissue interface in the lungs), which if ignored, could potentially result in an overestimated value of the diffusion coefficient. This method is based on the geometric mean of two separate sets of diffusion-weighted images, which only differ in the order of positive and negative lobes of bipolar diffusion gradients. For this purpose, a series of four HP gas breaths (\(^3\text{He}:O_2 = 4:1\)) were delivered to the rat at the designated tidal volume, and the fifth breath was held for 3 s to acquire the first series of ADC images for a given set of five diffusion gradient factors (\( b \) values) using the positive-negative ordering of bipolar diffusion-sensitizing gradients. The \( b \) value is given by \( b(j) = [2 - \pi \gamma G(j)/2] / (2 \xi - \delta/3) + \pi/30 - \delta \pi/6 \), where \( \gamma = 23.43 \text{ MHz/T} \) is the gyromagnetic ratio of \(^3\text{He} \), \( G(j) \) represents the diffusion-sensitizing gradient amplitude corresponding to the \( j \)th image in the series at time \( t(j) \), with \( \gamma \) the gradient ramp time, \( \delta \) the diffusion gradient duration (from the beginning of the ramp to the end of gradient flat top), and \( \Delta \) the diffusion time (defined as the time between the beginning of the first to the beginning of the second gradient lobe). This procedure was then repeated in an identical manner with the reverse (negative-positive) order of bipolar diffusion gradients in a second breath hold. The images corresponding to \( b \) values from each breath were pairwise multiplied and raised to the power of 1/2 to yield the geometric mean of the two sets of diffusion-weighted images, and then fit to the following equation to yield ADC values on a pixel-by-pixel basis:

\[ S(j) = S_0 \times \exp[N_{PE} \cdot \text{ln}(\cos(\alpha)) \cdot j - b(j) \times ADC - (t(j)/T_{1\text{rel}})] \]

where all other parameters represent the same quantities as in the fractional ventilation model.

Imaging equipment and parameters. \(^3\text{He} \) gas was hyperpolarized through spin-exchange collisions with optically pumped rubidium (Rb) atoms, as previously described (33), using a commercial polarizer (IGI9600, GE Healthcare, Durham, NC) to a level of \( \sim 30\% \) over 14 h. Imaging was performed on a 50-cm 4.7-T MRI scanner (Varian, Palo Alto, CA) equipped with 12-cm 25-G/cm gradients and a quadrature eight-leg birdcage body coil with ID = 7 cm (Stark Contrast, Erlangen, Germany) tuned to the \(^3\text{He} \) resonance frequency of 152.95 MHz. The animal was placed supine in the RF coil inside the MRI scanner, and RF pulse-width calibration was performed to estimate the applied imaging flip angle to the rat. This task was performed during a 2-s breath hold of HP \(^3\text{He} \). Ventilation imaging was performed using a fast-gradient-echo pulse sequence with the following parameters: field of view (FOV) = \( 6 \times 6 \text{ cm}^2 \), number of slices (NS) = 3, slice thickness (ST) = 5 mm, \( \alpha = 4-5^\circ \), matrix size (MS) = \( 64 \times 64 \text{ pixels} \), bandwidth (BW) = 50 kHz, repetition time (TR) = 6.6 ms (8.1 ms for ADC), and echo time (TE) = 3.3 ms (4.8 ms for ADC). The middle coronal slice was located by acquiring projection \(^3\text{He} \) images along the three primary axes and by ensuring that the trachea was included in the middle slice. The anterior and posterior slices were then positioned according to the middle slice with a 1-mm intraslice gap. ADC imaging was performed using a diffusion-weighted version of the same pulse sequence by adding a bipolar diffusion sensitizing gradient along the phase encode (PE) direction with diffusion time \( \Delta = 1.5 \text{ ms} \), gradient duration \( \delta = 200 \mu\text{s} \), and \( b \) values = 0, 5.27, 3.09, 1.41, and 0 \( \text{ cm}^2/\text{s} \) all with a fixed ramp time of \( \tau = 180 \mu\text{s} \). The initial and final zero \( b \) values were used to estimate the RF pulse flip angle.

Imaging data analysis. Data analysis was performed using custom MATLAB (Mathworks, Natick, MA) programs developed in the authors’ laboratory. Fractional ventilation and apparent diffusion coefficient analysis were performed on a pixel-by-pixel basis at a submillimeter planar resolution (approximately \( 0.94 \times 0.94 \text{ mm}^2 \)). The signal in the acquired images was bias-corrected for the background noise (17) according to:

\[ S = \sqrt{S^2 - \sigma^2} / \sigma \]

with the inherent noise in the MR image, calculated from \( \sigma = B \sqrt{2/\pi} \), where \( B \) is the average background signal intensity corresponding to a 10 \( \times \)10-pixel region far away from the lungs in the acquired image. To distinguish lung tissue from background, pixels with a signal-to-noise ratio (SNR) below a specific threshold were excluded from analysis. The SNR threshold was iteratively varied between 5 and 20 for each image and the highest threshold value was selected such that the entire lung parenchyma was retained after masking the low-SNR pixels. After excluding background pixels from the image, time evolution of signal intensity of valid pixels were fit to Eqs. 1 and 2 to yield maps of regional r and ADC values, respectively.

The cutoff threshold parameter \( r^* \) for fractional ventilation was defined as the \( r \) value above which 80% of the valid pixels in the lungs assume their values, i.e., \( r > r^* \). Correspondingly, the cutoff threshold parameter ADC* for apparent diffusion coefficient was defined as the ADC value below which 80% of the valid pixels in the lungs assume their values, i.e., ADC < ADC*. To calculate \( r^* \) and ADC*, the three imaged slices from each animal were combined to form one distribution, and pixels with \( r = 1.0 \) and ADC \( > 0.5 \text{ cm}^2/\text{s} \) were removed from this distribution. These values primarily correspond to major conductive airways that exhibit a nearly complete gas replacement per breath, in addition to having a near-free diffusion coefficient due to the absence of restrictive lung tissue present in parenchyma. Threshold curves for r or ADC maps were then generated from each distribution corresponding to percent population of pixels with \( r > r^* \) (with \( r^* \) varying from 0 to 1), and the percent population of pixels with ADC < ADC* (with ADC* varying from 0 to 0.5 \( \text{ cm}^2/\text{s} \)). The \( r^* \) and ADC* threshold quantities corresponding to 80% population for each distribution were then computed and reported as the cutoff threshold for each animal.

The rat and the some of the mechanical ventilation components (including the \(^3\text{He} \) reservoir) were affixed to the RF coil, which was pulled outside of the scanner in between the \( r \) and ADC acquisitions to refill the \(^3\text{He} \) bag. Even though care was taken to return the coil to its original position along the z-axis, on some occasions there was a slight variation in the axial position of the coil between the \( r \) and ADC acquisitions. Therefore to analyze the regional correlation of \( r \) and ADC, a simple rigid coregistration algorithm was utilized to align the two corresponding maps along the primary axis of the scanner. This goal was achieved by maximizing the normalized cross-correlation function of the two maps after translating one relative to the other. The results were visually inspected to ensure proper convergence of the algorithm. Each of the three imaged slices was then divided into four quadrants (approximately \( 16 \times 16 \text{ regions} \)) and the corresponding mean \( r \) and ADC values of each quadrant were correlated to each other across all rats in both groups.

For whole lung assessments, the relationship between threshold values and the relevant global PFT parameters was studied. Specifically, \( r^* \) was correlated to FEV\textsubscript{50}/FRC, \( R_p \), and \( C\text{dyn} \), whereas ADC* was correlated to FEV\textsubscript{50}/FRC, TLC, and \( C\text{dyn} \). In addition, the...
sensitivity of each PFT parameter to emphysematous changes in the rat lungs was analyzed in comparison to \( r^2 \) and ADC* values. For statistical analysis, a significance level of 0.05 was used. A separate one-way ANOVA test was performed for analysis of variance of fractional ventilation and apparent diffusion coefficient between the two cohorts of rats.

**RESULTS**

PFTs. Each group of rats initially consisted of five animals. One rat in the elastase cohort died \(<3\) mo after inducing the model, and before reaching the MRI session. The dead animal was not replaced due to the concern that introducing additional animals to this cohort could adversely affect the intersubject variation of the measurements. Table 1 summarizes the weight and PFT results for all animals. TV values are based on 15% variation of the measurements. Table 2 and 3 summarize the mean ± SD of \( r \) and ADC quantities were therefore included in the analysis. The lung volumes (FRC and TLC) were both larger in elastase rats (\( P = 0.001 \)), 11.5 ± 1.6 and 33.7 ± 1.9, compared with 6.2 ± 1.2 and 25.2 ± 2.4 in controls, respectively (means ± SD; all in ml). The lung compliance (\( C_{\text{dyn}} \)) was significantly higher in elastase animals compared with controls: 0.92 ± 0.16 vs. 0.61 ± 0.12 ml/cmH\(_2\)O (\( P = 0.018 \)). In contrast, the difference in airway resistance (\( R_1 \)) and FEV\(_{50} \) was insignificant between the two groups: 0.17 ± 0.02 vs. 0.20 ± 0.03 cmH\(_2\)O·s/ml (\( P = 0.947 \)), and 5.6 ± 1.4 vs. 4.5 ± 0.8 ml (\( P = 0.212 \)), respectively. Table 1 also shows the calculated overall ventilation coefficient, \( R = \text{TV/(TV + FRC)} \), and FEV\(_{50}/\text{FRC} \) for further comparison to regional fractional ventilation.

Regional distribution of \( r \) and ADC. The typical SRN of the acquired \( ^3\)He images ranged from 40 to 60 depending on the actual \( ^3\)He polarization level and on the ventilation efficiency of each animal. Figure 1 shows a representative set of multislice \( r \) and ADC maps for a healthy rat compared with the same parametric maps in a representative emphysematous rat shown in Fig. 2. Also shown in Figs. 1 and 2 are the respective frequency distribution histograms for each parametric map. The maps shown include all of the valid pixels. The large airways are clearly visible in ventilation maps with near-unity \( r \) values. They are however not as clearly visible in ADC maps, mostly due to the high diffusion-weighted attenuation of HP \( ^3\)He signal in these unrestricted regions. The pixels corresponding to large-conductive airways (\( r = 1.0 \) and ADC ≥ 5 cm\(^2\)/s) are therefore excluded from the histograms to remove the bias in mean value toward these high-value regions. Figure 3 shows the threshold analysis results for \( r \) and ADC values in both groups. Each curve in the left panels represents the distribution of pixels for a single animal from either the control or elastase groups. Figure 3A provides the percentage of pixels that fall above a given \( r^* \) threshold value in any given lung. Conversely, Fig. 3B provides the percentage of pixels that fall below a given ADC* threshold value. All \( r \) curves, therefore, by construct vary from 100% to zero corresponding to the minimum and maximum threshold values. This trend is opposite for ADC values ranging from zero to 100%. The right panels in Fig. 3 show the distribution of \( r^* \) and ADC* threshold values for all rats at the 80% threshold cutoff. The numerical values of these threshold values are reported in Tables 2 and 3, showing a significant difference between the two groups. \( r^* \) threshold values in controls were calculated as 0.42 ± 0.03 vs. 0.28 ± 0.06 in elastase-induced rats (\( P = 0.004 \)). Similarly, the ADC* threshold values were determined as 0.36 ± 0.01 vs. 0.27 ± 0.04 cm\(^2\)/s in emphysematous animals (\( P = 0.004 \)). All \( r \) and ADC curves show the same s-shaped pattern and mainly differ in the position of the inflection point. It is therefore evident that elastase rats have a larger number of pixels with lower \( r \) than the controls at a vast majority of the \( r^* \) values, which indicates a systematic ventilation decline in this model. In a similar fashion, elastase rats exhibit a larger number of pixels with higher ADC than the

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<th>Table 1. Pulmonary function testing measurements</th>
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<td>Healthy control rats</td>
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<td><strong>522 ± 46</strong></td>
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<td><strong>Elastase rats</strong></td>
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<td><strong>543 ± 25</strong></td>
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Boldface values are means ± SD for each rat group. \( P \) values are for healthy control vs. elastase rats. TV, tidal volume; FRC, functional residual capacity; TLC, total lung capacity; FEV\(_{50}\), forced expiratory volume in 50 ms; \( R_1 \), airway resistance; \( C_{\text{dyn}} \), dynamic compliance.
controls at a vast majority of the ADC* values indicating a
global enlargement of alveolar size.

**Global and regional correlation of imaging metrics.** Figure 4
shows the variation of threshold metrics, r* and ADC*, as a
function of PFT parameters for all animals. Specifically, r*
values are plotted as a function of FEV50/FRC, RI, and Cdyn,
and ADC* values are plotted as a function of FEV50/FRC,
TLC, and Cdyn, all with two different symbols for control and
elastase rats. The FEV50/FRC quantity is calculated from the
PFT measurements representing the clinically accepted metric
for staging emphysema. Each pair of threshold metrics and
PFT metrics partitioned the parameter space into two distinct
regions with varying degrees of overlap. Where there is no
overlap between the two groups of rats, dashed lines were
drawn to separate the two clusters in the parameter space. The
regional correlation of imaging metrics r and ADC was sepa-
rate calculated for all rats on a slice-by-slice basis. For a
given slice, the mean r and ADC values for each of the four
lung’s quadrants were calculated and plotted against each other
on the r-ADC parameter space. This process was then repeated
for the same slice on the rest of the animals, and results were
plotted on the corresponding r-ADC parameter space with
different symbols for control and elastase rats as shown in
Fig. 5. The best linear fit for each slice was then calculated and
plotted, with the resulting correlation coefficient reported on
the top of each panel.

**DISCUSSION**

**Global measurements of lung function and structure.**
Among the PFT measurements reported in Table 1 (apart from
the lung volumes), only dynamic compliance, Cdyn, showed a
significant increase in the elastase model compared with con-
trols. This observation is compatible with the current clinical
understanding of emphysema progression in humans where tissue deterioration leads to loss of lung recoil. However, no significant difference in the primary metric for diagnosis and classification of emphysema, FEV50/FRC, was observed between the two groups. The difference in airway resistance of the two groups was also nonsignificant and showed the smallest relative change in PFT metrics compared with healthy animals. Even though the decline in FEV50/FRC values for emphysema rats was nonsignificant, the 26% change in this quantity supports the successful induction of the emphysema-like changes in these lungs by elastase, and it can be expected that in a more severe model of emphysema the difference will become more substantial.

Elastase-induced tissue destruction in rat lungs leads to the enlargement of residual and total lung capacity, which in turn results in compromised lung tissue recoil and elevated lung compliance. The fact that only compliance along with lung volumes (FRC and TLC) exhibited a significant change can be linked to the severe structural remodeling of the lung tissue over the time period of 6 mo. It can be hypothesized that this change may not be as significant in an early stage model compared with the change in imaging metrics.

**Regional measurements of lung function and structure.** Information about simultaneous changes in lung function and structure can provide insight into the distribution of emphysematous changes in the lungs and a complementary set of measurements for confirming the presence and severity of the disease. Threshold curves have been shown to be effective tools to analyze the non-Gaussian distribution characteristics of image-based measurements (13, 34). As opposed to mean values, threshold cutoff values are a function of the skewness and shape of the distribution and therefore allow incorporating the heterogeneity of distributions in the analysis. As was shown in Tables 2 and 3, the $r^*$ value is significantly higher in

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**Fig. 2.** Multislice maps of fractional ventilation ($r$, top) and ADC (bottom) in a representative elastase-treated rat lung. Pixels with $r \approx 1.0$ and ADC $\approx 0.5$ (cm²/s) are masked in the histograms. The mean ± SD of each slice is shown above each histogram for the respective truncated distribution.

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Correlation of Lung Function and Structure by 3He MRI

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controls than in elastase rats ($P = 0.004$), and conversely, ADC* is significantly smaller in this group ($P = 0.004$). In comparison to ADC*, mean ADC values exhibited a significantly different difference between healthy and elastase rats ($P = 0.002$). This difference however was much smaller for fractional ventilation ($P = 0.012$) and could be attributed to a more homogeneous and symmetric distribution of ADC in the lungs compared with $r$.

Since all animals were mechanically ventilated, the mean $r$ value, regardless of the heterogeneity of distribution, was expected to remain unchanged among different animals provided that the overall ventilation coefficient, $R = TV/(TV + FRC)$, would be the same. As was shown in Table 1, $R$ is larger in controls than elastase rats, $0.39 \pm 0.03$ vs. $0.31 \pm 0.04$, which indicates an $\approx 22\%$ less effective mechanical ventilation of elastase rats compared with healthy ones for fractional ventilation measurements. This is mainly due to selecting the tidal volume for each animal based on its TLC value, and the fact that the $f = FRC/TLC$ ratio was proportionally larger in elastase animals, $0.34 \pm 0.04$ vs. $0.25 \pm 0.06$. Therefore, it can be argued that normalizing tidal volumes with respect to FRC would be a better practice in future studies to minimize the effect of relative hypoventilation among different test subjects.

Large-conductive airways were masked only by removing the pixels corresponding to $r > 1.0$ and ADC $\approx 0.5$ cm$^2$/s from $r$ and ADC distributions, respectively, and therefore it is possible that a number of pixels that technically belonged to conductive airways were not effectively removed by these binary maps. The fact that almost invariably a near-free-diffusion ADC and near-unity $r$ value is observed in the large airways provides a bimodal behavior between lung parenchyma and conductive airways (37) and serves as the basis for masking pixels purely based on their quantitative measurement values, as opposed to using a manual segmentation technique. Finally, it is not anticipated that any region in the lung parenchyma (even for severely diseased animals) would exhibit free-diffusion or free-flow characteristics, and therefore this masking technique was not expected to adversely affect the reported distributions.

Lung regions with severe ventilation defects in diseased animals (due to, for example, complete airway obstruction or flooding by edema) are certainly out of reach by HP gas contrast, which points to a fundamental limitation of HP gas MRI-based imaging techniques. Coupling these imaging techniques with complementary proton-based MRI techniques can assist in stratifying the missing regions in the HP gas images into unventilated airways, lung tissue, or pulmonary vasculature, which can improve the utility of regional imaging metrics for disease diagnosis. It has been shown however that for regions with highly compromised ventilation ($r = 0.1$ or less),

Table 2. HP $^3$He MRI measurements of fractional ventilation

<table>
<thead>
<tr>
<th>No.</th>
<th>$r$</th>
<th>$r^*$ (80% Threshold)</th>
<th>Ventral Slice</th>
<th>Middle Slice</th>
<th>Dorsal Slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.53 $\pm$ 0.16</td>
<td>0.40</td>
<td>0.56 $\pm$ 0.15</td>
<td>0.55 $\pm$ 0.16</td>
<td>0.44 $\pm$ 0.15</td>
</tr>
<tr>
<td>2</td>
<td>0.54 $\pm$ 0.15</td>
<td>0.42</td>
<td>0.57 $\pm$ 0.13</td>
<td>0.58 $\pm$ 0.15</td>
<td>0.47 $\pm$ 0.14</td>
</tr>
<tr>
<td>3</td>
<td>0.51 $\pm$ 0.13</td>
<td>0.41</td>
<td>0.53 $\pm$ 0.11</td>
<td>0.53 $\pm$ 0.13</td>
<td>0.45 $\pm$ 0.14</td>
</tr>
<tr>
<td>4</td>
<td>0.61 $\pm$ 0.15</td>
<td>0.47</td>
<td>0.60 $\pm$ 0.15</td>
<td>0.63 $\pm$ 0.15</td>
<td>0.59 $\pm$ 0.16</td>
</tr>
<tr>
<td>5</td>
<td>0.52 $\pm$ 0.17</td>
<td>0.40</td>
<td>0.52 $\pm$ 0.13</td>
<td>0.55 $\pm$ 0.17</td>
<td>0.50 $\pm$ 0.19</td>
</tr>
<tr>
<td>Elastase rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.47 $\pm$ 0.17</td>
<td>0.33</td>
<td>0.48 $\pm$ 0.15</td>
<td>0.51 $\pm$ 0.16</td>
<td>0.42 $\pm$ 0.18</td>
</tr>
<tr>
<td>2</td>
<td>0.33 $\pm$ 0.15</td>
<td>0.21</td>
<td>0.34 $\pm$ 0.16</td>
<td>0.34 $\pm$ 0.14</td>
<td>0.32 $\pm$ 0.14</td>
</tr>
<tr>
<td>3</td>
<td>0.47 $\pm$ 0.15</td>
<td>0.35</td>
<td>0.46 $\pm$ 0.16</td>
<td>0.48 $\pm$ 0.14</td>
<td>0.47 $\pm$ 0.16</td>
</tr>
<tr>
<td>4</td>
<td>0.37 $\pm$ 0.12</td>
<td>0.27</td>
<td>0.39 $\pm$ 0.12</td>
<td>0.36 $\pm$ 0.13</td>
<td>0.36 $\pm$ 0.13</td>
</tr>
<tr>
<td>5</td>
<td>0.41 $\pm$ 0.07</td>
<td>0.28 $\pm$ 0.06</td>
<td>0.40 $\pm$ 0.15</td>
<td>0.39 $\pm$ 0.16</td>
<td>0.38 $\pm$ 0.14</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.012</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD. Boldface values are means $\pm$ SD of each rat group. $P$ values are for healthy control vs. elastase rats. HP, hyperpolarized.

Table 3. HP $^3$He MRI measurements of apparent diffusion coefficient

<table>
<thead>
<tr>
<th>No.</th>
<th>ADC, cm$^2$/s</th>
<th>ADC*, cm$^2$/s (80% Threshold)</th>
<th>Ventral Slice</th>
<th>Middle Slice</th>
<th>Dorsal Slice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.24 $\pm$ 0.09</td>
<td>0.30</td>
<td>0.24 $\pm$ 0.09</td>
<td>0.25 $\pm$ 0.09</td>
<td>0.22 $\pm$ 0.09</td>
</tr>
<tr>
<td>2</td>
<td>0.25 $\pm$ 0.09</td>
<td>0.32</td>
<td>0.26 $\pm$ 0.09</td>
<td>0.26 $\pm$ 0.09</td>
<td>0.24 $\pm$ 0.08</td>
</tr>
<tr>
<td>3</td>
<td>0.21 $\pm$ 0.08</td>
<td>0.27</td>
<td>0.20 $\pm$ 0.07</td>
<td>0.21 $\pm$ 0.09</td>
<td>0.21 $\pm$ 0.07</td>
</tr>
<tr>
<td>4</td>
<td>0.18 $\pm$ 0.08</td>
<td>0.23</td>
<td>0.18 $\pm$ 0.07</td>
<td>0.17 $\pm$ 0.07</td>
<td>0.20 $\pm$ 0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.18 $\pm$ 0.07</td>
<td>0.23</td>
<td>0.17 $\pm$ 0.07</td>
<td>0.20 $\pm$ 0.08</td>
<td>0.19 $\pm$ 0.06</td>
</tr>
<tr>
<td>Elastase rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.31 $\pm$ 0.08</td>
<td>0.38</td>
<td>0.31 $\pm$ 0.08</td>
<td>0.30 $\pm$ 0.08</td>
<td>0.30 $\pm$ 0.09</td>
</tr>
<tr>
<td>2</td>
<td>0.28 $\pm$ 0.08</td>
<td>0.34</td>
<td>0.28 $\pm$ 0.08</td>
<td>0.28 $\pm$ 0.08</td>
<td>0.26 $\pm$ 0.08</td>
</tr>
<tr>
<td>3</td>
<td>0.30 $\pm$ 0.08</td>
<td>0.37</td>
<td>0.30 $\pm$ 0.07</td>
<td>0.31 $\pm$ 0.09</td>
<td>0.29 $\pm$ 0.08</td>
</tr>
<tr>
<td>4</td>
<td>0.29 $\pm$ 0.09</td>
<td>0.36</td>
<td>0.29 $\pm$ 0.08</td>
<td>0.29 $\pm$ 0.08</td>
<td>0.28 $\pm$ 0.10</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.002</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD. Boldface values are means $\pm$ SD of each rat group. $P$ values are for healthy control vs. elastase rats. ADC, apparent diffusion coefficient.

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using ~10 build-up breaths with a modest flip angle (α = 4−5°) can provide enough sensitivity to estimate \( r \) value with a relative error of <10%, provided that the SNR is at least 10 (11). These experimental conditions were therefore maintained in this study to limit the estimation errors even in poorly ventilated regions.

**Relative sensitivity of global and regional metrics to elastase-induced changes in lungs.** Table 4 shows the statistical significance in the difference between the key PFT and imaging metrics in elastase-treated rats compared with healthy controls. The overall similarity of trends in PFT and imaging metrics in the two groups supports the qualitative validity of \( r \) and ADC measurements in comparison to well-established clinical metrics. However, the more significant observation of this study for eventual benefit in staging and diagnosis of emphysema is the higher relative sensitivity of \( r^* \) and ADC* metrics to the presence of lung disease. Table 4 also summarizes the average percent change of elastase rats with respect to control groups for PFT and MRI metrics. Notable differences are a significant 33% increase in ADC* and a significant 31% decline in \( r^* \) for elastase rats compared with controls. For PFT, there is a significant 51% increase in C\(_{\text{dyn}}\), and a nonsignificant 26% decline in FEV\(_{50}/\text{FRC}\).

Correlation maps of Fig. 4 depict no systematic overlap in \( r^* \) and ADC* values between the two groups of rats. This observation effectively divides the rats into two separate clusters in the sample space. On the other hand, the characteristic metric FEV\(_{50}/\text{FRC}\), analogous to FEV\(_1/\text{FRC}\) in humans and, by far, the most commonly adopted metric in staging emphysema, shows a substantial overlap between the two groups (Fig. 4, A and B). The airway resistance is also considerably more variable between animals than fractional ventilation (Fig. 4C).

These observations confirm the higher sensitivity of the threshold imaging metrics compared with PFT parameters.

**Regional correlation of lung function and structure.** Referring to Fig. 5, there is a clear correlated trend of regional changes in \( r \) and ADC values in the lungs of all rats. Due to the potential error induced by imperfect coregistration in our
simple rigid alignment scheme such as rotational differences in the rat body orientation between the two acquisitions, the regional correlation study was limited to relatively large regions that loosely correspond to the four quadrants of each slice, resulting in 12 independent regions in each animal lung. Even though this limited number of regions did not provide a statistically meaningful relationship for the correlated changes of $r$ and ADC in lungs of healthy and elastase rats. The middle slice shows the best correlation ($R = -0.58$ vs. $-0.47$ and $-0.43$ for posterior and anterior slices, respectively). This observation is possibly due to the better coregistration of the $r$ and ADC maps, and inclusion of the residual conductive airways in the middle slice. It is likely that the anterior slice suffers the most from cardiac motion artifacts at the heart-lung tissue boundary. Regardless of the slice position, the $r$-ADC

Fig. 4. Correlation of global pulmonary parameters with relevant 80% $r^*$ (A, C, E) and ADC* threshold values (B, D, F). When possible the parameter space has been divided into 2 or 4 zones corresponding to a nonoverlapping set of parameters. Note that imaging parameters invariably allow for clustering the animals into 2 different zones. FEV$_{50}$, forced expiratory volume in 50 ms; FRC, functional residual capacity; $R_t$, airway resistance; $C_{dyn}$, dynamic compliance, TLC, total lung capacity.
clearly divides the sample space into two separate clusters comprised of healthy rats characterized by higher $r$ and lower ADC values, and elastase rats showing an inverse relationship between these two parameters. This observation supports the complementary nature of lung function and structure in both the healthy and diseased states and points to the inherent benefit of simultaneous measurement of these and other independent regional metrics for more reliable diagnosis and staging of emphysema.

**Challenges and potentials.** Simultaneous measurement of lung ventilation and gas diffusivity has the potential to provide a richer set of information in a clinical setting and may help to establish refined scoring systems for better staging of emphysema and other disease models, which would also have strong clinical applications. Adoption of these imaging techniques in pulmonary practice requires that several challenges be met and obstacles overcome. In this study, the disease model was examined in a steady-state condition where no significant change in disease pathology was expected during the course of the study. A continued steady state is not one that can be guaranteed in clinical settings. Therefore, to gain better insight into the kinetics of lung disease development and the underlying mechanisms relating structural and functional changes in the lung, it will be necessary to leverage the noninvasive nature of the ventilation and diffusion MRI techniques to perform longitudinal studies of a disease model in which progression is expected (such as smoke-induced models) and to acquire information about the temporal sensitivity of changes in these imaging metrics compared with standard PFT parameters. Additionally, from a practical standpoint, it will be important to perform MRI measurements in each animal with a fixed position inside the MRI scanner to eliminate the need for coregistration between different acquisitions. Finally, using 3D imaging pulse sequences in future studies will be advantageous for ensuring the inclusion of the entire lung in analysis and to improve the fidelity of the regional correlation analysis.

An outstanding challenge facing the present state of the technology is validation of the imaging techniques for regional quantitative measurements in lungs. Even though the comparison of mean or threshold values of imaging metrics with global PFT parameters does provide a crude validation of the MRI-based measurements, the sensitivity of these two families of techniques is very different and in most cases they measure fundamentally different physical or physiological phenomena. Explicit in vivo validation of the discussed imaging techniques is therefore necessary in order for these techniques to have real and absolute clinical utility, a topic that is the subject of both ongoing and future research.

**Table 4. Statistical significance of changes in MRI and PFT metrics**

<table>
<thead>
<tr>
<th>Marker</th>
<th>$P$ Value</th>
<th>Relative Change in Elastase Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC*</td>
<td>0.004</td>
<td>$+33%$</td>
</tr>
<tr>
<td>$r^{*}$</td>
<td>0.004</td>
<td>$-31%$</td>
</tr>
<tr>
<td>Cdyn</td>
<td>0.022</td>
<td>$+51%$</td>
</tr>
<tr>
<td>Rl</td>
<td>0.947</td>
<td>$-15%$</td>
</tr>
<tr>
<td>FEV$_{50}$/FRC</td>
<td>0.163</td>
<td>$-26%$</td>
</tr>
</tbody>
</table>

PFT, pulmonary function testing.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
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