Micro-CT imaging of rat lung ventilation using continuous image acquisition
during xenon gas contrast enhancement

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Lam WW, Holdsworth DW, Du LY, Drangova M, McCormack DG, Santyr GE. Micro-CT imaging of rat lung ventilation using continuous image acquisition during xenon gas contrast enhancement. J Appl Physiol 103: 1848–1856, 2007. First published August 9, 2007; doi:10.1152/japplphysiol.00009.2007.—We measured ventilation (V˙) in seven anesthetized, mechanically ventilated, supine Wistar rats. Images of the whole lung were continuously acquired using a dynamic, flat-panel volumetric micro-computed tomography (micro-CT) scanner during ventilation with a xenon/oxygen (Xe−O2) gas mixture. Forty time-resolved volumes consisting of eighty 0.45-mm-thick slices (covering the entire lung) were acquired in 40 s, using a gantry rotation rate of one rotation per second. The animals were ventilated at a respiratory rate of 60 breaths/min, matching the gantry rotation rate, and imaged without suspending ventilation. A previously published theoretical model was modified slightly and used to calculate the whole lung ventilation from volumes of interest generated by seeded region growing. Linear regression of calculated whole lung ventilation volumes vs. expected tidal volumes yielded a slope of 1.12 ± 0.11 (slope ± SE) and a y-intercept of −1.56 ± 0.42 ml (y-intercept ± SE) with 95% confidence intervals of 0.83 to 1.40 and −2.6 to −0.5 ml, respectively. The same model was used to calculate the regional ventilation in axial slices for each animal. Voxels were fit to the model to yield a map of V˙, which displayed an inhomogeneous distribution of regional V˙ with the temporal and spatial resolution necessary for rats.

functional imaging; in vivo imaging; small animal imaging; computed tomography

NON-INVASIVE IMAGING of ventilation (V˙, in units of ml/s) has been shown to be sensitive to a variety of lung diseases, including asthma in mice (17), emphysema in rats (39), pulmonary embolism in sheep (44), and chronic obstructive pulmonary disease in humans (43). Ventilation can be quantified from dynamic changes in image signals following application of an inhaled contrast agent. Ventilation imaging has been demonstrated with stable xenon-enhanced computed tomography (CT) (23, 28, 30), 13N wash-out with positron emission tomography (PET; Ref. 44), fluorescent microspheres using luminescence spectrometry (31), Te99m nuclear scintigraphy (12), and more recently with hyperpolarized 3He magnetic resonance (MR) imaging (5). However, these methods have not been used extensively in rodents because most rely on clinical imaging equipment that is not suitable for small animal imaging. There is a particular interest in using imaging to study murine models of lung disease to provide time course information on disease progression and regression in a single animal, which is of particular importance in drug development (35).

Recent advances in micro-CT imaging (15), such as faster detectors, slip-ring technology, and dedicated rodent scanners, have made it possible to obtain high-resolution CT images of rodents with an isotropic spatial resolution of 0.075–0.15 mm (16, 22). With the addition of mechanical ventilation and/or gating, it is possible to measure lung tissue density changes at different phases in the respiratory cycle for measurement of tidal volume (TV; Refs. 10, 20, 36, 45). The application of stable xenon (Xe; mol wt = 131) gas ventilation provides the opportunity to measure ventilation in rats and mice based on changes in CT number due to Xe attenuation of x-rays. Kreck et al. (23) used this technique to measure regional ventilation and perfusion, which are the volume of gas inhaled per unit time and volume of pulmonary blood flowing per unit time, respectively, in an arbitrary volume of interest in sheep. To our knowledge, micro-CT has not yet been used to measure regional ventilation in rodents. Part of the reason for this is that imaging speed ultimately dictates the dynamics that can be studied (i.e., ventilation) and previous slower CT technology has relied on prospective gating to synchronize the image acquisition (10, 45), which is challenging for the rapid respiratory rate typical of rodents (>100 breaths/min) within acceptable dose limits of radiation (11). Current slip-ring micro-CT scanners can continuously acquire 3D images of the rodent lung at a rate of one volume per second, facilitating time-resolved lung imaging for a respiratory rate of 60 breaths/min.

In this work, the application of micro-CT to imaging of ventilation in rat lungs using stable Xe gas is investigated. Measurements of total lung ventilation (which is the volume of gas inhaled per unit time), using ventilation imaging, were made in a group of normal rats and compared with the actual lung ventilation, based on the mechanical ventilator settings (i.e., TV and breathing rate). The implications of this method for the measurement of regional ventilation changes in rodents are discussed.

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THEORY

In this study, mechanically ventilated rats were given five to eight breaths of pure oxygen (O₂) to establish a baseline, followed by 32–35 breaths of Xe-O₂ mixture. The average CT number of voxels containing lung tissue was measured over each breath. A theoretical model of regional ventilation based on one previously described by Kreck et al. (23) was used for this study. This model describes the change in CT number of lung voxels containing lung tissue was measured over each breath relative to baseline CT number in continuous Xe-enhanced CT of rats is given by

\[ \Delta HU^{i}_{\text{vol}} = [f_A + \lambda_{\text{tissue/gas}}(1 - f_A)] \times \frac{(V_A + \lambda_{\text{tissue/gas}}V_{\text{tissue}} + V_D)\Delta HU^{i-1}_{\text{vol}} + (VT - V_D)\Delta HU^{i}_{\text{vol}}}{V_A + \lambda_{\text{tissue/gas}}V_{\text{tissue}} + VT + \lambda_{\text{blood/gas}}VT} \]  

where \( Q \) is the pulmonary blood perfusion, \( \lambda_{\text{tissue/gas}} \) and \( \lambda_{\text{blood/gas}} \) are the partition coefficients for Xe (the ratio of gas-phase to tissue- and blood-phase Xe at equilibrium, respectively), \( \Delta HU^{i}_{\text{vol}} \) is the change in Hounsfield units in the voxel containing the \( i \)th volume, and \( V_D \) is the dead space. In our study, we made the following modifications to reflect image acquisition under continuous breathing: 1) the change in airway CT number, \( \Delta HU^{i}_{\text{vol}} \), was made time dependent because the rise to maximum Xe concentration in the gas supply line is not instantaneous, 2) the recirculation of Xe from the vena cava was neglected (i.e., \( Q = 0 \) in the numerator of Eq. 2) because it does not alter lung density within the time scale of the experiment (19), and 3) the pulmonary blood perfusion \( Q \) was replaced with ventilation \( V \) to simplify the model since the ratio of ventilation is unity in healthy rats under normal ventilation conditions (1). Thus the change in CT number of a voxel for the \( i \)th breath relative to baseline CT number in continuous Xe-enhanced CT of rats is given by

\[ \Delta HU^{i}_{\text{vol}} = [f_A + \lambda_{\text{tissue/gas}}(1 - f_A)] \times \frac{(V_A + \lambda_{\text{tissue/gas}}V_{\text{tissue}} + V_D)\Delta HU^{i-1}_{\text{vol}} + (VT - V_D)\Delta HU^{i}_{\text{vol}}}{V_A + \lambda_{\text{tissue/gas}}V_{\text{tissue}} + VT + \lambda_{\text{blood/gas}}VT} \]  

The supine posture was chosen over the prone posture to achieve a greater gravitational ventilation gradient (28). The choice of supine or prone posture was not expected to affect the ability to perform continuous micro-CT imaging. Anesthesia was induced with an intraperitoneal injection of ketamine-xylazine in a 2:1 mixture (0.1 ml/100 g). A 24-gauge catheter was placed in the tail vein to allow for constant infusion of a maintenance dose (2.8–5.2 ml/h) of anesthetic using a syringe pump (38). Atropine (0.04 mg/kg) was injected subcutaneously to decrease bronchial and salivary secretions, and LaciRube (Allergan Canada, Markham, Canada) was applied to the eyes. Intubation was performed, and the animal was mechanically ventilated through a short 14-gauge catheter. Heart rate, oxygen saturation, and temperature were constantly monitored using 1) a pulse oximetry system (LifeSense Vet, MedAir, Delsho, Sweden) with the sensor taped to the left hindpaw and 2) a rectal temperature probe (RET-2, Physitemp, Clifton, NJ). The rats were euthanized after completion of the study using an intravenous pentobarbital sodium injection of 2 ml/kg (270 mg/ml).

\( V \) was maintained throughout the study with a custom ventilation circuit illustrated in Fig. 1, consisting of 1) a small animal ventilator (SAR-830/AP, CWE, Ardmore, PA), which controlled the inspiration rate, inspiration time, and peak inspiratory pressure and 2) a solenoid valve (L12BA452, Numatics, Highland, MI), which was used to switch between sources of 100% O₂ and an 80% Xe-20% O₂ mixture. When switching from O₂ to the Xe-O₂ mixture, the peak inspiratory pressure indicated by the ventilator did not change. When the Xe-O₂ mixture was not being supplied to the animal and during Xe-O₂ exhalation, it was vented to the room, thereby preventing pressure buildup in the gas supply line. The ventilator’s inspiratory, expiratory, and pressure-sensing ports were connected to the endotracheal catheter via a three-input Y-connector. The valved flowmeter in the ventilator was used to control the flow rate of O₂ and two external valved flowmeters (32460-40, Cole-Parmer Canada, Anjou, Canada) were used to control the flow rates of Xe and O₂ for the gas mixture from pressurized cylinders. The scales on the external flowmeters were independently calibrated by measuring the volume of the intended gas passing through the flowmeter into an inverted, water-filled graduated cylinder for each major division of the scales. The ventilator was set to a respiratory rate (RR) of 60 breaths/min and an inspiratory time of 500 ms. No positive end-expiratory pressure was applied. The gas flow rates and the inspiratory time determined the tidal volume and were adjusted such that the peak inspiratory pressure (PIP) was \(~8\) cmH₂O. O₂ (100%) was used to maintain a normal O₂ saturation of at least 96% because a RR of 60 breaths/min is lower than the normal respiratory rate of rats and was found to decrease.
blood O₂ saturation below acceptable levels (<93%) in this study. A RR of one breath per second was chosen to coincide with the frequency of continuous volumetric data acquisition by the CT scanner.

Scanning Protocol

Three scans per animal were performed using a dynamic flat-panel volumetric preclinical micro-CT scanner (eXplore Ultra, GE Healthcare, London, Canada; Ref. 32). Forty volumes (80 slices each) were acquired in an exposure time of 40 s at 80 kVp and 60 mA, with the scanner acquiring a whole lung volume once per second. Each 80-slice volume covered 3.6 cm in the axial direction, with a slice thickness of 0.45 mm. The center 512 × 512 pixels were reconstructed using an in-plane pixel size of 0.15 mm × 0.15 mm. The nominal voxel volume was therefore 0.010 mm³. The entrance dose given to the animal during each 40-s scan was 27 cGy. The rats breathed 100% O₂ for at least 2 min before the scan; as soon as the scan started, breathing was switched to the 80% Xe-20% O₂ mixture. The first five to eight volumes were not enhanced by Xe because of the dead space in the system; these volumes provided baseline measurements of CT number. The tidal volumes of the baseline (O₂) and tracer (Xe-O₂) gases may be slightly different because when gases of different density and viscosity are flowing, the pressure driving the flow of the Xe mixture into the same resistance at the same flow rate will be higher. However, the tidal volume for xenon is constant. Each volume was obtained in synchrony with the animal breathing over an entire respiratory cycle; there was no requirement to suspend respiration during the scan. The voxel intensities in each volume were calibrated to HU by linear regression postscanning using air and a sample of water deliberately introduced within the field of view.

Data Analysis

Identification of lung parenchyma and trachea lumen. A volume of interest (VOI) containing both lungs, but excluding the major airways, was generated by seeded region growing (MicroView version 2.2.a5, GE Healthcare, London, Canada) of the second baseline volume of each scan, using thresholds set to exclude voxels with >82% or <23% air space. These thresholds were determined by applying seeded region growing with a range of thresholds to a series of images and maximizing the volume while preventing region growth beyond the lung boundaries. The mean baseline CT number was −912 HU for trachea lumen and 58 HU for the heart, yielding typical thresholds of −737 and −165 HU. These criteria effectively excluded the trachea and chest wall from the VOI. The second baseline volume was used instead of the first to avoid any possible misregistration that might occur from manually switching the ventilator from pure O₂ to the Xe-O₂ mixture. The second baseline lung VOI was used to construct a mask that was applied to each of the 40 volumes in that scan; calculating the mean value within the VOI yielded values for ΔHU/VOI.

A second VOI, containing only the major airways, was generated by setting the software threshold to accept only voxels with >82% air space. Equation 2 requires the CT number of the inhaled gas, ΔHUtrachea. The airway VOI of the baseline image was applied to the baseline and steady-state images to obtain the CT number of the O₂ and Xe-O₂ mixture in the airways, respectively. To determine the Xe rise time, the CT number of the inspired gas from the supply tube of the ventilator was measured, which has the same rise time as ΔHUtrachea. The signal from the trachea lumen is not representative of the transient value of the inspired gas because the signal measured there is an average of the inspired and expired gas. The rise time of the CT number of the supply tube gas was found to be two breath periods. From the baseline and steady-state CT numbers and the rise time, the absolute value and shape of ΔHUtrachea was approximated by fitting to a ramp function, which is more accurate than assuming a square wave input.

Following Kreck et al. (23), alveolar lung fraction was calculated from

\[ f_A = \frac{H_{U0} - H_{U_{heart}}}{H_{U_{trachea}} - H_{U_{heart}}} \]  

where \( H_{U0} \) was defined as the mean lung CT number, \( H_{U_{heart}} \) as the heart CT number, and \( H_{U_{trachea}} \) as the CT number of the air in the major airways, all from the baseline image. The heart was chosen to be representative of non-Xe-enhanced tissue and blood.

Parameter matching. Using the relationship described in Eq. 2, values for total V in units of milliliters per second were determined using the Levenberg-Marquardt least-squares fitting algorithm (as implemented by Scilab version 4.0, INRIA ENPC) that best fit the measured lung VOI, ΔHU/VOI, and calculated trachea lumen CT numbers, ΔHUtrachea, over the period in which the Xe-O₂ mixture was perfused. Following Kreck et al. (23), Xe partition coefficients of \( k_{A_{blood/gas}} = 0.10 \) and \( k_{A_{tissue/gas}} = 0.13 \) were used. The 95% confidence interval on \( V \) was calculated by applying the Monte Carlo methods of Simon et al. (37), applying noise to the measured parameters and refitting for \( V \). There was <1% change in the estimated confidence interval limits above 300 trials, thus this value was used. The reduced \( \chi^2 \) was also calculated for all fits as a measure of goodness of fit. The ratio of anatomic dead space to end-expiratory alveolar volume, \( f_D \), from Eq. 2, was taken to be 25% from literature values including dead space, functional residual capacity, and total lung capacity in similar rats (7, 47, 48).

Identification of lung parenchyma for parametric map. Maps of V were also produced for an axial slice immediately superior to the diaphragm and for another axial slice at the midlevel of the heart, where the main branch of the right bronchus enters the lung in the first scan of each animal. The slice superior to the diaphragm afforded the largest axial cross section of lung parenchyma. It has been shown in dogs that there is a significant difference in gradient between the base and apex levels (28), but the slice at the midheart level was chosen because it has more cross sectional area than that at the apex. Voxels in the plane of the image were combined into groups of \( 3 \times 3 \times 3 \) and their intensities averaged to yield larger, isotropic voxels (0.45 mm × 0.45 mm × 0.45 mm). The aggregated voxels of the first baseline image were compared with those in all subsequent baseline images and voxels were excluded if the difference was greater than ±100 HU. This gross filtering removed misregistered voxels at the edges of the lungs. Averaged voxels were also ignored if ≥50% of the original voxels had intensities outside of the air/lung and air/tissue thresholds used to form the previously discussed whole lung VOIs. The same Levenberg-Marquardt fitting algorithm as described above was em-
ployed for each group of averaged voxels over the course of 40 breathing cycles. V˙ calculation was not performed if any of the averaged voxels from a set of 40 breaths had been previously discarded to minimize the effect of noise. Fitted values were discarded if the reduced $\chi^2$ value of the fit exceeded five or the calculated V˙ exceeded the V˙ value calculated for the trachea lumen, which was taken to be the maximum possible. The maps were each analyzed for evidence of a vertical (gravitational) gradient in V˙.

**Statistical analysis.** The precision of whole lung VT (ventilation multiplied by the breath period, which should be equivalent to tidal volume for whole lung analysis and equivalent to regional ventilation for voxel-by-voxel analysis) measurements was determined by calculating the per animal and mean coefficients of variation (CV). A repeated measures one-way ANOVA with Tukey’s multiple comparison post test (GraphPad Prism version 4.0a for Macintosh, GraphPad Software, San Diego, CA) was applied to the whole lung VT measurements over the three scans per animal to test for significant differences between scans. Accuracy was determined by the comparison of whole lung VT measurements with the tidal volume setting of the mechanical ventilator by linear regression and a paired t-test. Paired t-tests were also employed to verify whether significant differences existed between the slopes of any ventilation gradients found in the ventilation maps at the diaphragm and heart levels and between VT values when Xe uptake by blood was included or neglected from the model.

**Effect of noise on V˙.** Simulated whole lung CT numbers were generated using Eq. 2 with typical values for HU₀, HŪheart, HŪtrachea, ΔHU₂, and V. Normally distributed noise, which mimics the noise in a CT image, similar to the standard deviation of the whole lung and major airway baseline CT numbers was added to the input and the effect on V˙ was explored by fitting the noisy parameters to the model (Scilab version 4.0).

**RESULTS**

The use of an 80% Xe-20% O₂ mixture allows for a 200 HU increase in the trachea from a baseline value of −910 HU. Within the lung parenchyma, which has a typical alveolar fraction of 55%, the typical rise is 120 HU (110 HU from the alveolar fraction and 10 HU from the tissue fraction) from a baseline value of −470 HU. These were readily measurable above the airway VOI voxel noise (SD of baseline CT numbers) of ±10–20 HU and whole lung VOI voxel noise of ±6 HU.

Continuous imaging, in synchrony with the breathing cycle, provided consecutive time resolved images with few registration artifacts. Figure 2 demonstrates the inhalation of Xe during an entire scan in one of the rats: the volume images are represented as projections in the coronal direction. The first panel is a minimum intensity projection of the baseline image. The baseline image was subtracted from each subsequent image and the remaining panels in Fig. 2 are average intensity projections of these subtracted volumes, demonstrating the inhalation of Xe during the first 16 s of the scan. There is some minor misregistration due to the movement of the diaphragm at the bottom of the images. The enhancement is presented

![Fig. 2. Top left frame shows a minimum intensity projection of an oxygen-filled rat lung. Subsequent frames show evolution of xenon with baseline image subtracted at 1-s intervals. The field of view for each frame has been cropped for presentation purposes and is 36.5 mm × 36.5 mm.](http://jap.physiology.org/Downloaded from http://jap.physiology.org/ by 10.220.33.4 on May 30, 2017)
quantitatively in Fig. 3A, which shows the rise in average lung CT number for three consecutive scans on one animal. Similarly, Figs. 3, B and C, show the rise in the gas supply line and major airway CT numbers, respectively. Figure 3C also shows a ramp function with a two-breath rise time overlaid on top of the measured airway CT numbers; this ramp function was used as the major airway enhancement, ΔHU^1^, in the model.

Whole lung VOI data were supplied to the model, which returned whole lung values for ventilation, V. The whole lung ventilation value multiplied by the breathing period, T, should be equivalent to the tidal volume. Table 1 lists the VT values for each scan on each animal and the CVs for the VT values. The mean CV of the VT measurements was 24%. The method of Glüer et al. (13) indicates that the upper and lower 90% confidence limits are 50% of the estimate of the precision error of the mean (i.e., 12–36% for the mean CV of the VT measurements). Repeated measures one-way ANOVA reported that the mean VT values from the three scans were not significantly different from each other (P = 0.13). Tukey’s multiple comparison post test also reported that pairs of scans also did not have significantly different means (P > 0.05).

Linear regression analysis of VT calculated from scan 1 vs. the tidal volume set on the ventilator (Fig. 4) yielded a best-fit slope of 1.12 ± 0.11 (slope ± SE) and a y-intercept of -1.56 ± 0.42 ml (y-intercept ± SE) with 95% confidence intervals of 0.83 to 1.40 and -2.6 to -0.5 ml, respectively, and a correlation coefficient of 0.98 (P < 0.0001). This shows that calculated VT from scan 1 is directly proportional to the tidal volume. However, linear regression of scans 2 and 3 yielded best-fit slopes of -0.03 ± 0.621 and -0.34 ± 0.28, respectively, indicating a decrease in accuracy with repeated scanning (to be discussed later).

When Xe uptake was removed from the model, the mean VT value decreased 25% from 2.57 to 1.93 ml. Linear regression analysis of VT without Xe uptake calculated from scan 1 vs. the tidal volume set on the ventilator yielded a best-fit slope of 0.73 ± 0.07 (slope ± SE) and a y-intercept of -0.77 ± 0.28 ml (y-intercept ± SE) with 95% confidence intervals of 0.54 to 0.92 and -1.5 to 0.0 ml, respectively, and a correlation coefficient of 0.98 (P < 0.0001). This shows that the uptake of Xe by blood is a nonnegligible aspect of the model.

A baseline and Xe-enhanced image of an isotropic-voxel size slice from immediately superior to the diaphragm are shown in Fig. 5. A and B, respectively. The ventilation map generated through fitting Eq. 2 to data from the entire scan is shown in Fig. 5C. The criterion that includes only voxels where the baseline does not deviate >100 HU from the second baseline image drops 20% of the lung parenchyma voxels. Figure 6 is a plot of mean regional V values as a function of vertical height measured from the dorsal surface of the lungs from supine animals (the images of the 7th animal had excessive motion artifact near the diaphragm and were not included). Ventilation maps were also produced for a slice from all seven animals at the midlevel of the heart. V displayed a vertical (gravitational) gradient of (-3.9 ± 1.8) × 10^-6 ml·s^-1·cm^-1 for slices immediately superior to the diaphragm and (-6.0 ± 2.4) × 10^-6 ml·s^-1·cm^-1 for slices at the midlevel of the heart (mean ± SD). Paired t-tests showed that the gradients at these two locations were significantly different (P = 0.0096).

Effect of noise on V. Simulated whole lung CT numbers were generated using Eq. 2, with the following typical whole lung parameters: HU_0 = -471 HU, HU_heart = 58 HU, HU_trachea = -912 HU, steady-state trachea CT number = -712 HU, V = 4 ml/s, and T = 1 s. When normally distributed noise similar to the standard deviation of the baseline whole lung CT numbers (±6 HU) was added to the whole lung CT numbers, the fitted V for 1,000 trials was 4.01 ± 0.29 ml (mean ± SD). Similarly, when Gaussian noise (±21 HU) was added to the major airway baseline CT numbers, the fitted V for 1,000 trials was 4.08 ± 0.51 ml. When both sets of noise were added to their respective whole lung and major airway CT numbers, the fitted V for 1,000 trials was 4.12 ± 0.59 ml.
DISCUSSION

Advantages of Continuous Xe-CT

Continuous CT image acquisition using Xe contrast enhancement accurately measures ventilation in the whole lung of rats in vivo with low voxel noise. To our knowledge, micro-CT has not yet been used for dynamic functional lung imaging in the rat with inhaled xenon gas. The lack of interscan delay allows the monitoring of Xe enhancement in a single scan without the requirement for breath holding and repeat scans. Direct measurements of the gas in the trachea lumen and ventilator supply tube allowed determination of the average signal enhancement introduced over the duration of each breath of Xe, allowing the model to account for any changes in delivery of Xe to the lung, which may have been introduced by the ventilation system. This is equivalent to the arterial input function used for quantitative blood flow measurements with intravenous contrast agents (24).

Comparison to Other Work

Our work measured absolute regional ventilation in real time using continuous Xe-enhanced CT in the whole rat lung with 0.45 mm isotropic resolution. This work builds on similar measurements done on partial sheep lungs with end-inspiratory breath holds with 2.5 mm and 10 mm isotropic resolution (23, 31). The use of continuous scanning negates the need for breath holds that do not occur during normal respiration at the cost of increased blurring. The lack of interscan delay in our experiment leads to decreased anesthesia time for the subject. Experiments using specialized equipment include dynamic Xe concentration profile and regional specific ventilation measurement during end-expiratory breath holds using synchrotron CT in rabbits with 0.35 mm in-plane resolution in whole lung anterior/posterior projections (2, 30) and using electron-beam cardiac-gated CT in pigs with 0.39 mm\(^3\) resolution (41). Normalized regional ventilation has also been measured at end-expiratory breath hold using PET in a single

Table 1. Ventilator tidal volume settings and calculated values for VT

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Mass, g</th>
<th>Tidal Volume, ml</th>
<th>VT (95% CI, ml)</th>
<th>CV of VT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan 1</td>
<td>Scan 2</td>
<td>Scan 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>294</td>
<td>3.47±0.21</td>
<td>2.23 (1.99–2.37)</td>
<td>11%</td>
</tr>
<tr>
<td>2</td>
<td>377</td>
<td>4.17±0.21</td>
<td>2.93 (2.55–3.20)</td>
<td>18%</td>
</tr>
<tr>
<td>3</td>
<td>356</td>
<td>3.54±0.21</td>
<td>2.38 (2.15–2.59)</td>
<td>6%</td>
</tr>
<tr>
<td>4</td>
<td>446</td>
<td>4.50±0.21</td>
<td>3.34 (2.87–3.70)</td>
<td>26%</td>
</tr>
<tr>
<td>5</td>
<td>427</td>
<td>4.50±0.21</td>
<td>3.73 (3.03–4.06)</td>
<td>30%</td>
</tr>
<tr>
<td>6</td>
<td>292</td>
<td>2.92±0.21</td>
<td>1.70 (1.52–1.80)</td>
<td>44%</td>
</tr>
<tr>
<td>7</td>
<td>303</td>
<td>3.30±0.21</td>
<td>2.30 (2.11–2.47)</td>
<td>24%</td>
</tr>
</tbody>
</table>

The error for the tidal volume is derived from the reading error of the valved flowmeters and is representative of the error in the ventilation system. VT represents whole lung ventilation over one breath.

Fig. 4. Calculated VT vs. tidal volume for scan 1 of all animals. The solid line represents the best fit by linear regression.

Fig. 5. Baseline (A) and Xe-enhanced images (B) and a regional V map of an axial slice immediately superior to the diaphragm (C). The width and height of each subfigure are 31.9 mm and 23.5 mm, respectively. Voids representing the heart (h) and vena cava (vc) are also labeled.
axial slice in sheep lungs with 2 mm × 2 mm × 5 mm resolution (18) and using CT in dogs at four axial lung locations with 0.43 mm × 0.43 mm × 10 mm resolution (28). However, our technique allows for comparatively high-resolution isotropic coverage of the entire rat lung.

Regional normalized lung ventilation has also been measured using hyperpolarized 3He MR imaging in guinea pigs (5), rats (27, 40), and an asthmatic mouse (17). Unfortunately, 3He is one order of magnitude more expensive than natural abundance Xe. Because the signal intensity of the MR image depends on the polarization of the gas at the time of measurement, it is difficult to measure the mass of tracer gas in the lungs to get an absolute value of ventilation; therefore only relative ventilation can be measured. Absolute ventilation values are easier to extract from CT images because the change in signal strength is linearly proportional to the mass of tracer gas, whereas absolute MR ventilation measurement requires depolarization and relaxation effects to be taken into account. CT methods can therefore be used to validate and refine hyperpolarized MR imaging of regional ventilation.

Isogravitational Differences

In our work, absolute regional ventilation was measured and a gravitational gradient was evident with more dependent regions of the lung (those lower in height as measured from the CT scanner bed) having greater ventilation. A comparison between the right and left sides of the images revealed no isogravitational differences. Increased ventilation in the dependent lung regions was previously reported (28) and is a consequence of the less negative intrapleural pressure and resulting decreased transpulmonary pressure gradient in this area. This results in alveoli in dependent regions being on the steeper portion of the pressure-volume curve, therefore being more compliant. Regional ventilation data were normalized to total lung ventilation to give a ventilation index, which is used to allow comparison between animals. Normalization of our slope of ventilation vs. height to ventilation index vs. height yields values of −0.048 ± 0.022 cm⁻¹ for axial slices immediately superior to the diaphragm and −0.056 ± 0.056 cm⁻¹ for axial slices at the midheart level, similar to reported values (27, 28).

Potential Limitations

Decreasing correlation over multiple scans. The mean CV of our VT measurements, 24%, is favorable to that of Kreck et al. and indicates that continuous Xe-enhanced CT measurement of rat ventilation is reasonably robust. The accuracy and precision of the measurement was confounded by the fact that repeated measurement in an animal has the possibility to change the physiological state of that animal; thus our mean CV is a conservative value. The saturation of body tissues with Xe is unlikely to cause this decrease in measured ventilation over time because saturation would lead to higher baseline Xe levels in blood and cause more Xe to remain within the lungs and increase the measured ventilation. The gradual decrease of calculated whole lung ventilation vs. the tidal volume set on the ventilator over the course of three scans per animal could be due to atelectasis (alveolar collapse) of the lungs due to breathing of pure O₂ (9). Deep sighs of the animal, which can mitigate the effect (33), were not given between scans in this study. Other possibilities include a change in lung performance due to continuous breathing of dry gas, similar to exercise-induced asthma (29). They could also be due to leakage between the trachea and endotracheal tube, which tend to increase with time (i.e., multiple ventilation experiments).

Motion-induced blurring. Images in this work were acquired during continuous acquisition. Movement during CT acquisition induces blurring into the image, which lowers effective resolution. The blurring potentially restricts the ability to calculate a precise boundary for whole lung VOIs and the edges of parametric maps. This may significantly reduce the number of usable voxels in animals smaller than rats. However, the blurring is the same from image to image. Because the ventilation time is equal to the scan time, there is no global misregistration from image to image unless the animal gasps or shifts position or if the trajectory of the chest wall is not identical for every breath.
V/Q ratio. In Eq. 2, the pulmonary blood perfusion $Q$ from Eq. 1 was replaced with ventilation $V$ to simplify the formula since the ratio of ventilation is unity in supine, normoxic rats (1). However, Kreck et al. found empirically that in supine sheep, $V/Q$ was 1.34. When $Q$ from Eq. 1 was substituted with $V/1.34$ and the resulting equation was used to fit the scan 1 data, linear regression analysis of $V$ vs. the tidal volume set on the ventilator yielded a best-fit slope of $1.00 \pm 0.09$ (slope $\pm$ SE) and a y-intercept of $-1.31 \pm 0.35$ ml (y-intercept $\pm$ SE) with 95% confidence intervals of 0.76 to 1.23 and $-2.2 \text{ to } -0.41$ ml. This change in the slope toward a value of unity may suggest that the $V/Q$ ratio is closer to 1.34 than unity.

Future Improvements

Leakage of inspired gas from lungs. The y-intercept of the linear regression of whole lung $VT$ vs. expected tidal volume for scan 1 was $-1.56 \pm 0.42$ ml. This systematic error may arise from leakage of Xe gas from the trachea around the endotracheal tube. The calculated ventilation volume of the lungs $VT$ would have a negative offset from the tidal volume setting on the ventilator if a constant fraction of the inspired gas were to escape before reaching the lungs. After insertion of the endotracheal tube, the mouth of the animal was bound tightly with 1/8-in. braided polyester umbilical tape (Hallowell EMC, Pittsfield, MA). Subsequent leak testing of this configuration using a water manometer during a breath hold maneuver identified a leak. Additional testing showed that three loops on silk suture tied around the trachea after intubation is sufficient to eliminate this leak with tightening of these loops after several hours.

The current experimental setup utilizes a set of flowmeters to ensure each of the O2 and Xe-O2 breaths is identical to the after several hours. Additional testing showed that three loops on silk suture tied around the trachea after intubation is sufficient to eliminate this leak with tightening of these loops after several hours.

Potential Applications

The technique presented in this paper is ideally suited for measuring changes in regional ventilation that result from heterogeneous lung disease in rat models. Such models include anhydride-induced asthma (4), occupational asthma (21), alerger-induced airway hyperresponsiveness (3, 6), cigarette smoke-induced COPD (46), sulfur dioxide-induced chronic bronchitis (8), and autoimmune emphysemia (42). Longitudinal studies are possible to track any gradual impairment and restoration of lung function associated with these conditions and potential treatments. The real-time aspect of this technique allows for monitoring of fast-acting disease models, such as a methacholine challenge test, which causes temporary (<1 min) bronchoconstriction (14, 26, 34).


