Mild loss of lung aeration augments stretch in healthy lung regions

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INVESTIGATORS AGREE THAT VENTILATOR-induced elevated tissue stretch (i.e., tidal deformation) can cause pulmonary damage de novo (23, 46) and worsen preexisting acute respiratory distress syndrome (ARDS) (1). Yet the mechanisms by which mechanical ventilation can generate injurious stretch in lungs without preexisting injury (39) (e.g., during general anesthesia) remain unclear. The pressure-causing tidal stretch (i.e., the "driving pressure") (3) and peak inspiratory distension of the lungs (18) are each relatively low, although mild stress disrupts the extracellular matrix within only 4 h of ventilation and can initiate injury in healthy lungs (34, 37). Furthermore, general anesthesia causes lung deflation below the normal functional residual capacity (FRC), which promotes atelectasis (26, 42) and increases stress at re-inflation (6, 40, 49). In fact, laboratory studies in isolated lungs have shown that atelectasis due to unimpeded deflation to a volume below FRC is harmful (38, 50). Although positive end-expiratory pressure (PEEP) maintains lung volumes and prevents atelectasis (26), clinical trials have produced ambiguous results on whether PEEP benefits the lungs of healthy patients undergoing surgery (41a) and those with ARDS (7).

Uncertainty about how and to what degree atelectasis contributes to stretch-related injury has been caused in part by the use of conventional imaging methodologies that may not capture the subtleties of ventilator-induced damage in some normal lungs. Computed tomography (CT) indicates a distribution of aeration between atelectatic vs. overstretched regions (24) when a substantial amount of tissue is not aerated and CT density is ≥100 Hounsfield units (HU) (43). However, ventilated subjects frequently exhibit areas of intermediate aeration (32), which may represent microscopic or partial atelectasis (25). In these regions, CT voxels of intermediate gray scale may contain a mixture of ventilated and atelectatic air spaces that cannot be separated from uniformly sized air spaces due to the necessity of signal averaging in the setting of low spatial resolution. A mixed aeration pattern and heterogeneity of localized inflation have been associated with increased mortality in injured lungs (i.e., ARDS) (13).

Our group has begun using hyperpolarized gas magnetic resonance imaging (HP-MRI) in an attempt to capture relationships between atelectasis and elevated tissue stretch that may be undetectable via CT. The apparent diffusion coefficient (ADC) derived from HP-MRI information measures diffusivity restriction within the alveoli and the alveolar ducts (58) and is proportional to their dimensions (56), although the dimensions are smaller than the voxel resolution of the scanner. This volume-weighted metric is particularly suited to studying the effects of atelectasis on residual ventilated air spaces because it is sensitive to enlarged microstructures (31). We showed that ADC is elevated in rats with atelectasis (8) or lung injury (9), suggesting overdistension. This gas distribution pattern was also observed in healthy lungs with microatelectasis due to suboptimal recruitment (10). On the basis of these results, it appears that sparse loss of aeration may increase the propensity to damage of neighboring alveolar units (57).

However, static measurements of air space dimensions may not fully describe the susceptibility to ventilator-induced lung injury, which results from excessive tidal stress in addition to overdistension (41). As such, this study sought to better understand the stretch of peripheral air spaces during suboptimal recruitment in noninjured lungs by regionally correlating air space dimensions as measured by ADC with fractional ventilation (FV; a measurement of gas turnover in peripheral airspaces) (33), gas volume, and CT tissue density. We hypothe-
esized that lung regions with overall loss of gas due to derecruitment would be characterized by elevations in both FV and ADC, the latter due to the presence of ventilated air spaces in close proximity to collapsed alveoli within each voxel. This combination (colocalized increases in ADC and FV) could indicate fully recruited air spaces being overstretched due to nearby, persistent derecruitment. This hypothesis was tested in ventilated, healthy rats by analyzing the spatial distribution of HP-MRI parameters in dorsal and ventral quadrants of the lungs.

METHODS

Study Protocol

Animal preparation and care. Studies were performed on 14 male Sprague-Dawley rats weighing 390 ± 48 g and with approval from the Institutional Animal Care and Use Committee of the University of Pennsylvania. Animals were anesthetized with pentobarbital (40–60 mg/kg ip initially, then 10–20 mg/kg hourly for maintenance), the trachea was intubated (14-gauge catheter; BD Biosciences, Franklin Lakes, NJ), and the glottis was sealed with putty (DAP Products, Baltimore, MD) (8). Animals were paralyzed using pancuronium bromide (1 mg/kg iv; Abbot Laboratories, North Chicago, IL) injected through a 24-gauge tail-vein catheter. Rats received hydration with normal saline (30 ml/kg) injected subcutaneously in the lateral neck after induction of anesthesia. After anesthetic induction and intubation, all animals were placed in the scanner in the supine position and ventilated using a MRI-compatible small-animal ventilator capable of delivering mixtures of helium, nitrogen, and oxygen (8). Airway pressure was continuously recorded using a fiber-optic sensor (Samba Sensors, Sweden); peak inspiratory pressure (PIP) and dynamic respiratory compliance (Cdyn) were measured. Heart rate and peripheral oxygen saturation (SpO2) levels were monitored by a veterinary pulse-oximeter (Nonin Medical, Plymouth, MN) attached to the hind foot. The animal’s rectal temperature was maintained at 37°C by a heating pad (Gaymar Industries, Orchard Park, NY) placed under the body. After the last set of measurements, animals were removed from the scanner and euthanized by lethal pentobarbital injection.

Ventilation protocol. Rats were ventilated in volume-control mode with a constant tidal volume ($V_t$) of 10 ml/kg, respiratory rate of 60 breaths/min, and FiO2 0.5. PEEP was set at 9 cmH2O (ZEEP) except during alveolar recruitment maneuvers, which were performed by applying PEEP at 9 cmH2O for 1 min. This method of recruitment was during alveolar recruitment maneuvers, which were performed by limiting time-dependent 3He signal buildup during 10 back-to-back hyperpolarized gas breaths (79% 3He, 21% O2), with images acquired during 500-ms end-inspiratory breath-holds using an HP-MRI methodology developed by our group (20). Analysis was performed on a voxel-by-voxel basis with a planar resolution of 0.47 × 0.47 mm². In each voxel, we measured the growth of signal after each inspiration (due to the volume of “fresh” hyperpolarized gas) and indexed it by the total signal generated by residual (expiratory) and by fresh gas volume: $V_{resid}/(V_{fresh} + V_{resid})$ (15). Time evolution of signal intensity was then fitted to equations (20) yielding values of FV for each valid voxel.

Air space dimensions were estimated by measuring the apparent diffusion coefficient (ADC) for 3He, as described in our previous studies (8). Briefly, ADC mapping was performed with a planar resolution of 0.94 × 0.94 mm² at the same slice position as in the fractional ventilation imaging using interleaved diffusion-weighted gradient echo imaging pulse sequence (9). End-inspiratory images were obtained following ventilation with a mixture of hyperpolarized 3He and oxygen (79% 3He, 21% O2). Each ADC acquisition was obtained during a single breath hold and consisted of six diffusion-weighted images with $b$ values of 0, 5, 3, 2, 1, and 0 cm²/s, each corresponding to a different diffusion-sensitizing gradient. Images were analyzed by fitting the time evolution of each pixel’s signal intensity to a standard equation (8) to yield maps of regional ADC values. A threshold of signal-to-noise ratio (SNR) >20 was used to identify the location of the lungs in the last image of each ventilation series and in the ADC image with zero $b$ value. The average SNR in these two images was typically above 60, and very few low SNR voxels were identified in the lung parenchyma. The last $b$-value image in the series of ventilation acquisitions was also used to estimate global and regional lung gas volume ($V_e$) at end-inspiration, which was quantified by counting voxels in images of both slices.

Computed tomography. In six rats, high-resolution, end-inspiratory CT scans were acquired using an eXplore 120 micro-CT scanner (eXplore CT120 system; Northridge Tri-Modality Imaging, Chatsworth, CA). Settings used for imaging were as follows: 80 kVp, 32 mA, 16-ms exposure time, 220 projections (half-scan), and 100-µm isotropic resolutions. To avoid blurring due to respiratory motion, imaging was ventilator-gated and performed during 500-ms end-inspiratory and end-expiratory breath-holds. Images were reconstructed to three-dimensional whole-lung maps with 200-µm isotropic resolution using a proprietary program supplied by the scanner manufacturer (MicroView 2.2; GE Healthcare). For lung gas content determination, image thresholding was used to obtain images of all relevant three-dimensional regions of interest (ROI), including both lungs in their entirety. All pixels with CT density higher than −300 HU were excluded, thereby ensuring an adequate delineation of the aerated lung parenchyma from the surrounding chest wall and from other nonpulmonary tissue in healthy lungs. End-inspiratory lung gas volume (EILV) and end-expiratory lung volume (EELV) were calculated using CT density analysis methodology on each ROI (16). $V_f$ was computed as EILV − EELV. Because ventilator settings were identical and airway pressures were similar to those of rats receiving HP-MRI, we assumed that inflation volumes were comparable between animals receiving CT and HP-MRI.

Statistical Analysis

For analysis, ADC, FV, and 3He density maps of the ventral and dorsal slices were binned to four ROIs corresponding to upper, lower, right, and left
“lobes” of each slice, which resulted in $2 \times 2 \times 2$ replications for each rat (spatial pseudoreplications). Means and standard errors in each ROI were calculated and used in the regional statistical analysis as mean and heterogeneity of that lobe, respectively. This step was performed to maintain regionality of information. A repeated-measures (nested) ANOVA was performed to test differences between the three conditions in the imaging markers (FV, ADC, and V). The lobe term was nested within the slice term, which was nested within the animal identification term. A priori power analysis was performed in G*power (21) before the experiments on the basis of observed effect size of increased ADC in rats. To achieve a minimum power of 80% for a repeated measure within-factor analysis with three measurements (three conditions in the same animal), eight rats are sufficient to detect the required effect size [Cohen’s $d = 0.5$ from (8)]. Because we used a nested (pseudoreplication) methodology, eight spatial samples were used for each rat and a total of 64 samples guarantees the power of this study. ANOVA was performed, followed by a post hoc Tukey’s test to identify the statistically significant differences between conditions. A variance component analysis was then performed, as detailed in the APPENDIX. A mixed-effect regression model was fit to the data to describe the mean ADC in each lobe ($\text{ADC}_\text{m}$) by a linear model containing all the other imaging variables and their interactions at a regional level. Retained fixed-effects in the final minimal adequate model included mean FV ($\text{FV}_\text{m}$), heterogeneity of FV ($\text{FV}_\text{h}$), V, and a slice term. The random effects were as explained above for the nested analysis (rat/slice/lobe). Statistical analysis was performed using R (R Foundation for Statistical Computing; Vienna Austria, http://www.r-project.org). An experiment-wide type I error level of 0.05 was used.

RESULTS

Hyperpolarized MRI

Ventilation at ZEEP for 1 h was associated with higher values for both FV and ADC, changes that are evident in HP-MRI images in the ventral and dorsal lung slices of a representative animal (Fig. 1). Frequency distributions in this individual rat suggest increased heterogeneity of FV (Fig. 1B) at 1 h. Changes in FV and ADC were mostly reversible after recruitment. Figure 2 (left) shows coregistered maps of FV and ADC in the ventral slice of a representative animal, together with mean values calculated in each of the four binned lobes.
increases in FV and ADC at 1 h were obvious in all lobes. In contrast, regional gas volume (V) declined in each lobe at 1 h but increased again after recruitment (Fig. 2). Figure 3 shows mean slice values of HP-MRI variables in the three conditions studied: the dorsal slices displayed a larger increase in ADC and FV after 1 h of ventilation than the ventral lung. The increase in PIP observed at 1 h (Fig. 3D, P < 0.01) was reversible after recruitment; dynamic compliance decreased from a baseline of 0.43 ± 0.05 to 0.29 ± 0.05 ml/cmH₂O (P < 0.01) at 1 h, then returned to 0.43 ± 0.05 ml/cmH₂O.
ml/cmH2O after recruitment, confirming that this maneuver improved lung mechanics.

The nested ANOVA results are shown in Table 1. There were significant differences in FV, FV heterogeneity, ADC, and V among the three conditions (in all cases, $P < 0.05$) and between the ventral and dorsal slices (in all cases, $P < 0.01$) (Fig. 3, Table 1). The post hoc tests consistently confirmed significant change between 1 h and the other two conditions (in all cases, $P_{\text{Tukey}} < 0.005$), except for FV between 1 h and recruitment ($P_{\text{Tukey}} = 0.083$). Mean ADC, FV, and V values were not different between baseline and after recruitment (in all cases, $P_{\text{Tukey}} > 0.1$), whereas lungs still had significantly higher FV heterogeneity after recruitment (Table 1, $P_{\text{Tukey}} = 0.025$).

To further illustrate regional changes in FV, ADC, and V, differences in these values between each condition and the baseline are shown in each lobe after combining the ventral and dorsal slices (Fig. 4). The post hoc tests consistently confirmed significant change between 1 h and the other two conditions (in all cases, $P_{\text{Tukey}} < 0.005$), except for FV between 1 h and recruitment ($P_{\text{Tukey}} = 0.083$). Mean ADC, FV, and V values were not different between baseline and after recruitment (in all cases, $P_{\text{Tukey}} > 0.1$), whereas lungs still had significantly higher FV heterogeneity after recruitment (Table 1, $P_{\text{Tukey}} = 0.025$).

Table 2 shows the results of the mixed-effect regression analysis. Details of the model generation are presented in the Appendix. All covariates not listed were found to be insignificant. The constant term chosen was the overall mean ADC in all the ventral ROIs for all the conditions ($ADC_m = 0.178$). The next fixed-effect variable (slice term) showed a position-dependent gradient, with $ADC_m$ rising by $0.06$ in the dorsal slices ($ADC_m = 0.235 + 0.178 + 0.057$). The volume was a significant covariate with a negative strong effect on the $ADC_m$ (i.e., a 5% decrease in V increased $ADC_m$ by an average of $14\%$ in ventral slices). The interaction between the remaining two effects, mean FV ($FVm$) and heterogeneity of FV ($FVh$, the standard error of FV), showed a significant positive effect on $ADC_m$. Random effects explain the variance in the slopes of the regression terms. Approximately 24% of the variance is explained by the variability among rats and 53% by regional differences between lobes in the lung.

### Computed Tomography

CT showed no macroscopic evidence of atelectasis after ventilation with ZEEP (Fig. 6A, top). However, a subtle increase in tissue density (indicating reduced aeration) was present at this time vs. baseline, with a gradual ventral (non-dependent) to dorsal (dependent) distribution that was evident in the lung views with narrowed density window (Fig. 6A, bottom). Quantitative CT analysis showed that EILV decreased by $8.46 \pm 0.03\%$ after 1 h of ventilation at ZEEP (Fig. 6B) and was restored to near-normal values after recruitment. $V_T$, measured by CT, was constant in all conditions ($3.61 \pm 0.08$ ml at baseline, $3.56 \pm 0.10$ ml at 1 h, and $3.61 \pm 0.09$ ml after recruitment) and was consistent with the value set on the ventilator ($3.62 \pm 0.08$ ml).

### Table 1. Repeated-measures ANOVA (nested) for differences between FV, ADC, and volume

<table>
<thead>
<tr>
<th>Source</th>
<th>df*</th>
<th>FVm $F$</th>
<th>$P$</th>
<th>FVh $F$</th>
<th>$P$</th>
<th>ADC $F$</th>
<th>$P$</th>
<th>Volume $F$</th>
<th>$P$</th>
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<td>Conditions</td>
<td>2</td>
<td>8.72</td>
<td>0.002</td>
<td>15.25</td>
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<td>8.04</td>
<td>0.003</td>
<td>4.15</td>
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<tr>
<td>Rats in conditions</td>
<td>21</td>
<td>0.95</td>
<td>0.542</td>
<td>2.39</td>
<td>0.111</td>
<td>0.74</td>
<td>0.754</td>
<td>0.16</td>
<td>0.999</td>
</tr>
<tr>
<td>Slices in rat</td>
<td>24</td>
<td>1.97</td>
<td>0.008</td>
<td>0.62</td>
<td>0.201</td>
<td>3.82</td>
<td>&lt;0.001</td>
<td>4.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lobes in slice</td>
<td>144</td>
<td>Z</td>
<td>$P_{\text{Tukey}}$</td>
<td>Z</td>
<td>$P_{\text{Tukey}}$</td>
<td>Z</td>
<td>$P_{\text{Tukey}}$</td>
<td>Z</td>
<td>$P_{\text{Tukey}}$</td>
</tr>
<tr>
<td>Post hoc?</td>
<td>2</td>
<td>4.11</td>
<td>&lt;0.001</td>
<td>11.08</td>
<td>&lt;0.001</td>
<td>3.16</td>
<td>0.004</td>
<td>1.312</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 h recruitment</td>
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<td>0.083</td>
<td>8.48</td>
<td>&lt;0.001</td>
<td>3.205</td>
<td>0.004</td>
<td>1.233</td>
<td>&lt;0.001</td>
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<td>Baseline recruitment</td>
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<td>1.312</td>
<td>0.083</td>
<td>2.60</td>
<td>0.025</td>
<td>0.037</td>
<td>0.999</td>
<td>0.079</td>
<td>0.775</td>
</tr>
</tbody>
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ADC, apparent diffusion coefficient; FV, fractional ventilation; $FV_m$, mean fractional ventilation; $FV_h$, heterogeneity of fractional ventilation (i.e., the standard error of FV). *Degree of freedom. †Using Tukey’s test.
DISCUSSION

We have provided evidence that mild loss of gas content, but with no apparent macroscopic atelectasis, increases airspace dimensions, fractional ventilation, and ventilation heterogeneity in ventilated healthy rats under general anesthesia. This finding suggests that ventilator-induced injury might occur in lungs without preexisting lesions whereby suboptimal recruitment results in maldistribution of ventilation, and this in turn leads to an increase in local lung stretch (35).

The paradoxical presence of higher values for both ADC and FV in areas with modest reduction of aeration is not intuitive. On its own, elevation in FV in a region of interest after ZEEP ventilation could indicate the influx of a larger volume of “fresh” hyperpolarized gas, smaller “residual” alveolar volume in the same region, or both of these factors together, assuming that minute volume is constant. In our study, although ventilation with ZEEP caused a heterogeneous response in FV, the predominant effect was the increase of its mean values (globally and regionally) and a comparable decrease in lung gas volume. Volume loss could suggest that smaller alveoli were responsible for the higher FV because total ventilation was constant for the whole lung. However, concomitant larger mean ADC points to a prevalence of dilated rather than contracted ventilated air spaces (29, 56), because ADC is sensitive to enlargement of subacinar microstructures (31).

Alveolar collapse can explain this dissociation between smaller lung volume and larger air spaces (10). Under conditions of poor recruitment, the amount of ventilated lung is smaller and inspired gas is redirected to residual open air spaces that are consequently overdistended (8). Because lung tissue is finite and total ventilation is constant, these open air spaces receive more fractional ventilation than when the lungs are optimally recruited.

Our battery of HP-MRI parameters revealed that gas volume reduction, increased air space dimensions, and FV maldistribution were colocalized after ventilation with ZEEP. Lung regions with larger loss of gas volume had higher ADC and higher and more heterogeneous FV. Using a mixed-model regression analysis (Table 2) we confirmed that mean values and heterogeneity of FV were positively correlated with ADC in each lobe. Despite a significant topographic variability in responses, the observed FV changes were more pronounced in the dependent (dorsal) lung regions. Although other researchers (5) have shown greater ventilation within poorly recruited areas of injured lungs, this is the first study to document colocalized elevations in both FV and air space dimensions in healthy lungs with suboptimal aeration. Our previous studies measured ADC as an estimate of the state of regional air space dilatation (8–10), but ADC provides a static estimate of the dimensions of ventilated peripheral air spaces at the end of inspiration; it does not quantify their dynamic behavior. Elevated air space dimensions and high FV do not necessarily coexist: the air-trapping characteristic of emphysematous lungs, for example, is associated with substantially higher ADC and lower FV (29). A means of assessing both air space dimensions and regional ventilation in mechanically ventilated subjects is extremely valuable because static deformation is less harmful than large dynamic stretch (28).

The notion that microscopic derecruitment in a given lung region causes abnormal distension and stretch of local air spaces is lent further support by our CT results. No macroscopic atelectasis was visible after ventilation at ZEEP (Fig. 6A), which is at variance with work showing regional hyperinflation secondary to discrete atelectasis after lung injury (51) or after prolonged ventilation (19) in rats. However, we ob-
Fig. 6. A: axial CT scans in a representative rat. B: trend in end inspiratory lung volume expressed as a fraction of baseline values (EILV*) (means ± SD of five rats) measured at baseline, after 1 h of ventilation at zero PEEP, and after recruitment maneuver. No macroscopically visible atelectasis was detected in the raw CT scans; however, a greater anterior-posterior density gradient was appreciated in the narrowed density windows (see color map beneath A) suggesting decreased aeration in the dorsal lung regions.

served gravity-dependent gradients of CT attenuation and reversible increases in PIP (Fig. 3) at 1 h, which suggest the presence of microatelectasis. Such sparse alveolar collapse is too small to be resolved by CT at the resolution we adopted (25). Under such conditions, subtle recruitment alters the distribution of inspired gas at the peripheral (subvoxel) level (35). The presence of such a mechanism is supported by the fact that FV was ubiquitously increased in our study, suggesting gas redistribution locally within each lobar ROI rather than between macroscopically distant regions of the lungs. Furthermore, the greater heterogeneity in FV in the form of both hypoventilated and hyperventilated air spaces suggests a maldistributive pattern of aeration, which can be generated by close proximity of collapsing and open alveoli in areas of recruitment (36). Finally, the effect of recruitment on FV and ADC was more prominent in the dorsal (dependent) regions, where density as depicted by CT was higher. Our finding of microatelectasis could be related to our use of FIO2 0.5: imaging studies have shown that higher inspired oxygen results in more substantial atelectasis (17, 43, 45), which could macroscopically redistribute (e.g., from dorsal to ventral regions) FV and ADC at ZEEP.

Although our study does not address whether marginally aerated lung tissue proceeds to ventilator-induced injury, it does present evidence of abnormal alveolar mechanics in these areas. The effect of colocalized microatelectasis and overdistribution on the clinical course of ventilated patients is unknown, but recent evidence in humans suggests that heterogeneities of inflation and borderline aeration are associated with greater ARDS mortality (13), which could be due to local concentration of mechanical stress (2). Supporting this interpretation, recent studies in ventilated animals showed injury in lung tissue surrounding isolated foci of atelectasis (44) and regional aeration heterogeneities (14, 57). Maldistributions of ventilation resulting from borderline aeration are probably common during mechanical ventilation in anesthetized surgical patients: in that setting, a recent survey showed that ZEEP is used by many clinicians in combination with large VT (30). Recent studies showed that lung protective ventilation with low VT and higher PEEP improved outcomes, including rates of postoperative respiratory failure and pulmonary infections in patients undergoing high-risk abdominal surgery but with no preexisting injury (23, 46). However, studies testing higher vs. lower PEEP separately from VT size during general anesthesia had negative results (41a), clouding the analysis of the role of recruitment in surgical patients.

This study has several limitations. Most broadly, we did not measure lung stretch directly, but rather inferred the presence of abnormal inflation characteristics from the observed elevations in air space dimension and fractional ventilation after loss of gas volume at ZEEP. This approach is supported by studies that have found high correlations between local stretch, calculated as the fractional change in lung volume (22) and regional ventilation (53). In these studies, ventilation was measured as specific ventilation (sV'), defined as $V_{fresh}/V_{residue}$ (range 0 to infinity) (53); however, FV and sV' are linked by a transform, and their physiological meanings (dynamic change of gas content) are superimposable. Both these metrics are related to the rapidity of gas turnover in the alveolar environment, with their largest values found in major conducting airways, and they do not represent the contributions of each individual voxel to the total ventilation of the whole lung.

Ventilation has been measured using numerous imaging techniques, and the hyperpolarized gas method we used here has its own idiosyncrasies. Compared with studies that have measured regional ventilation through the washout of inhaled tracer (11, 27, 54), the inspiratory imaging that we used is more prone to artifacts due to fresh tracer gas in the conducting airways (47). Authors using injected 13NH3 positron emission tomography described decreased ventilation in poorly aerated regions of lungs compared with the increased fractional ventilation reported here (55). This apparent discrepancy is likely related to the fundamental difference between the measurements. Partial or transient atelectasis may reduce overall airflow to the affected region, thus reducing ventilation while increasing the fractional replacement of the gas with each breath (e.g., during alveolar collapse). Some of the discrepancy may also be due to the differing regional sensitivities of the two methods. Washout of intravenously injected tracer has low sensitivity in poorly perfused but well-ventilated lung. Con-
versely, wash-in techniques such as ours are subject to low signal in air spaces with reduced gas clearance due to distal airway collapse (54). Each technique is therefore potentially subject to bias if the signal in the low-sensitivity regions is too low to quantify, but the nature and direction of bias is different. Nevertheless, we found heterogeneity of FV to be a key characteristic of poorly recruited lung regions, suggesting that FV detected at least a portion of the alveolar units that were hypoventilated. Additionally, because rats have a pliable chest wall, our results may be hard to extrapolate to large animals and to humans in whom vertical gradients in pleural pressure are larger and chest wall recoil supports alveolar opening. However, postural changes in FV, observed by others using HP-MRI in supine rats (33), suggest vertical gradients in lung mechanics in rats as well.

ADC is weighted in favor of larger air spaces and is not affected by partial volume effects because areas of the voxels outside the lung contribute no signal. However, our HP-MRI estimates of gas volume were based on pixel counting, and as such, they were prone to overestimation where lungs were thinner than the voxels (e.g., in the lung periphery). Despite this technical limitation, changes in lung volume were similar when estimated by HP-MRI and CT in identical conditions of recruitment and derecruitment.

We performed recruitment by increasing PEEP to 9 cmH₂O for a short time. Although this single recruitment maneuver was sufficient to restore baseline values of ADC and lung volume, the decrease in FV and its heterogeneity were not significant after recruitment. This was probably due to incomplete recruitment during the short duration of high PEEP, and it is possible that higher or more prolonged airway pressure elevations (48) could have restored baseline FV distributions.

Inadequate recruitment of residual dorsal microatelectasis could also explain the higher baseline values of ADC in the dorsal vs. ventral slices (Fig. 3), which we did not observe in our previous study in which rats received longer recruitment maneuvers (10). We studied only one large VT value and, because of this design, we cannot assess the effects of VT selection on FV and ADC. However, lower VT in combination with better recruitment is likely to improve the maldistribution of regional ventilation in healthy lungs (54). Finally, we used a nested methodology to retain the regionality of the information (not sacrificing it to whole-lung statistics). Whereas in ANOVA tests and regression analyses a single error term is typically assumed, in our nested experiment we found that the error variance differed for each spatial and temporal scale within the same animal. In these circumstances, namely having both temporal (condition) and spatial (slices and lobes) pseudoreplications, a linear mixed-effects model may be the optimal choice for analysis (12).

Conclusions

We have provided in vivo evidence that when aeration subtly declines in previously normal lungs, fractional ventilation and air space dimensions are increased in those regions with more lung volume loss. This abnormality is undetectable by conventional monitoring or imaging and suggests the presence of elevated stretch in regions where poorly aerated lung tissue predominates. These mechanisms may have a role in the development of ventilator-induced injury.

APPENDIX

Variance Component Analysis

To study the relationships between the measured regional continuous variables and factors (i.e., conditions, anatomical position), and to test the main hypothesis of our study, a variance component analysis was performed (23a). In the first step of the analysis, decision trees were used to explain variances in the data (41b). A tree-based model can be constructed by a stepwise principle. The algorithm sorts out which of the available variables explain most of the variance observed in the response variable (determined a priori), then determines a threshold value (for each identified variable) that best partitions the variance in the response. The process is repeated for values of the identified variable that are larger and smaller than the threshold until no residual explanatory variable remains.

On the basis of preliminary tree results, we chose a mixed-effect model (4) to predict lobar air space dimensions (ADCₘ, in each ROI as response variable) from the regional stretch (FVm), aeration (Vₐ as local volume), and unevenness of ventilation in each lobar ROI (FVₜ). Slice term also entered the model as fixed effect because we found (in the tree analysis and from ANOVA results) that ADC was always significantly higher in the posterior slices. The random effects were chosen as in the ANOVA nested model (Rat/Slice/Lobe) to extract the variance components for different rats and anatomical positions.

This analysis was performed using the nlme package in R. The model was fit using a maximum likelihood technique and backward elimination based on Akaike information criteria (AIC) (1a). Once the significant covariates were determined, the final model was constructed using the restricted maximum likelihood technique. The first maximal model contained all the variables and all their interactions (up to four-way interactions between FVm, FVₜ, V, and Slice). Any

![Fig. 7. Quality of the mixed-model regression fit showing residuals in each rat and resulting quantile (QQ) plots.](japplphysiol.org)
factor/variable that was not significant was removed from the model; in each step the reduced model was compared with the original one with an ANOVA test and based on the change in AIC. All the four-way and three-way interactions were nonsignificant and were removed from the model. The only two-way interaction that remained significant was between $F_{V_m}$ and $F_{V_h}$. We retained all the fixed effects in the final model. The mixed-effect model results are presented in Table 2.

The continuous variables were scaled and centered to attain interpretable coefficients; with centering the data, the overall mean $\text{ADC}_m$ in all the anterior ROIs (for all the conditions) was selected as the constant term. Therefore, the first fixed-effect variable ($\text{slice}$ term) yielded the overall average in posterior slice ROIs for all the conditions. The volume ($V$) was a significant term with a negative strong effect on the $\text{ADC}_m$. The $V$ term was scaled such that a 5% decrease (100 voxels in a lobar ROI with the overall average of 2,000 voxels) increased $\text{ADC}_m$ by about 14.1% (0.0251/0.178) in that same ROI in anterior slices. The interaction between the other two main effects ($r_m$ and $r_n$) was a significant, strong positive explanatory term for $\text{ADC}_m$; when both $r_m$ and $r_n$ increased in a lobe, $\text{ADC}_m$ also increased in that same lobe. Random effects explained the variance in the slopes of the regression terms. At the end of the analysis, 20% of the variance remained unexplained. The residuals in each rat and the resulting QQ-plot ($Q$ stands for quantile) (54a) are shown in Fig. 7, which indicates the quality of the mixed-model regression fit. The residuals do not show any trend, and the linear QQ plots show the similarity and normality of the distributions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.C., Y.X., S.K., B.P.K., and R.R.R. conception and design of research; M.C., Y.X., H.H., N.M., J.Z., and H.P. performed experiments; M.C., H.H., and N.M. analyzed data; M.C., Y.X., S.K., B.P.K., and R.R.R. interpreted results of experiments; Y.X. prepared figures; M.C. and J.C. drafted manuscript; M.C. and R.R.R. approved final version of manuscript; Y.X., J.C., S.K., B.P.K., and R.R.R. edited and revised manuscript.

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