Early changes of lung function and structure in an elastase model of emphysema—a hyperpolarized $^3$He MRI study

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Early changes of lung function and structure were studied in the presence of an elastase-induced model of emphysema in 35 Sprague-Dawley rats at mild (5 U/100 g) and moderate (10 U/100 g) severities. Lung ventilation was measured on a regional basis (at a planar resolution of 3.2 mm) by hyperpolarized $^3$He MRI at 5 and 10 wk after model induction. Subsequent to imaging, average alveolar diameter was measured from histological slices taken from the centers of each lobe. Changes of mean fractional ventilation, mean linear intercept, and intrasubject heterogeneity of ventilation were studied during disease progression. Mean fractional ventilation was significantly different between healthy controls (0.23 ± 0.04) and emphysematous animals at both time points in the 10-unit group (0.06 ± 0.02 and 0.12 ± 0.05, respectively). Changes in average alveolar diameter were not statistically observable until the 10th wk between healthy (37 ± 10 $\mu$m) and emphysematous rats (73 ± 25 and 95 ± 31 $\mu$m, for 5 and 10 units, respectively).

Assessment of function-structure correlation suggested that the majority of the decline in fractional ventilation occurred in the first 5 wk, while enlargement of alveolar diameters appeared primarily between the 5th and 10th wk. A thresholding metric, based on the 20th percentile of fractional ventilation over the entire lung, was utilized to detect the onset of the disease with confidence, independent of whether the regional ventilation measurements were normalized with respect to the delivered tidal volume and estimated functional residual capacity of each individual rat.

lung physiology; early detection of emphysema; regional fractional ventilation; hyperpolarized helium-3 magnetic resonance imaging

DESPITE BEING LARGELY preventable, chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States, and it is now the most common form of chronic lung disease (3). The high morbidity and mortality rates for COPD highlight both our failure to identify at-risk patients early in the disease process and the lack of effective intervention and treatment. Emphysema, a primary subclass of COPD, is characterized by abnormal enlargement of lung components distal to the terminal bronchioles (2), accompanied by destruction of the alveolar walls and loss of tissue density (49). This disease results principally from inhalation of 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 (36).

Emphysema symptomatology can be arrested, or possibly even reversed, 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). The most common clinical pulmonary marker used to assess the presence and progression of this disease is decline in forced expiratory volume in 1 s (11), and its ratio to forced vital capacity. These tests measure the increased time required to force the air out of the lungs due to the effects, either separately or in combination, of increased resistance of small airways (59) and increased lung compliance due to diminished lung elastic recoil (32). These global markers, along with conventional chest radiology, only provide a gross assessment of the state of emphysema, and their application is largely limited to qualitative, clinical diagnosis. Furthermore, it has been shown that global pulmonary markers are generally insensitive to early, mild changes in lung function due to emphysema (54), and up to 30% of functional lung capacity can be lost before changes appear in PFTs (52). Finally, assessment of disease progression using PFTs requires a reproducible forced expiratory effort over a long period of time.

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 (10). Emphysema-induced tissue destruction, however, results in lower X-ray attenuation per unit volume compared with healthy lungs, adversely affecting the sensitivity of this technique. Other disease processes, such as reduced perfusion and air trapping (55), can also reduce attenuation, and these effects may not be directly separable from emphysematous changes. Newell et al. (35) argued that precise separation of a fully expanded healthy lung from mild emphysema using CT is difficult, given that the diagnostic criteria for emphysema (abnormal enlargement of air spaces) presupposes knowledge of normal air space size and that “normal” varies among individuals. Finally, the high radiation dose of CT scans limits its

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use in longitudinal studies and in cases in which continuous clinical follow-up is required to monitor therapeutic response (5).

Various researchers have demonstrated a strong link between lung structure and function (4, 18). In addition to structural deterioration, emphysema also directly affects lung function through irreversible airflow limitation (36). This fact signifies the value of simultaneous measurement of structural and functional changes in the lung during the progression of this disease. Regional pulmonary ventilation has been measured from CT images using certain contrast agents, most commonly the radiodense tracer gas xenon (25). Ventilation measurements with high spatial resolution and a high degree of anatomic localization may be derived from the measured time constant of the change of regional lung attenuation during the washin and subsequent washout of xenon in a series of scans acquired at a specific point in the respiratory cycle (28). This technique, however, requires repeated measurements and, therefore, repeated radiation exposure, at each imaging plane. Additionally, xenon has anesthetic and sedative properties that limit the concentration, the achievable contrast enhancement, and, consequently, the signal-to-noise ratio (SNR) (26).

During the past decade, hyperpolarized (HP) helium-3 magnetic resonance imaging (3He MRI) has emerged as a new technique capable of providing physiological insight into both structure (42, 58) and function (14, 27) of the lung. Inhaled 3He distributes in the lung air space and is detected with MRI techniques. This technique thus provides images of the distribution of gas within the lung, making this imaging modality a strong candidate for screening patients at risk for emphysema. Beyond the potential for identifying patients with early emphysema, its attractive safety profile makes this sensitive, nonionizing, and noninvasive modality a useful tool for evaluating emphysema progression after therapeutic intervention. Despite the recent advances in HP 3He MRI technology, the regional correlation of lung structure and function has not been well characterized, especially in early pathogenesis. Originally, gas density HP 3He MR images were used as a qualitative tool to assess the ventilation deficiency in both emphysematous (44) and asthmatic patients (1). More recently, dynamic HP 3He MRI techniques have been developed that provide quantitative regional ventilation maps (12). Dynamic imaging methods have been compared with PFT measurements in a study of changes in lung ventilation in cystic fibrosis patients (24). A few recent works have explored the correlation between functional (22) and structural (43) HP 3He MRI biomarkers with PFT. However, the HP 3He MRI probe most commonly used to study early emphysematous changes in the lung is 3He apparent diffusion coefficient (13, 53), which is strictly a structural marker. Additionally, studies using HP 3He MRI have not yet established whether this modality can be utilized to quantitatively differentiate between severities of emphysema.

In this study, early alterations of lung function and structure in the presence of emphysema have been, for the first time, examined in rats. A well-established rodent emphysema model induced by intratracheal administration of porcine pancreatic elastase (PPE) (16, 50) was utilized. Instillation of PPE results in rapid and significant air space enlargement, followed by acute neutrophil and subacute macrophage accumulation within the lung. Earlier studies utilizing a similar emphysema model (47) report that, by following a careful procedure, it is possible to induce a reasonably homogeneous tissue destruction throughout each lobe. However, since the elastase instillation is carried out through the trachea, the delivery rate to each lobe is not directly controlled, and therefore each lobe in a rat lung may experience a different level of tissue destruction induced by elastase exposure.

For analysis, the time evolution of regional lung function and structure was studied by measuring an MRI-based ventilation marker and the gold standard histological marker of average alveolar diameter, as well as the underlying relationship between the two quantities. Furthermore, a metric with higher sensitivity to heterogeneity of ventilation distribution to better differentiate between healthy and emphysematous lungs was introduced and utilized to detect the onset of the disease.

**THEORY**

**Regional measurement of fractional ventilation.** Fractional ventilation, \( r \), 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 \( V_f \), to the total gas space of that ROI at the end of inspiration, \( V_i \) (comprising \( V_f \) and the residual volume \( V_r \)):

\[
 r = \frac{V_f}{V_i} = \frac{V_f}{V_f + V_r} \tag{1}
\]

A voxel’s gas content at end inspiration under breathhold pressure is assumed to be divided between \( r \), consisting of the delivered fresh gas, and \( q = 1 - r \), representing the residual capacity of the ROI. A measurement of \( r = 0 \) indicates no gas replacement and \( r = 1 \) indicates complete gas exchange for each breath.

A HP 3He MRI method for direct measurement of the fractional ventilation has been previously demonstrated by Deninger et al. in guinea pigs (12). This method consists of performing MRI with increasing numbers of HP 3He gas breaths (Fig. 1) (17). The time interval between two consecutive breaths is equal to \( \tau \). Over a succession of HP 3He breaths, signal intensity in highly ventilated regions grows at a faster rate than in the poorly ventilated regions. Theoretically, after an infinite number of helium breaths, and in the absence of any polarization decay mechanism, the signal in all regions will converge to a steady-state value (\( S_{ss} \)) specific to each region. Therefore, \( S_{ss} \) is proportional to the ventilated gas volume present in the respective ROI. In practice, however, the maximum number of helium breaths will be finite but sufficient to wash out a substantial portion of the gas from previous inhalations of normal air; this maximum is limited by the onset of hypoxia.

The resulting 3He signal within a given voxel after a certain number of HP gas breaths is a function of the magnetization of the fraction \( r \) of the fresh 3He and magnetization of the fraction \( q \) of the 3He remaining from previous breaths of the same cycle. The first fraction is subject to spin-lattice relaxation in the external HP 3He reservoir, \( T_{1, ext} \), while the second fraction is mostly affected by oxygen-induced relaxation in the lung, \( T_{1, O_2} \). Oxygen-induced relaxation time constant of HP 3He is governed by (41):

\[
 T_{1, O_2} = \frac{\xi}{P_{A_0}} \tag{2}
\]

with the proportionality constant \( \xi \approx 2.6 \text{ bar·s} \) at normal body temperature, and where \( P_{A_0} \) is alveolar partial pressure of oxygen. Neglecting the uptake of oxygen into the blood during
each HP $^3$He step, the oxygen-induced relaxation time of $^3$He will, in practice, be a function of the number of breaths $j$ in a given step, $T_{1,O_2}(j)$. Therefore, in this approximation, $T_{1,O_2}(j)$ is influenced by the washout of $PAO_2$ as:

$$T_{1,O_2}(j) = \frac{\xi}{PAO_2(j)} = \frac{\xi}{(P_0q^j)}$$

(3)

where $P_0$ is the initial $PAO_2$ value in the ROI. To estimate the $T_{1,ext}$ decay time constant for a given study, two normalization sequences were acquired: one immediately before the actual ventilation sequence, and the other immediately after (Fig. 1). Assuming an exponential decay of polarization in the reservoir, and given the signal level in the normalization steps and the elapsed time between sequences, the relaxation time of the external HP $^3$He reservoir, $T_{1,ext}$, can be estimated.

Having acquired the signal values for $n$ steps of the ventilation sequence, and correcting for external relaxation of HP $^3$He, the signal buildup in a given ROI as a function of number of helium breaths can be expressed as (12):

$$S(n) = S_0 \cdot \psi \cdot (1 - q) \sum_{j=0}^{n-1} \phi \cdot q^j \exp \left[ -\frac{P_0 \cdot \xi \cdot q^j (1 - q^j)}{\frac{\xi}{1 - q}} \right]$$

(4)

where $\phi = \Delta^{n-j-1} \cdot \psi = \Delta^{B(n)}$, with $\Delta = \exp (-\pi/T_{1,ext})$ representing the external polarization decay over one breath. $B(n)$ is the total number of breaths enclosed between the very first helium breath and the $n$th step, which also includes the number of air breaths between each HP $^3$He step. Equation 4 is fit to the experimental data points to yield $S_0$ and $r$ as free parameters. When normalized with respect to the $S_0$, all ventilation curves converge to unity, allowing the comparison of lung voxels with different air space volumes. This procedure is carried out on a voxel-by-voxel basis to generate the regional ventilation map for the given slice.

**Regional and global fractional ventilation and volumes.** Each animal’s tidal volume ($V_T$) during spontaneous respiration was used for ventilation during the imaging experiment, and thus an absolute change of $r$ could be caused by a change in $V_T$. To minimize the dependence on $V_T$, $r$ was normalized by the global fractional ventilation, $R = V_T/(V_T + FRC)$, a function of $V_T$ and functional residual capacity (FRC). The motivation for this procedure is justified in the following. The global fractional ventilation, $R$, can be expanded in terms of fractional volumes as:

$$R = \frac{V_T}{FRC + V_T} = \sum_i \left( V_{f,i} + V_{r,i} \right)$$

(5)

It is assumed that the entire set of ROIs $i$ encompass the full volume of the lung. Assume that the volume of fresh gas in an ROI is related to $V_T$ by:

$$V_{f,i} = f_i \cdot V_T$$

(6)

that is, independent of $V_T$, the same fraction of the $V_T$, denoted $f_i$, is added to the ROI. This approximation breaks down when the conductive airways are considered. A similar relationship is assumed for FRC:

$$V_{r,i} = g_i \cdot FRC$$

(7)

Note that if $f_i = g_i$, then $r_i = R$. Because a constant $r_i$ is not generally observed across the lung, it is concluded that, in general, $f_i \neq g_i$. The fractional ventilation of the ROI can be expanded as a power series in $V_T/FRC$ to obtain:

$$R = \sum_i \left( f_i + g_i \right) V_T$$
Therefore, and residual volumes. These additional terms related to intra-ellipsis points in
which by substitution in Eq. 10
where \( V_{\text{VT}} \) and the average end-inspiratory volume is \( \langle V_i \rangle \) for each ROI \( i \) as follows:
\[
\langle r \rangle = 1 + \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{V_{\text{VT}}}{\langle V_i \rangle} \left( 1 - \frac{(V_j - V_{\text{VT}})}{\langle V_j \rangle} \right)
\]
\[
\langle r \rangle = 1 + \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{\sum_{i=1}^{N} \sum_{j=1}^{N} (\Delta V_j)}{\langle V_i \rangle}
\]
where \( V_{\text{VT}} \) is the end-inspiratory volume of total gas in voxel \( i \) and the average end-inspiratory volume is \( \langle V_i \rangle \). \( \Delta V_j \) is a measure of heterogeneity of airway volume across different regions of the lung. Using \( V_{\text{VT}} = V_{\text{VT}} + V_{\text{f}} \), the regional airway heterogeneity can be expressed as:
\[
\Delta V_i = \langle V_i \rangle - V_{\text{f}} + V_{\text{f}} - V_{\text{f}}
\]
which by substitution in Eq. 10 gives:
\[
\langle r \rangle = 1 + \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{V_{\text{VT}}}{\langle V_i \rangle} \left( 1 + \frac{\Delta V_j}{\langle V_j \rangle} + \cdots \right) = \frac{V_{\text{VT}}}{\langle V_i \rangle}
\]
\[
\langle r \rangle = 1 + \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{\sum_{i=1}^{N} \sum_{j=1}^{N} (\Delta V_j)}{\langle V_i \rangle}
\]
where \( \sigma \) and \( \text{cov} \) represent SD and covariance functions, respectively. This shows that \( \langle r \rangle \) is a function of \( R \) and the two quantities differ by terms that relate to lung heterogeneity, such as the SD of fresh volumes \( \sigma(V_i) \) and the correlation of fresh and residual volumes. These additional terms related to intra-subject heterogeneity of fractional ventilation cannot be measured on a plethysmography system as a global quantity, and therefore \( \langle r \rangle \) and \( R \) are distinct quantities containing different pieces of information. Although the terms indicated by the ellipsis points in Eq. 12 are expected to be small corrections, the argument does not depend on that; the truncated terms have the form:

For \( j \geq 2 \), meaning that all additional terms depend on the heterogeneity of the partial volume difference.

**METHODS**

**Study animals.** All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Five groups of seven male Sprague-Dawley rats were used to perform the studies: healthy controls; rats with a mild model of emphysema, defined as those administered with 5 units of intratracheal elastase per 100 g body mass (referred to hereafter as the SU group), of which one-half of the population was studied at 5 wk and the rest at 10 wk; and rats with a moderate model of emphysema, defined as rats administered with 10 U/100 g of elastase (referred to hereafter as the MU group), similarly studied at 5 and 10 wk. 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. All rats were carefully age-matched and maintained under very similar environments and dietary conditions to allow uniform development of emphysema over the period of either 5 or 10 wk.

**Emphysema model induction.** Under an inhaled general anesthesia of isoflurane, 8-wk-old rats were placed supine and intubated with a 14-gauge angiocatheter. They were briefly ventilated with a rodent ventilator (CWE, Ardmore, PA) using supplemental oxygen. The designated dose of PPE (ICN Pharmaceuticals, Irvine, CA) diluted in 0.60 ml of saline was instilled through the catheter, and the animals were gently rocked from side to side to evenly distribute the elastase throughout the lungs. Three large, positive pressure breaths at 25 cmH2O were given to move the elastase into the most distal airways. The animals were then recovered from anesthesia.

**Baseline respiratory measurements.** Respiratory rate (RR) and \( V_t \) for each rat were measured during light sedation in an unrestrained plethysmographic measurement system (Buxco Electronics, Wilmington, NC) over a period of stable condition for 5 min. These measurements were conducted on each rat no earlier than 2 days before the imaging session. During imaging, the measured RR and VT for each rat were used for mechanical ventilation, both for air and HP \(^3\)He breaths.

**Preparation of animals for imaging.** Animals were sedated with 0.1 g/kg intraperitoneal ketamine and 10 mg/kg xylazine. The dose was repeated every 90 min or upon observation of movement. The rats were intubated with a 2-in.-long, 14-gauge angiocatheter (BD, Franklin Lakes, NJ), modified with a sealant (UHU Tac adhesive putty; Saunders Manufacturing, Readfield, ME) to create a tight seal around the entrance to the trachea, enabling a breath hold of up to 25 cmH2O for 5 s with negligible leakage. Animals were then temporarily paralyzed with 1 mg/kg intravenous pancuronium bromide (Abbott Laboratories, North Chicago, IL) and were immediately placed on an MRI-compatible ventilator (GE Healthcare, Durham, NC). Ventilation was maintained with a VT and RR specific to each rat, previously measured on the plethysmography system. Heart rate was monitored using an ECG attached by subdermal electrodes on one forelimb and the opposite hindlimb (SA Instruments, Stony Brook, NY). 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. Blood oxygen saturation level was monitored using a veterinary pulse oximeter (Vet/Ox, Heska, Loveland, CO) with the optical probe attached to the rat’s paw.

**Hyperpolarization of helium.** The imaging helium gas (Spectra Gases, Branchburg, NJ) has a nominal concentration of 99.19% \(^3\)He and 0.81% \(^4\)He. This mixture was hyperpolarized through spin exchange collisions with optically pumped rubidium (Rb) atoms, as previously described (39), using a commercial polarizer (IGL9600.He, GE Health-
Four tissue sections from each lobe. The slides were acquired near the respective lobe. Briefly, a 1-mm-wide slide was obtained from each of the right lobes, as shown in Fig. 4. The corresponding mean \( L_m \) values for the respective lobe.

**Heterogeneity analysis.** Due to absence of histological information at the same resolution of regional ventilation, the functional-structural correlation analysis was limited to the central regions of the three lung lobes, namely the left, upper right, and lower right lobes, as shown in Fig. 4. The corresponding mean \( r \) for each lobe, \( \langle r \rangle \), was calculated by averaging \( r \) values for a group of 4 or 6 bins in the center of the respective lobe, either as a 2 × 2 or 3 × 2 grid, depending on the size of the lobe. To consider different levels of elastase destruction in different lobes of an emphysematous lung, all lobar regions were treated as independent data points, only for the functional-structural correlation study. Finally variation of lobar \( \langle r \rangle \) with respect to lobar \( L_m \) values was studied to analyze the underlying relationship between the two quantities during the progression of disease. The average change in \( r \) values between two time points (i.e., 0 to 5, or 5 to 10 wk) was calculated as:

\[
\langle \Delta r \rangle_{1 \rightarrow 2} = \frac{1}{N_1 N_2} \sum_{i=1}^{N_1} \sum_{j=1}^{N_2} \frac{r_i - r_j}{r_i}
\]

where \( N_1 \) and \( N_2 \) are the number of data points at each time point, respectively. The average change in \( L_m \) values between the two time points was calculated in a similar fashion.

**Heterogeneity analysis.** Reducing a distributed measurement, e.g., fractional ventilation in the imaged slice, to its mean value can...
potentially mask important information regarding the heterogeneity of the distribution. Therefore, it is of extra value to explicitly consider distribution variation of ventilation within the lung of each animal, especially because emphysematous lungs are expected to exhibit a higher level of heterogeneity. In this study, coefficient of variation especially because emphysematous lungs are expected to exhibit a higher level of heterogeneity. In this study, coefficient of variation was used as a measure of intrasubject heterogeneity, where \( \sigma(r)/\bar{r} \) is the standard deviation of \( r \) over the entire slice.

In addition to the coefficient of variation, another metric was proposed that is a function of both the regional fractional ventilation value and the uniformity of its distribution. The proposed quantity is the percentile threshold population of regional ventilation maps. Thresholds in parametric maps are commonly used in segmentation and assessment of lung images, especially those acquired with CT scans (23, 34). Here, the same approach is used by adopting a 20th percentile threshold in the regional ventilation maps. In other words, the proposed marker is the quantity such that 80% of the pixels in the imaged lung have a higher fractional ventilation value. Threshold curves for regional fractional ventilation were generated using bin-by-bin \( r \) maps. Bins with \( r > 0.5 \) were masked from these maps. For each lung, the \( r \) threshold value was varied from 0 through 1, and the percent population of bins with \( r \) value above the threshold was calculated and further used to determine the 20th percentile threshold values, \( r_{\text{threshold}} \) and \( (r/R)_{\text{threshold}} \).

Statistical analysis. A generalized two-way ANOVA test was used for analysis of variance with unequal sample size for each group of rats. Time and elastase dosage were considered as independent factors. The time factor can assume either of the three levels: 0, 5, and 10 wk. Similarly, the dosage factor can assume either of the three levels: 0, 5, and 10 units. In this study, separate two-way ANOVA were performed on each quantity of interest between all five groups of rats: lobar \( (r) \), lobar \( (L_m) \), \( \sigma(r)/\bar{r} \), \( r_{\text{threshold}} \), and \( (r/R)_{\text{threshold}} \). Both main effects and the two-way interactions were considered.

Post hoc pairwise statistical comparisons of means (simultaneous inference) were performed using Tukey’s honestly significant difference criterion, which retains the main effects while eliminating the interaction terms. If there is a statistically significant difference across \( n \) means, the multiple-comparison method will determine the specific differences between pairs of groups. Multiple comparison also allows comparison of all of the possible pairs of means and avoid the type I error that may be seen if a two-sample methods such as \( t \)-test is used for these comparisons. The significance level of \( \alpha = 0.05 \), corresponding to a confidence level of 95%, was considered for all the statistical analyses.

RESULTS

Population of rats in each group. Each group initially consisted of seven rats. The goal was successful completion of the study with a minimum of five animals in each group, allowing for two accidental deaths in each cohort. The difference in number of rats in each group (Table 1) reflects the number of successful imaging sessions and/or accidental death of rats between model induction and imaging. The fifth group (10U rats at 10 wk) with only four survived animals was the only cohort that did not meet this condition. Since there was the concern that introducing additional animals to the cohort could adversely affect the intersubject variation of our measurements, the dead animal was not replaced.

Regional distribution of fractional ventilation. Figure 5 shows a representative set of MRIs acquired during a ventilation experiment for an increasing number of helium breaths. The external depolarization time constant of HP \( ^3 \)He, \( T_{1,\text{ext}} \) was generally observed to be in the range of 10 ± 2 min. The typical SNR of the acquired images ranged from 40 to 60, depending on the actual \( ^3 \)He polarization and ventilation efficiency of each animal (signal was typically lower in emphysematous rats). The SNR threshold was varied to ensure the inclusion of all pixels enclosed in the lung boundary and the exclusion of any background pixels. Regional fractional ventilation was measured in the middle coronal slice of the rat lungs. Figure 6 shows regional maps of fractional ventilation in representative rats from each group superimposed on grayscale MRIs of each lung. Also shown are the respective frequency distribution histograms for each ventilation map. In the maps, regions with \( r > 0.50 \) are masked with white to allow greater contrast in the main regions of the lung. The masked regions primarily represent conductive airways. Fractional ventilation in the trachea and major bronchi should be close to unity, because the \( V_T \) exceeds the volume of the conductive airways, and, therefore, the gas in these regions is completely
Table 1. Five study groups of rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Weight, g</th>
<th>RR, breaths/min</th>
<th>VT, ml</th>
<th>FRC, ml</th>
<th>R</th>
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<td>0.22</td>
</tr>
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<td>2.9</td>
<td>13.3</td>
<td>0.18</td>
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</table>

The five study groups of rats are shown: 5U, rats administered 5 units of intratracheal elastase per 100 g body mass; 10U, rats administered 10 units of elastase per 100 g body mass. Weight, respiration rate (RR), and tidal volume (VT) are measured within 2 days before the helium MRI session. The last column shows the calculated global fractional ventilation, R.

replaced in each breath. The fractional ventilation in the healthy rats was consistently high \((\bar{r}) = 0.19 \sim 0.27\) and homogeneous. Emphysematous rats, on the other hand, exhibit a heterogeneous distribution of fractional ventilation throughout their lungs. As can be seen in Fig. 6, certain areas of emphysematous lungs show near normal r values, whereas other regions exhibit highly degraded fractional ventilation \((r < 0.1)\). Emphysematous lungs were also, in general, larger than healthy lungs. Table 2 shows the \((r)\) values in the three lobar regions of lungs (left, lower right, and upper right) of all of the rats, along with respective SDs in the grouped bins within each lobe. The left lobe of the 5U emphysematous rat at 5 wk was excluded from r analysis due to poor ventilation and a subsequently low SNR. The average lobar \((r)\) value for normal rats was calculated as \((0.23 \pm 0.04)\) mean \pm SD, compared with \((0.09 \pm 0.05)\) and \((0.10 \pm 0.04)\) for 5U rats at 5 and 10 wk, and \((0.12 \pm 0.05)\) and \((0.06 \pm 0.02)\) for 10U rats at 5 and 10 wk, respectively.

Figure 7 shows the intrasubject \(r\) heterogeneity \(\sigma(r)/r\) values evaluated over the entire coronal slice of each lung as the percentage of \((\bar{r})\) value for the respective animal. Each group is identified with a distinct symbol throughout this paper. Values corresponding to the same time points are slightly offset for clarity. The mean value for each group is shown with a thicker symbol. The error bars in Fig. 7 represent SE of the mean within each group. Even though the average heterogeneity of \(r\) is smaller in healthy animals (38.8 \(\pm\) 11.2\%) vs. emphysematous ones, ranging from 78.1 \(\pm\) 18.2 to 96.7 \(\pm\) 40.5\%, the two-way ANOVA of \(\sigma(r)/r\) showed that this difference is not significant for any of the time points or dosages.

Lobar variations of \(L_m\). The calculated \(\langle L_m \rangle\) values for the five groups of rats are presented in Table 3, along with their respective SDs across the 12 intercept readings within each lobe. The average alveolar diameter calculated from \(L_m\) in the lungs of healthy rats is 37 \(\pm\) 10 \(\mu\)m, compared with \(44 \pm 6\) and \(73 \pm 25\) \(\mu\)m for 5U rats at 5 and 10 wk, and \(45 \pm 5\) and \(95 \pm 31\) \(\mu\)m for 10U rats at 5 and 10 wk, respectively.

Time evolution of \(r\) and \(L_m\). Lobar data points for the five groups of rats are plotted as \((\bar{r})\) and \(\langle L_m \rangle\) time evolution curves in Fig. 8, A and B, respectively. The error bars represent the corresponding SE of the mean within each group. For clarity, the grouped data points and mean values are slightly offset on the abscissa. The overall decline of lobar \((r)\) with respect to time is apparent from healthy to emphysematous rats, whereas lobar \(\langle L_m \rangle\) shows an overall rise for the same timeline.

The two-way ANOVA analysis of lobar \((r)\) showed that there was a significant main effect of time \((P < 0.035)\), but no significant main effect for dosage. A significant interaction effect between time and dosage factors \((P < 0.001)\) was also observed. Post hoc tests (all Tukey’s honestly significant difference criterion) revealed that lobar \((r)\) in healthy rats is significantly different from that of all the emphysematous rats \((P < 0.05)\). For the time factor, the difference is statistically significant for 10U rats only \((P < 0.05)\). There was no significant difference in the time factor for 5U rats.

![Fig. 5. Representative ventilation images of an emphysematous rat lung acquired during breath holds after an increasing number of hyperpolarized 3He breaths. Imaging was performed on a 4.7-T small-bore MRI scanner using a gradient echo pulse sequence with the following imaging parameters: field of view = 5 \times 5 \text{ cm}^2, slice thickness = 4 \text{ mm}, \alpha = 10^\circ, \text{matrix size} = 128 \times 128 \text{ pixels}, \text{repetition time} = 6.6 \text{ ms}, \text{and echo time} = 3.3 \text{ ms.}](http://jap.physiology.org/)
indicated by the two-way ANOVA test, no significant difference was observed for the dosage factor at either of the time points.

The two-way ANOVA analysis of lobar \( \langle L_m \rangle \) showed that there was a significant main effect for time \( (P < 0.001) \), as well as for dosage \( (P < 0.02) \). There was also a significant interaction effect between time and dosage factors \( (P < 0.014) \). Post hoc tests revealed that at 5 wk, lobar \( \langle L_m \rangle \) is not significantly different between the two elastase dosages, 5U and 10U, or the healthy rats. For both time and dosage factors, the lobar \( \langle L_m \rangle \) difference is significant at 10 wk for all three groups of healthy, 5U, and 10U rats \( (P < 0.05) \).

Correlation of \( r \) and \( L_m \) during elastase-induced emphysema progression. Figure 9 shows the lobar \( r-L_m \) correlation for all five groups of rats, along with mean values of each group. Also shown in this graph are vertical and horizontal error bars that correspond to SD of \( r \) and \( L_m \) within each group, respectively. The average decline in \( r \) value from 0 to 5 wk was calculated as \( (\Delta r)_{5-10} = -55 \pm 25% \) vs. that of 5 to 10 wk as \( (\Delta r)_{5-10} = +7 \pm 93% \). The corresponding average change in \( L_m \) values were calculated in a similar fashion as \( (\Delta L_m)_{5-10} = +34 \pm 31% \) and \( (\Delta L_m)_{5-10} = +81 \pm 68% \), respectively. One-way ANOVA test of the four different slopes followed by a Tukey’s simultaneous inference test showed that they are all statistically different from each other \( (P < 0.01) \).

Normalization by respiratory parameters. Table 1 summarizes the measured pulmonary parameters, including RR and \( V_r \), for all rats in the five groups. Also reported are the estimated FRC values for each rat. Based on these values, the global fractional ventilation \( R = \frac{V_r}{\text{FRC} + V_r} \) is calculated and further used as a normalization parameter for fractional ventilation among different rats.

Deviation of mean fractional ventilation from global fractional ventilation. Figure 10A shows the \( r \) and \( R \) correlation for all rats using the estimated FRC values (Table 1). These two quantities show a better correlation when considering healthy rats only \( (R^2 = 0.87) \), compared with emphysematous ones \( (R^2 = 0.27) \). Figure 10B shows the difference between \( R \) and \( r \) values for all five groups of rats. The difference between the mean regional and global fractional ventilation is smaller in healthy rats \( (0.09 \pm 0.04) \) compared with the emphysematous cohorts (ranging from \( 0.11 \pm 0.04 \) to \( 0.13 \pm 0.04 \)). The difference in \( R - \langle r \rangle \) value between the five groups is not statistically significant, as determined by the two-way ANOVA.

Threshold analysis. Figure 11A shows the calculated threshold curves from the ventilation maps of all rats. By construction, all curves start from 100%, at \( r_{\text{threshold}} = 0 \), and end at 0%, at \( r_{\text{threshold}} = 0.5 \). The curves for healthy animals (in black) remain near 100% up to higher values of \( r_{\text{threshold}} \), indicating healthier function throughout these lungs compared with emphysematous rats. Next, the ventilation map for each lung was normalized by the respective global ventilation \( R \), and similar threshold curves were generated for all animals. Normalized \( r/R \) threshold curves are shown in Figure 11B.

DISCUSSION

Survival of rats through the study. All of the unexpected deaths occurred within 3 days of instillation of elastase. Earlier studies have shown that deaths are not caused by a more severe disease, but are rather due to the idiosyncratic development of hemoptysis in certain rats (47, 48). Some animals, for reasons that are unclear, develop hemoptysis soon after elastase instillation, and this almost invariably leads to death by airway obstruction. Therefore, it is not expected that the animals’ deaths bias the results.

Effect of oxygen-induced depolarization of HP \(^3\)He on \( r \) calculations. Specific imaging techniques have been developed for measuring regional \( P_{A\text{O}_2} \) in the lung using HP \(^3\)He MRI (14). However, these techniques are fundamentally designed for larger species capable of holding their breaths for several tens of seconds and are, consequently, impractical for rodents. Therefore, for this study, we used an estimate of the global \( P_{A\text{O}_2} \) value in entire lung of a healthy rodent based on the standard alveolar gas equations reported in literature (33). The initial \( P_{A\text{O}_2} \) before the first \(^3\)He breath and after a sufficient number of normal air breaths was assumed to be \( P_{A\text{O}_2} = 130 \) mbar. This assumption gives \( T_{1,\text{O}_2} \approx 20 \) s. Although alveolar oxygen tension may differ somewhat from this physiologically normal value in our disease model, we do not anticipate a large error in the calculated \( r \) value, since the effect of inaccurate
Table 2. Mean and SD of fractional ventilation in three lobes of five groups of rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>L Mean</th>
<th>SD</th>
<th>LR Mean</th>
<th>SD</th>
<th>UR Mean</th>
<th>SD</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>0.01</td>
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<td>0.02</td>
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<td>0.01</td>
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<td>0.00</td>
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<td>0.22</td>
<td>0.02</td>
<td>0.30</td>
<td>0.03</td>
</tr>
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<td>0.17</td>
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<td>0.01</td>
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<tr>
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<td>0.01</td>
<td>0.24</td>
<td>0.01</td>
<td>0.29</td>
<td>0.00</td>
</tr>
<tr>
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5U Elastase @ 5 wk: 0.09±0.05

<table>
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<th>LR Mean</th>
<th>SD</th>
<th>UR Mean</th>
<th>SD</th>
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<tr>
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<td>0.09</td>
<td>0.01</td>
<td>0.08</td>
<td>0.00</td>
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<tr>
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<td>0.02</td>
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<tr>
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</table>

5U Elastase @ 10 wk: 0.10±0.04

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<th>LR Mean</th>
<th>SD</th>
<th>UR Mean</th>
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<td>0.00</td>
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<td>0.00</td>
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<tr>
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10U Elastase @ 5 wk: 0.12±0.05

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<th>L Mean</th>
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<th>LR Mean</th>
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10U Elastase @ 10 wk: 0.06±0.02

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<th>L Mean</th>
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<th>LR Mean</th>
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<td>0.06</td>
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Values are means and SD of fractional ventilation in three lobes of five groups of rats, along with the overall mean and SD of each group. L, left lobe; LR, lower right lobe; UR, upper right lobe.

Estimation of $P_{A0}$ has been shown to be relatively small in this mathematical model of fractional ventilation (12).

Time evolution of $r$ and $L_m$. The time changes of lobar fractional ventilation and $L_m$ are shown in Fig. 8, A and B, respectively. Lobar $(r)$ value of healthy rats progressively declines toward emphysematous groups at 5 and 10 wk. However, the 5- to 10-wk change is significant only for the 10U rats, i.e., the higher severity of the disease. The time evolution curve of lobar $(L_m)$ depicts the overall increase of alveolar diameter as time progresses. As indicated in results, $(L_m)$ is not statistically different between healthy rats and the two elastase dosages at 5 wk. The difference between $(L_m)$ in healthy and emphysematous rats only becomes significant at 10 wk. The fact that lobar $(r)$ value of healthy rats is not statistically different from that of the 5U emphysematous rats at either time point indicates that $(r)$, for the duration of this study, did not elucidate less severe emphysematous changes in lung function, and, therefore, the onset of elastase-induced emphysematous changes in lung function was only detectable for the 10U group. This change was, however, not detectable by $(L_m)$.

Correlation of $r$ and $L_m$ during elastase-induced changes. Studying correlated changes of fractional ventilation and the average alveolar diameter in each lobe can be an important tool for the onset of emphysematous changes in lung function.
to understand the evolution of lung function and structure during early stages of emphysema (Fig. 9). The average change of \( r \) in 0–5 wk (for all rats combined) was calculated as \(-55 \pm 25\%\) vs. that of 5–10 wk at \(+7 \pm 93\%\). A one-sample \( t \)-test showed that the slope of \( \Delta r_{5-10} \) is not significantly different from zero, and, therefore, \( r \) decline was substantially attributed to the first phase, i.e., 0–5 wk. Furthermore, the one-way ANOVA showed that \( \Delta r \) slopes were also significantly different from the corresponding \( \Delta L_m \) values, \(+34 \pm 31\%\) and \(+81 \pm 68\%\), respectively. A proper correlation analysis during disease progression requires coregistered measurements of independent lung function and structure and perhaps a larger sample size. These findings, however, suggest that, in this model of emphysema, the first phase (0–5 wk) is dominated by decline in lung ventilation, whereas the second phase (5–10 wk) is dominated by a rise of \( L_m \). Therefore, lung function and structure can be affected by elastase through different mechanisms or on a different time scale.

**Normalization of regional ventilation measurements.** Quantitative pulmonary measurements are generally affected by the inhaled gas volume (7, 37). Changes in \( V_t \) affect the number of recruited alveoli and/or the overall elastic deformation of the lung, thereby altering regional gas delivery. The impact of changes in \( V_t \) is even more significant when these measurements are compared among various subjects, each of which possessing a unique lung physiology, volume, and gas exchange efficiency. It is, therefore, necessary to normalize the regional measurements of fractional ventilation, \( r \), with respect to global fractional ventilation, \( R \), calculated based on the plethysmographic measurements of \( V_t \) and estimated/measured FRC values in each rat. It was shown that the difference between \( r \) and \( R \) values are, in general, a function of the heterogeneity of \( r \) distribution and due to higher uniformity, these two quantities correlate better in healthy rats than emphysematous ones (Fig. 10).

**Threshold analysis.** There is clinical evidence that emphysema progression can lead to heterogeneous distribution of this disease, at least in certain areas of the lung (20). Even though the coefficient of variation of ventilation \( (r/r)_{\text{threshold}} \) was smaller in healthy rats, the differences between the groups were not significant. Therefore, a threshold-based metric was utilized as a function of the distribution of fractional ventilation values in the lung. Using the fractional ventilation threshold curves (Fig. 11), the specific values of \( r_{\text{threshold}} \) and \( (r/R)_{\text{threshold}} \) were calculated above which 80% of imaged lung bins were included. These values were denoted as \( r_{80\%} \) and \( (r/R)_{80\%} \),
respectively. Further work with larger data sets will be needed to determine if this is the best possible threshold and whether it is applicable to other models of emphysema. For the present data set, the exact threshold value was relatively unimportant, and any value in the range of 75 - 85% yielded very similar results. Figure 12, A and B, shows \( r_{80\%} \) and \( (r/R)_{80\%} \) threshold values, respectively, for each animal.

The two-way ANOVA analysis of \( r_{80\%} \) and \( (r/R)_{80\%} \) values showed that there was a significant main effect for time (\( P < 0.05 \)). A significant effect for neither dosage nor interaction was observed. Post hoc tests revealed that both \( r_{80\%} \) and \( (r/R)_{80\%} \) are significantly different between healthy rats and the rest of the groups (\( P < 0.05 \)). No significant difference between emphysematous groups was detected.

It should be noted that, since \( r/R \) distribution is normalized with respect to the delivered and residual volumes of each animal, the differences between groups of animals are primarily functions of deviation in regional ventilation and heterogeneities of its distribution. This metric may, therefore, yield a more independent measure of ventilation difference between animals caused by elastase-induced emphysema compared with overall \( r \) or coefficient of variation \( \sigma(r)/r \). It is also interesting to note that there is a much smaller overlap between healthy and emphysematous rats for both \( r_{\text{threshold}} \) and \( (r/R)_{\text{threshold}} \) values compared with \( r \) or \( \sigma(r)/r \). The difference in normalized ventilation curves among various animals appears to be less than the differences in the unnormalized threshold curves, but a larger study is needed to identify the optimum test.

**Effect of anatomical dead space on \( r \) calculation.** Several earlier studies of regional lung ventilation derived from tracer kinetics modeling during washout examined the contribution of dead space (DS) in measurement of specific ventilation in lung parenchyma. Valind et al. (56) utilized a three-compartment model of human lung consisting of 1) the common DS shared by all regions (i.e., trachea and major bronchi), 2) the regional DS specific to each region, and 3) the alveolar space representing parenchyma air spaces. They estimated the ratio of combined anatomical DS, to acinar airway volume at rest (VA), using Weibel’s morphometric model of airways (57), as DS/VA \( \approx 0.08 \). Subsequently assuming DS/V \( T \) \( \approx 0.25 \), they calculated that the errors associated with the airway DS in a normal lung ranges from an overestimation of 3% to an underestimation of 8%, in ventral to dorsal regions, respectively. A more recent study by Richard et al. (38) utilized \(^{13}\)N-N\(_2\) washout and PET to measure regional ventilation in pigs. They concluded that the effect of anatomical DS could result in underestimation of regional alveolar ventilation by up to 30% due to rebreathing of radioactive tracers.

To incorporate the DS factor in this rat study, and to estimate the associated error in measurements of alveolar fractional ventilation, we derived a simple two-compartment model for \(^{3}\)He signal buildup in alveoli. This system comprises two compartments in series: 1) DS, conductive airways DS; and 2) VA, the volume of lung parenchyma. Since Deninger’s fractional ventilation model only gives meaningful values for the inflated regions of lung parenchyma (the conductive airways have \( r = 1 \) by definition), this error analysis only pertains to the second compartment. A volume of V\( T \) of the polarized \(^{3}\)He enters the conductive airways at each breath. Therefore, the gas
entering the lung parenchyma will be a mixture of the fresh delivered gas and the gas sitting in conductive airways from the previous breath. On the other hand, the gas present in conductive airways at each breath necessarily has the same concentration as that of alveolar gas in the previous breath. Therefore, the fraction \( r_{DS} = DS/V_T \) of the inhaled gas at each step is rebreathed from the previously exhaled gas. Ignoring the \(^3\)He polarization decay over the course of a series of breaths, the signal kinetics can be modeled as a recursive relationship of the form:

\[
\begin{align*}
S_A(j) &= r \cdot S_C(j) + (1 - r) \cdot S_A(j - 1) \\
S_C(j) &= (1 - r_{DS}) \cdot M_S + r_{DS} \cdot S_A(j - 1)
\end{align*}
\]  

where \( S_A(j) \) and \( S_C(j) \) represent signal at \( j \)th breath in alveoli and conductive airways, respectively. \( M_S \) is the available magnetization of polarized \(^3\)He in the ventilator reservoir. Figure 13A shows an example signal buildup in the trachea and alveoli for \( r = 0.3 \) and \( r_{DS} = 0.25 \), with an arbitrary \( M_S = 1 \). Both alveolar and tracheal signals start from an initial condition of zero, i.e., no \(^3\)He. It is evident that, due to rebreathing, it takes several breaths before the signal in trachea becomes equal to \( M_S \). This effect also diminishes the effective fractional ventilation of the alveoli compared with the case of no DS, \( r_{DS} = 0 \).

Figure 13B shows the effect of DS on the relative error of the estimated \( r \) value when ignoring DS, and by assuming average values of \( V_T = 3 \) ml and FRC = 7 ml. The DS of the ventilation device (including valves and connectors between the respirator and trachea) is ~250 \( \mu \)l. In addition, the combination of trachea and major bronchi in a healthy rat is estimated at 0.5 ml resulting in a total \( r_{DS} = 0.25 \). Therefore, \( r \) is expected to be underestimated by 25%. The error associated with assuming an incorrect DS value in estimating \( r \) is shown in Fig. 13C. Using an \( r_{DS} \) value higher or lower than 0.25 results in a relative over- or underestimation of \( r \) value, respectively.

It is fair to assume that the variation of DS volumes is negligible compared with lung volumes across different cohorts. The resulting error in \( r \) estimation can, therefore, be considered a bias error and is not expected to affect the differentiation of healthy and emphysematous rats.

**Relationship of elastase model to human disease, and future research.** The fundamental differences between the elastase model and human disease have been investigated (8, 45). The elastase model does not exactly recapitulate the pathogenesis of the human disease, which is produced over decades by gradual injury to the lung structure, rather than by a single massive injury. This model, however, remains a useful model of emphysema, since it is relatively simple to perform, replicates many aspects of the disease, and produces the most consistent and impressive air space enlargement in a large array of animals (50). Other emerging emphysema models on the other hand, such as cigarette smoke exposure (21, 30) and genetic murine models (29, 46), are becoming more important, especially because of great similarity of mouse and human air spaces with respect to emphysema (19). An important avenue for future research will be the study of whether alternative emphysema models behave similarly. Ultimately, these questions could be most directly addressed in longitudinal human studies, if a method for fractional ventilation measurement with fewer helium breaths can be demonstrated.

**Conclusions.** Mean fractional ventilation measurements in the three lobes of rat lungs were larger in healthy rats (0.23 ± 0.04) than in emphysematous rats (ranging from 0.06 ± 0.02 to 0.12 ± 0.05). Correlated measurements of regional fractional ventilation, \( r \), and mean alveolar size, \( L_m \), in three lobes of each lung induced with the elastase instillation described how lung function and structure evolve during the progress of disease over the time period of 10 wk in five cohorts of rats. The lobar \( \langle r \rangle \) values decline with progression of elastase-induced changes, whereas lobar \( \langle L_m \rangle \) values increase monotonically, each at a rate significantly different between 0–5 and 5–10 wk. The lobar \( \langle r \rangle \) is significantly different between the controls and two time points of the 10U elastase model. Changes in alveolar diameter were not statistically observable until the 10th wk. These findings suggest that functional and morphological changes induced by elastase in the lung evolve through different mechanisms and possibly at different time scales, and
therefore different aspects of disease progression can be manifested in either of the metrics in a complementary manner. The noninvasive simultaneous measurement of lung function and structure (e.g., through measuring \(^{3}He\) diffusivity), therefore, is of utmost value in assessment of emphysema and monitoring response to therapy. Nevertheless, the findings of this work can serve as the starting point for designing a longitudinal study for simultaneous monitoring of lung function and structure by providing the necessary information for determining the statistical power and sample size.

Heterogeneity of ventilation distribution, as quantified by coefficient of variation \(\sigma(r)/\langle r \rangle\), even though lower in healthy rats, was insignificant among all groups. In contrast, a threshold analysis based on the 20th percentile of regional fractional ventilation yielded a significant difference between healthy and emphysematous rats in the studied sample. The sensitivity, specificity, and optimum value of the threshold are important subjects for further study. A threshold measurement based on normalized fractional ventilation showed similar discriminatory power.

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