Effect of tidal volume on distribution of ventilation assessed by synchrotron radiation CT in rabbit


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The influence of tidal volume (Vt) on the overall nonuniformity of ventilation has been extensively studied (9, 18, 24, 38). However, most of the available data are based on analyses of multiple-breath washout of inert tracer gases, which do not provide information on the anatomic site of ventilation nonuniformity (36). Larger Vt may allow recruiting less ventilated lung zones. However, high Vt may produce alveolar overdistention and ventilator-induced lung injury (5). On the other hand, lung damage may also arise at the lower end of the lung’s pressure-volume range because of repeated collapse and reopening of alveoli within the respiratory cycle (10). Quantitative determination of ventilation distributions with a high spatial resolution would therefore be useful in the determination of optimal ventilation parameters in experimental models of lung disease.

Clinically, ventilation imaging has been performed by use of radionuclide scintigraphic techniques, including radioactive gases, radiolabeled aerosols, and positron emission tomography with positron-labeled gases (20), but these methods have been limited by their poor spatial and temporal resolution and nonquantitative nature. Laser-polarized 129Xe and 3He gases were introduced as enhanced sources of signal for ventilation scanning by MRI, and 3He imaging has been applied for functional studies of lung ventilation (2, 8, 11, 30, 35). Another method for the assessment of regional ventilation by MRI is the use of O2 for signal enhancement (15). The signal from paramagnetic O2 is far inferior to that from spin-polarized 3He, but the method is less complex and provides clinically useful information (29). At the same time, there has been a growing interest in computed tomography (CT) imaging techniques as a means of measuring regional lung ventilation, particularly using stable Xe gas for contrast enhancement (22, 23, 33, 42, 44). Xe-enhanced CT is a method for noninvasive measurement of regional pulmonary ventilation, determined from the washin and washout rates of nonradioactive Xe gas, as measured in serial CT scans. This method allows imaging of the lung with higher spatial resolution compared with other techniques. Recently, Xe-enhanced CT imaging has been used to study the effect of body position on regional ventilation distribution in anesthetized dog and pig (33, 44) with a spatial resolution on the order of a few millimeters. With the use of this technique, the entire lung can be imaged by spiral CT acquisition.

Ideally, determination of ventilation distributions should be based on a method in which a signal proportional to the concentration of the inhaled gas is imaged with the resolution of the most advanced CT techniques. Recently, we introduced a respiration-gated CT technique that fulfills these requirements (6). This technique uses X-rays from a synchrotron radiation (SR) source, which, as opposed to standard X-ray sources, allows selecting monochromatic X-ray beams from the full spectrum while conserving enough intensity for imaging. Stable Xe gas is used as the contrast agent, and when two images are acquired simultaneously using two energies that bracket the K-absorption edge of Xe, their subtraction yields the distribution of the Xe gas. One advantage of this so-called K-edge subtraction (KES) method over CT techniques using standard X-ray sources is that it allows both visualizing stable Xe gas used as a tracer and directly quantifying its absolute...
concentration at any given point of a lung CT image. Another advantage is that contrast resolution is markedly improved by avoiding nonselective contrast and beam hardening, which occurs with conventional X-ray sources (12). Currently, the use of SR technology has allowed imaging with microscopic spatial resolutions (1, 41). Short image-acquisition time makes it possible to evaluate regional changes in ventilation during mechanical ventilation. Validation of this technique has been performed previously (16). Imaging was performed with two monochromatic beams with slightly different energies. Beams were produced from the continuous SR spectrum by using a bent silicon crystal. A liquid nitrogen cooled high-purity germanium (Ge) dual line detector (432 elements per line, Erisys Measuring Systems, Lingolsheim, France) was used for simultaneous X-ray transmission measurements of the two beams. The beams were focused and crossed at the animal holder position, beyond which they diverged and were recorded by the dual-line Ge detector. The horizontal pixel size of the detector was 0.35 mm, and the height of the beams was 0.7 mm at the animal position. Because the incident energy beam was stationary, the animal had to be moved vertically or rotated about an axis perpendicular to the plane defined by the beams. A high-precision computer-controlled stage with seven degrees of freedom (Spretec, Grenoble, France) allowed both positioning of the animal and the motions during image acquisition. For acquisition of anteroposterior radiographs, the rabbit was moved vertically through the beams at a constant speed of 25 cm/s. The detector was read out at 1.4-ms intervals, which makes the vertical pixel size equal to the horizontal one, 0.35 mm. For tomographic acquisition, the animal was rotated about an axis perpendicular to the plane of the incident beams. Rotation was performed by the precise computer-controlled stage with an angular velocity of 180°/s. A full rotation cycle with a 40° acceleration ramp took 2.2 s, after which the animal was rotated back to the start position. The essential differences between SRCT and conventional CT are the narrow angular width of the SR fan due to a distance to the source of 150 m and very limited scattering noise because of the large animal-to-detector distance of 6 m. For tomographic images, 720 projections were acquired with both beams simultaneously, over a 360° rotation in 2 s and with a readout interval of 2.78 ms. All images were reconstructed with filtered backprojection algorithm, using the Matlab programming package (MathWorks).

**METHODS**

**Animal preparation.** Animal care and procedures of the experiment were in accordance with the *Guiding Principles in the Care and Use of Animals* provided by the American Physiological Society and approved by the local institutional authorities. The experiments were performed on male New Zealand rabbits (average weight: 2.0 kg, Elevage Scientifique des Dombes, Chatillon sur Chalaronne, France). A 22-gauge catheter was inserted in the marginal ear vein (Cathlon IV, Ethicon, Rome, Italy). Anesthesia was induced by intravenous injection of thiopental sodium (25 mg/kg iv; Nedsol, Rhone-Poulenc-Rohrer, Paris, France), after local anesthesia using 5% topical lidocaine (Emla, Astra-Zeneca, Paris, France). The animal was tracheostomized and an endotracheal tube (no. 3, Portex, Berck sur Mer, France) was inserted and secured with a gas-tight seal. A 22-G catheter was inserted in the marginal ear vein (Cathlon IV, Ethicon, Rome, Italy). Anesthesia was induced by intravenous injection of thiopental sodium (25 mg/kg iv; Nedsol, Rhone-Poulenc-Rohrer, Paris, France), after local anesthesia using 5% topical lidocaine (Emla, Astra-Zeneca, Paris, France). The animal was slowly placed in the vertical position on a rotation stage with its ventral side toward the Spretec motor (Spretec, Grenoble, France) allowed both positioning of the animal and the motions during image acquisition. For acquisition of anteroposterior radiographs, the rabbit was moved vertically through the beams at a constant speed of 25 cm/s. The detector was read out at 1.4-ms intervals, which makes the vertical pixel size equal to the horizontal one, 0.35 mm. For tomographic acquisition, the animal was rotated about an axis perpendicular to the plane of the incident beams. Rotation was performed by the precise computer-controlled stage with an angular velocity of 180°/s. A full rotation cycle with a 40° acceleration ramp took 2.2 s, after which the animal was rotated back to the start position. The essential differences between SRCT and conventional CT are the narrow angular width of the SR fan due to a distance to the source of 150 m and very limited scattering noise because of the large animal-to-detector distance of 6 m. For tomographic images, 720 projections were acquired with both beams simultaneously, over a 360° rotation in 2 s and with a readout interval of 2.78 ms. All images were reconstructed with filtered backprojection algorithm, using the Matlab programming package (MathWorks).

**KEE.** A detailed description of this method has been published elsewhere (6). In this technique, two simultaneous transmission im-

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**Fig. 1.** Experimental setup. A single solenoid valve (v) is used for mechanical ventilation. The rabbit inspires air during resting periods and Xe-O2 mixture during imaging. Three solenoid valves are used to switch from 1 inspired gas to another. Gas flows are controlled using mass flowmeter controllers (r). All valves and the animal rotation-translation stage are synchronized and remote controlled by the computer system. Ptr, tracheal pressure.
ages are acquired at two slightly different energies, above and below the K-edge of a contrast element such as Xe. The mass attenuation coefficient (μ/ρ) of Xe increases by a factor of 5.4 when the K-edge at 34.56 keV is crossed, whereas the change in the attenuation coefficients of cortical bone and lung tissue are negligible. Subtraction of the high-energy image from the low-energy image on a logarithmic scale eliminates the contributions of bone and tissue to absorption and yields the distribution of the contrast agent. The absolute Xe concentration can be directly calculated for tomographic images as a part of the reconstruction routine. There is no misalignment of the two images, because they are recorded simultaneously, so that motion artifacts are eliminated. Figure 2 presents a transmission image and the KES subtraction image from Xe-filled lungs.

Experimental protocol. Animal position was checked from an anteroposterior thoracic reference image. On the basis of the reference image, three different levels were selected at the fourth, sixth, and eighth thoracic vertebral levels for tomographic image sequences. To limit misregistration errors in between images in a sequence, all CT images were obtained at the same level of lung inflation, at functional residual capacity (33). Although cardiac gating was not used, very little blur artifact was observed in the lungs and a result of cardiac motion on the sequential CT images was negligible, as reported previously (6). Measurements of regional ventilation were based on the dynamics of Xe washin. The imaging sequence started by switching of the inhaled gas from air to Xe (60–70%) and O2 (40–30%) mixture, and a sequence of tomograms was recorded at each level. After a preset number of respiratory cycles, ventilation was paused at end expiration for 3 s, during which a tomographic image was acquired. The imaging cycle was repeated 15 times, and at the end an equilibrium concentration of Xe was reached in the lungs. The duration of an entire image sequence acquisition was 2.5 min. At the end of the imaging sequence, inhaled gas was switched back to air. Imaging sequence and a washin image series are presented in Fig. 3.

The imaging sequence was repeated at all three levels using three or four different VT values. The preset number of respiratory cycles and the respiratory rate were decreased with the increasing VT to maintain the absolute minute ventilation (V̇) as constant as possible. The values of target and measured VT, the preset number of respiratory cycles, overall V̇, P02, and the relative change in expiratory P02 due to the imaging sequence are summarized in Table 1. A small increase in V̇ was observed with increasing VT. This may have been due to the fact that the respiratory system was not volume controlled and that the obtained VT results were slightly different from target settings (Table 1). As a result of this difference between target and measured VT, overall V̇ during imaging dropped by 10.2, 19.7, and 13.6%, respectively, with VT 2, VT 3, and VT 4, compared with VT 1. Minute ventilation was reduced on the average by 43% during the imaging sequence, i.e., 1.4 min at a time. The percent change in expiratory P02 between the immediate pre- and postimaging periods, produced by the relative hypoventilation during the imaging sequence, was not significantly different among the different VT values. In addition, there was some variation in V̇ between the rabbits at the same VT level, which were corrected for by using a normalization term (V̇/V̇m), where V̇ is the minute ventilation and V̇m is the average value for all rabbits in the same VT group. In this study, it was decided not to impose PEEP, to observe the effects of VT alone on lung recruitment; however, a small spontaneous PEEP of 1.0 ± 0.7 cmH2O was observed, which may have been due to the resistance of the expiratory branch of the ventilation circuit.

Image analysis. Image processing was performed using the Matlab programming package (MathWorks). Tomographic images were reconstructed using the filtered backprojection algorithm. Lung tissue was selected within the CT images, by segmentation-based Xe concentration thresholds. In this fashion, major blood vessels were excluded from the analyses. As previously shown, Xe concentration for a given region of interest (ROI) increases in the washin sequence after monoexponential dynamics (33, 42, 44). The local time constant was calculated by fitting the following function to the absolute Xe concentration [c(t) as a function of time (t)]

\[ c(t) = c_A[1 - e^{-\tau t}]. \]

Here cA is the asymptotic concentration and τ is the time constant.

The arrival time t0 includes the transfer time from the gas valve to the trachea and the transfer time from the trachea to the entrance of the acini in the ROI. Because of the structure of the bronchial tree the arrival time varies, being later in the distal parts of the lung. The time scale is directly proportional to the number of breaths, because the apnea pauses during imaging were subtracted. As an example, a fitted c(t) used for parameter estimation is shown in Fig. 4. For comparison, the density change due to Xe, in a single-energy transmission image is given in Hounsfield units (HU). The X-ray energy above Xe K-edge is chosen to maximize the effects of absorption due to Xe.

The time constant τ can be calculated for every point of the CT image and is equal to the inverse of local specific ventilation (sV̇; ventilation normalized to the regional lung air content) (33, 42, 44). To minimize the effects of statistical truncations and image registration errors, images were smoothed with a 5 × 5 pixel moving average window. Exponential fit was performed with multidimensional unconstrained nonlinear minimization (Nelder-Mead), and the goodness of fit was assessed by examination of the normalized summed squared residuals (SSR).

In the above fashion, a map of τ values was calculated. The instrumental resolution is 0.35 × 0.35 mm, but for the present purposes smoothed maps of 1.75 × 1.75 mm resolution reveal sufficient detail and eliminate the effects of small differences (maximum of two pixels) in the animal position between successive images.

**Fig. 2.** A: single-energy transmission image. Plastic parts of the animal holder and tubing carrying Xe and O2 to the animal are visible laterally. B: K-edge subtraction (KES) image: only Xe-carrying structures (the bronchi and the lung parenchyma) and the tubing lumen are visible. Reference bar = 1 cm. The coefficient of variation was 10.0% for the simple transmission image obtained at the energy above Xe K-edge and 7.0% for the KES image. Variability was calculated in a lung tissue “mask” defined by using the KES computed tomography image, by segmentation-based Xe concentration thresholds, without noise reduction filtering. HU, Hounsfield units.
The frequency distribution of the \( \tau \) values in a map is skewed with a tail toward higher values. The histograms are best described by the log-normal distribution. This functional form is of fundamental importance for correct description of many statistical distributions met in nature (32). The advantage of presenting the data in logarithmic scale is that the standard tools for characterizing Gaussian distributions can be used. Log-normal distributions are characterized by their mean values (\( \tau_0 \)) and their multiplicative standard deviations. However, to perform comparisons with the more familiar distributions in a linear scale, the peak values of the \( \tau \) histogram (\( \tau_{\text{max}} \)) and the widths at the midheight (\( \Delta \tau_w \)) were also calculated from the fitted log-normal distribution functions. These parameters are used to compare ventilation between lung slices, or between VT groups for the same lung level. The inverse value of \( \tau_0 \) is called the average specific ventilation (sV\( \dot{O}_2 \)). For a comparison of regional ventilation between dorsal and ventral regions at one level in the lungs, the maps of sV were divided into two equal ventral and dorsal areas, and the mean sV\( \dot{O}_2 \) was determined separately for each of the two subsections.

**Statistics.** The exponential fit and the log-normal fit were performed by using multidimensional unconstrained nonlinear minimization (Nelder-Mead) by examination of the SSR. The average goodness-of-fit for the log-normal distributions was \( R^2 = 0.95 \pm 0.05 \) (mean \( \pm \) SD). The mean values of the physiological variables and the average specific ventilation are given at each VT with the standard error of the mean (SE). The paired \( t \)-test was used to compare data between the groups, and \( P < 0.05 \) was considered as significant.

**RESULTS**

sV\( \dot{O}_2 \) values are at different VT values at the fourth (apical), sixth (middle), and eighth (basal) dorsal vertebral levels presented in Table 2. Results are means \( \pm \) SE of five rabbits, and they are also shown graphically in Fig. 5. At all three levels, increase in VT produced a significant rise in sV\( \dot{O}_2 \) compared with the baseline VT 1 (see Table 2). In two animals, an additional larger VT (12.7 \( \pm \) 1.5 ml/kg) was used; however, this did not increase sV\( \dot{O}_2 \) further. sV\( \dot{O}_2 \) was smaller in the apical lung level than in the middle and basal lung levels, and the difference was significant with small VT values (VT 1 and VT 2). Figure 6 illustrates a series of representative sV or local \( \tau \) (inverse scale) maps in one rabbit, obtained at the apical, middle, and basal lung levels, at three different VT values. These images demonstrate differences in regional sV, which were most apparent at the basal level of the lungs. At this level, ventilation was distributed preferentially to the dorsal lung region. As VT increased, despite the small drop in overall V\( \dot{O}_2 \), the \( \tau \) of Xe washin became smaller and sV increased in all lung zones. Figure 7 shows frequency distributions of \( \tau \) obtained from the same animal as in Fig. 6. The histogram peak (\( \tau_{\text{max}} \)) shifted toward smaller values, and the distribution became narrower when VT was increased. However, on the relative logarithmic scale, the width of the distribution remained nearly the same.

For a quantitative comparison of regional ventilation between dorsal and ventral regions, the sV maps were divided in two equal areas, ventral and dorsal. These results are presented in Fig. 8. At the basal lung level, sV\( \dot{O}_2 \) was significantly smaller in the ventral area compared with the dorsal area (see Fig. 8). There was no significant difference between dorsal and ventral sV\( \dot{O}_2 \) in the apical and middle lung levels.

With the increase of VT, the absolute scattering in \( \tau \) decreased. As shown in Fig. 7, when VT increased, the \( \tau \) histograms became narrower. The \( \Delta \tau_w \) was calculated as an indicator of scattering in \( \tau \) values. Values are presented in Table 3. Figure 9A represents \( \Delta \tau_w \) as a function of VT. At all slices, \( \Delta \tau_w \) decreased significantly when VT was increased from VT 1 to

**Table 1. Measured and target VT, number of preset RC administered before each image within an imaging sequence, \( \dot{V} \) administered during the imaging sequence, P\( \text{tr} \), and \( \Delta \text{P}_{\text{ECO}} \), between the immediate pre- and postimaging periods**

<table>
<thead>
<tr>
<th>Target VT, ml/kg</th>
<th>Measured VT, ml/kg</th>
<th>RC</th>
<th>( V, \text{ml/min} \cdot \text{kg}^{-1} )</th>
<th>P( \text{tr}, \text{cmH}_2 \text{O} )</th>
<th>( \Delta \text{P}_{\text{ECO}_2} % )</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT 1</td>
<td>4.8</td>
<td>4.9( \pm )0.2</td>
<td>6</td>
<td>294( \pm )13</td>
<td>6.4( \pm )0.5</td>
<td>1.0( \pm )0.5</td>
</tr>
<tr>
<td>VT 2</td>
<td>7.2</td>
<td>6.6( \pm )0.4</td>
<td>4</td>
<td>264( \pm )16</td>
<td>8.1( \pm )0.5</td>
<td>1.3( \pm )0.5</td>
</tr>
<tr>
<td>VT 3</td>
<td>9.6</td>
<td>7.9( \pm )0.4</td>
<td>3</td>
<td>236( \pm )11</td>
<td>10.8( \pm )0.9</td>
<td>1.4( \pm )0.7</td>
</tr>
<tr>
<td>VT 4</td>
<td>14.4</td>
<td>12.7( \pm )na</td>
<td>2</td>
<td>254( \pm )na</td>
<td>15.2( \pm )na</td>
<td>0.8( \pm )na</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n \) = no. of tested animals. VT, tidal volume; RC, respiratory cycles; \( \dot{V} \), minute ventilation; P\( \text{tr} \), tracheal pressure; \( \Delta \text{P}_{\text{ECO}_2} \), percent change in expiratory \( \text{PO}_2 \); na, not applicable.
V T 3, but in the largest tidal volume (V T 4) no further decrease in ΔτV was observed (see Table 3). However, the normalized width of the histogram (ΔτV/τ0) was almost independent of V T, as shown in Fig. 9B.

To obtain a tangible measurement of the accuracy of the specific ventilation, maps of the normalized SSR of the fitted c(t) in 5 × 5 pixel ROIs, were calculated for the basal slice shown in Fig. 6 and presented in Fig. 10. The SSR maps were calculated both in a KES image and in a single energy transmission image in HU. The highest SSR values were found in the proximity of the heart. The mean values over the entire slice were 5.2% (mode: 4.0%) for the KES and 18.7% (mode: 10.0%) for the single-energy image.

**DISCUSSION**

The objective of this study was to test the feasibility of a new X-ray CT imaging technique for the quantitative assessment of regional lung ventilation in upright anesthetized and mechanically ventilated rabbits. We tested the effect of V T on the regional distribution of ventilation and its nonuniformity. The results are discussed below along two main lines: the effects of the body position and anatomic constraints and the origin of the changes in distributions when V T is increased. As concluding remarks, the methodological aspects, possible sources of error and improvements, and applications in physiology and medicine are discussed.

**Regional differences in lung ventilation.** Values of sV measured in this study were similar to those previously reported (44), ranging from 0 to 3.5 min⁻¹ in basal pig lung, although comparison is difficult because of differences in body position and overall lung ventilation between the two studies. We observed both gravitational and isogravimetric differences in ventilation distribution. Regional ventilation increased along a vertical gradient from nondependent to dependent lung zones. In the horizontal plane, a very reproducible ventral-dorsal gradient in regional ventilation was observed at the basal level of the lung. We are not aware of previous reports of such isogravimetric gradients in regional lung ventilation in upright rabbit. Melsom et al. (34) found vertical heterogeneity to be approximately two-thirds the isogravitational heterogeneity in regional ventilation measured with technetium aerosols, by a destructive technique, in lung pieces of −1.5 cm³ in upright goat and sheep. Kreck et al. (31) using a traditional Xe/CT method in sheep, Hoffman (26) using the dynamic spatial reconstructor in dog, as well as Marcucci et al. (33) have reported both vertical and isogravimetric gradients in ventilation. These studies suggest that gravity is not the predominant factor determining regional ventilation in the models studied. Altemeier et al. (3) obtained measurements of regional venti-

**Table 2. sV₀, its change from baseline V T 1, and the paired t-test P values compared with the baseline V T 1**

<table>
<thead>
<tr>
<th></th>
<th>Apical</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sV₀, min⁻¹</td>
<td>ΔsV₀, %</td>
<td>P</td>
<td>sV₀, min⁻¹</td>
<td>ΔsV₀, %</td>
<td>P</td>
</tr>
<tr>
<td>V T 1</td>
<td>2.3±0.5</td>
<td>0</td>
<td>2.6±0.4</td>
<td>0</td>
<td>2.7±0.5</td>
<td>0</td>
</tr>
<tr>
<td>V T 2</td>
<td>2.9±0.3</td>
<td>26</td>
<td>3.6±0.5</td>
<td>36</td>
<td>3.5±0.4</td>
<td>29</td>
</tr>
<tr>
<td>V T 3</td>
<td>4.0±0.8</td>
<td>74</td>
<td>4.3±0.6</td>
<td>65</td>
<td>4.4±0.6</td>
<td>63</td>
</tr>
<tr>
<td>V T 4</td>
<td>4.4±na</td>
<td>89</td>
<td>4.6±na</td>
<td>74</td>
<td>4.8±na</td>
<td>79</td>
</tr>
</tbody>
</table>

Values are means ± SE. sV₀, average specific ventilation; ΔsV₀, change sV₀ from baseline V T 1.
lation with inhaled fluorescent microspheres in pig. From their data, it appears that dorsal caudal lung zones have higher regional ventilation. Also, Hoffman’s study shows larger lung expansion in dorsal caudal lung zones in dog. Both of the above studies were performed in supine and prone animals. Robertson et al. (39), using the fluorescent microsphere technique in prone pig, did not find any significant gravitational gradient in ventilation. Isogravimetric differences in ventilation may be due to regional differences in lung mechanical properties. Close correlations between regional ventilation and nonuniformity in lung compliance and airway resistance have previously been proposed as mechanisms for observed regional differences in ventilation (4, 7). Examination of the raw CT images in our study revealed that the largest lobar airways are dorsally located at the middle and basal levels in the studied animals. This suggests that the path length for air flow from major conducting airways to alveoli is maximal in the ventral regions of the basal lung zones. Chest shape and the interaction between the lung and chest wall, heart, and abdominal content may all cause differences in regional lung expansion (48). The relative displacement of the diaphragm vs. the rib cage in these anesthetized and paralyzed animals may also affect regional ventilation. Inspiration may preferentially induce caudal and basal expansion of the lungs, considering that the diaphragm and intra-abdominal organs are more easily displaced than the less compliant rib cage. With the use of Xe-enhanced CT, it has been observed that regional ventilation increased from apex to base in supine dogs, despite the absence of differences in gravitational force (33). Interestingly, such apex-base differences were reduced in the prone position, suggesting predominantly lung-thorax-abdomen mechanical interaction, rather than mechanical properties of the lung itself, as the mechanism (26, 27, 48).

Effect of VT on regional lung ventilation and its nonuniformity. As VT was increased, regional lung ventilation increased in all three studied lung levels, despite the fact that the overall lung ventilation was not increased. It is well known that by increasing VT, alveolar ventilation is maximized vs. anatomic dead space ventilation. In the present study, this increase in
alveolar ventilation could be quantified, and a relationship between VT and sV̇ was established in dependent and nondependent lung zones, on the basis of the imaging data (Fig. 8).

The sV̇ increased approximately linearly with VT in the range from 5 to 8 ml/kg but seemed to saturate beyond this range in two animals. This larger VT value of 12.7 ml/kg, which was applied in two animals, may have produced an elevation in sV̇ small enough to be countered by the drop in the measured overall ventilation. Estimation of mean functional residual capacity and total lung capacity based on body weight (45) in these two animals yields 31 and 83 ml, respectively. Therefore, at the larger VT, lung inflation in each respiratory cycle reaches ~70% total lung capacity. The increase in sV̇ induced

Fig. 8. sV̇ in the ventral and dorsal parts of each lung slice at different VT levels. Values are means \pm SE for 5 rabbits. VT 4 was tested in 2 animals only. At the basal level, the dorsal lung had significantly larger sV̇ compared with the ventral lung (VT 1: P = 0.0499; VT 2: P = 0.024; VT 3: P = 0.014). There was no significant difference in sV̇ between the dorsal and ventral parts at apical and middle levels.

Table 3. Widths of \( \tau \) histograms at midheight and the paired t-test P values compared to the baseline VT 1

<table>
<thead>
<tr>
<th></th>
<th>Apical</th>
<th>Middle</th>
<th>Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta \tau_w, \text{s} )</td>
<td>VT1: 12.3±2.7</td>
<td>VT1: 11.3±1.7</td>
<td>VT1: 14.0±2.6</td>
</tr>
<tr>
<td></td>
<td>VT2: 7.1±0.7</td>
<td>VT2: 6.7±0.4</td>
<td>VT2: 9.7±1.3</td>
</tr>
<tr>
<td></td>
<td>VT3: 5.5±0.7</td>
<td>VT3: 5.0±0.5</td>
<td>VT3: 8.4±0.8</td>
</tr>
<tr>
<td></td>
<td>VT4: 5.4±na</td>
<td>VT4: 7.0±na</td>
<td>VT4: 7.1±na</td>
</tr>
<tr>
<td>P</td>
<td>0.04</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

Values are means \pm SE. \( \Delta \tau_w \), width of time constant (\( \tau \)) histograms at midheight.

Fig. 9. A: \( \Delta \tau_w \) at different VT levels for the apical, middle, and basal lung levels. Values are means \pm SE for 5 rabbits. VT 4 was tested in 2 animals only. B: \( \Delta \tau_w \) significantly decreased at all lung levels when VT increased from VT 1 to VT 3 (Table 3). B: relative width of the histogram \( \Delta \tau_w/\tau_0 \) (where \( \tau_0 \) is mean \( \tau \)) was nearly independent of VT.

Fig. 10. Maps of normalized summed squared residuals (SSR) of the fitted Xe concentration as function of time \( t [c(t)] \) in 5 × 5 pixel ROIs, calculated for the same basal slice as in Fig. 6, both in a KES image, and in the corresponding transmission image obtained at the energy above the Xe K-edge (\( E > E_K \)) in HU. SSR distributions for the entire slice are given in bottom panels. The SSR is normalized to the asymptotic concentration of \( c(t) \) (\( c_A \)) for the KES image, and to \( (\text{HU}_{\text{final}} - \text{HU}_{\text{initial}}) \) in the single-energy image to obtain comparable maps.
by the elevation of $V_T$ is therefore curbed by the physiological limits in lung expansion.

The scatter of the Xe washin $\tau$ values has two distinct features. First, there are clear differences between local ventilation in dependent and nondependent lung regions. These differences persist even when $V_T$ increases, suggesting mechanisms other than derecruitment at small $V_T$. On the other hand, in each part of the lungs, apical, middle, basal, ventral, and dorsal, the absolute scatter of $\tau$ decreases with increasing $V_T$, as evidenced by the smaller $\Delta \tau_{in}$ of the $\tau$ frequency distributions. These observations indicate that the interregional differences remain even when the intraregional homogeneity of ventilation increases with increasing $V_T$. This is in agreement with the results of human studies that have shown that increasing $V_T$ tends to increase the uniformity of gas distribution among small air spaces on the scale of acini, despite an increase in the differences in ventilation between larger separate lung regions (9, 24, 37).

The relative importance of the intra-acinar component on overall ventilation nonuniformity and the effect of $V_T$ can be quantitatively assessed in further studies using Xe/SRCT imaging in the experimental model used here, owing to a resolution scale that is not accessible by standard Xe/CT techniques. Ventilation heterogeneity is scale dependent in the regime in which the airway structures are self-similar, as shown by Altmeier et al. (3); in other words, heterogeneity increases as resolution is improved. Theoretically, ventilation heterogeneity increases up to a certain limit, which defines the size of the functional unit of gas exchange. The minimum voxel size achieved by the Xe/SRCT is $\sim 0.1$ mm$^3$, which is an order of magnitude smaller than the acinar volume in small animal lungs (40). Preliminary analysis, in which the volume of the ROI is varied from 0.1 to 100 mm$^3$ by binning voxels, indicates that the standard deviation increases with decreasing ROI but levels off when the ROI is $< 1$ mm$^3$. This may be associated with the so-called volume of ventilation unit (3). The ROI used in this study is somewhat larger than the above limit, so the regional specific ventilation is mapped in length scales where the fractal structure of the bronchial tree prevails. Given the wealth of data, a full analysis of the regional ventilation distributions by varying the size of the ROI is necessary.

An interesting new aspect is revealed when the relative widths of the washin $\tau$ values are examined. The relative width $\Delta \tau_{in}/\tau_{in}$ is shown in Fig. 9B, and in each case it turns out to be almost independent of $V_T$. This means that the response of the lungs to the increase in $V_T$ is uniform, so that the scatter in $\tau$ scales to the average value. In Fig. 7, the $\tau$ distributions follow the log-normal shape, and in the logarithmic, relative scale the distributions retain their shape, only moving to smaller values on the log($\tau$) scale when $V_T$ increases. The log-normal shape of the distributions may be explained by the fractal structure of the bronchial tree (3, 47), which implies that the Xe-O$_2$ mixture reaches each position in the lung through a path that is characteristic of that position. The gas flow in the airways is a sequence of redistributions at each airway bifurcation, and the concentration at a given position is proportional to the product of the probabilities of the gas following the path leading to the position considered. Such a process leads to the log-normal distribution of $\tau$, when the probabilities of gas redistribution in a given bifurcation are not 50/50 (32). These distributions are very common in nature, probably much more so than the normal (Gaussian) distribution. In the present case, the standard deviation of the log-normal distribution is almost independent of $V_T$. This observation may be explained by the fact that the airway structure does not change with $V_T$, in normal lung, and that there is no or very little derecruitment at small $V_T$ values used in this study. The washin rate of the gas is proportional to the “driving pressure” $P_{tr}$, and, if this pressure is equally exerted in all parts of the imaged lung slice, the distribution in the relative scale is unchanged. It is concluded that this is characteristic of normal lung function in anesthetized rabbit and may be an important benchmark for comparison in models of lung pathology.

Methodological considerations. KES imaging allows the observation of small anatomic structures carrying a tracer element, while removing practically all features due to other structures. This advantage of the KES method is illustrated in Figs. 4 and 10. The fit of Eq. 1 to the observed Xe concentration $c(t)$ is nearly perfect in the KES case, and the mean normalized SSR is 5.2% (mode: 4.0%) in the KES case (SSR/c$_A$), when the ROI is $5 \times 5$ pixels. In a single-energy CT, particularly at energies below Xe K-edge, absorption in Xe gives only a relatively small contribution to the total absorption in lung, and the statistical noise is seen as much larger deviations from the fitted curve than in the KES case. However, the use of an X-ray energy just above the K-edge enhances absorption in Xe, so that the radiographic density increases by more than 200 HU between the initial and final concentrations of Xe. The mean SSR of the single-energy map is $\sim 18.7\%$ (mode: 10.0%) of $(H_{\text{final}} - H_{\text{initial}})$. When the residuals in different methods are compared, it must be borne in mind that they depend on the imaging protocol, the Xe concentration of the inhaled gas, and, above all, the size of the ROI. In this work, imaging was performed during apnea at end expiration to reduce motion artifacts, whereas in some studies with conventional Xe/CT imaging is done during breath hold at end inspiration to maximize the Xe signal (31). Given these differences, it can be expected that the errors would be considerably larger for an image acquired using a broad-band X-ray source.

Because KES requires narrow energy bands bracketing the K-absorption edge of the contrast element, sufficient flux is available only with the high-intensity SR sources. Unlike CT with standard X-ray sources, results are totally independent of changes in the lung tissue density because the signal intensity on subtracted images depends only on Xe concentration, resembling scintigraphic methods using radioactive Xe but with much higher spatial resolution. KES imaging gives the possibility to directly measure local lung ventilation, but also local lung volume. The ability to quantify lung volume locally with simultaneous measurements of airway pressure can theoretically allow measurements of regional mechanical properties. Kreck et al. (31) have described a model allowing for simultaneous measurements of ventilation and perfusion using traditional Xe/CT that could be employed with higher resolution Xe/SRCT. Furthermore, when a tracer such as iodine is introduced in the blood stream, perfusion or blood-air barrier permeability can be directly quantified (1). Therefore, SRCT can potentially offer a nearly complete instrument for the experimental study of regional lung structure and function. Another advantage of using KES imaging is that image quality is improved, because there are no motion or deformation artifacts in the images. This gives the possibility to quantita-
tively image lung function in smaller animal models such as rabbits, rats, or mice. With the rapid progress of SRCT technology, spatial resolution is constantly improved and functional in vivo imaging is currently feasible with a pixel size of 0.049 mm and a spatial resolution, defined by the width at half-maximum of a point source, of 0.10 mm (1).

Xe is relatively insoluble in blood, with a blood-to-air partition coefficient of 0.13 (28, 43). Very small amounts of Xe, ~8% of the concentrations in conducting airways, appeared in a ROI selected in the inferior vena cava during the short time interval where a complete image sequence was acquired. Kreck et al. (31), using traditional Xe/CT, found that accurate analysis of low-SV regions required extended washin periods, and therefore the inclusion of this recirculation effect was necessary. Given the excellent contrast sensitivity of KES, this was never the case in the present study. Xe blood diffusion and recirculation were therefore considered negligible. Moreover, the amount of blood in the ROIs where specific ventilation was mapped was minimized by excluding major blood vessels, by segmentation based on Xe concentration values.

We used 60–70% Xe concentrations to obtain a strong contrast on the CT images; however, concentrations of 35% can be used with an optimal signal for regional ventilation measurements. Xe was chosen as an inhaled contrast agent because it is a stable, nonradioactive, inert gas that is also an inhaled anesthetic currently in clinical use (13). Sedation or other anesthetic side effects start with concentrations above 35% (25, 49). Xe is an expensive gas, however, and other investigators have used commercial devices that allow Xe recycling by rebreathing in a closed circuit (33). Because the higher density of Xe may itself affect homogeneity of ventilation (19), care was taken to administer the same inhaled Xe concentration in all experiments by using accurate mass flowmeters for Xe and O2.

In this study, no effort was made to minimize radiation dose fluxes applied for image acquisition. However, radiation dose delivered with SR sources is compatible with clinical application, and SR has been extensively tested in human patients for intravenous coronary angiography (14, 17, 46). The feasibility of Xe-enhanced imaging has previously been demonstrated in a human subject (21). We estimate that the mean noise level (SSR) is 5.2% in a reconstructed KES-CT image, whereas the noise level is 18.7% in a corresponding image acquired by the traditional Xe/CT technique. Because the statistical noise is proportional to the square of the incident intensity, the radiation dose used in clinical imaging may be reduced by an order of magnitude by the use of the KES method, while maintaining the image quality. Optimization of the CT imaging sequence could allow substantial reduction in radiation dose also in the present case.

Summary. In the present study, we assessed the impact of VT on regional lung ventilation and its nonuniformity by using SRCT. Our findings are in concordance with previous findings obtained with other methods. The results indicate that the increase of VT decreases the nonuniformity of intraregional gas exchange. The application of SR technology to functional lung imaging offers unique possibilities for the noninvasive investigation of regional lung mechanics and physiology, which include direct quantification of tracer elements, and dynamic respiration-gated image acquisition with excellent spatial resolution, allowing the exploration of regional lung function in smaller animal models such as rabbits and rodents. This technique has the potential to address several unresolved issues of respiratory physiology, including the size of the functional unit of ventilation, the relative contribution of acinar heterogeneity to overall ventilation heterogeneity, and the application of Xe/SRCT to the simultaneous measurement of lung ventilation and perfusion.

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REFERENCES


