Effect of Positive End-expiratory Pressure on Regional Ventilation Distribution during Mechanical Ventilation after Surfactant Depletion

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ABSTRACT

Background: Ventilator-induced lung injury occurs due to exaggerated local stresses, repeated collapse, and opening of terminal air spaces in poorly aerated dependent lung, and increased stretch in nondependent lung. The aim of this study was to quantify the functional behavior of peripheral lung units in whole-lung lavage-induced surfactant depletion, and to assess the effect of positive end-expiratory pressure.

Methods: The authors used synchrotron imaging to measure lung aeration and regional specific ventilation at positive end-expiratory pressure of 3 and 9 cm H2O, before and after whole-lung lavage in rabbits. Respiratory mechanical parameters were measured, and helium-washout was used to assess end-expiratory lung volume.

Results: Atelectatic, poorly, normally aerated, hyperinflated, and trapped regions could be identified using the imaging technique used in this study. Surfactant depletion significantly increased atelectasis (6.3 ± 3.3 [mean ± SEM]% total lung area; \( P = 0.04 \) vs. control) and poor aeration in dependent lung. Regional ventilation was distributed to poorly aerated regions with high (16.4 ± 4.4%; \( P < 0.001 \)), normal (20.7 ± 5.9%; \( P = 0.03 \) vs. control) and poor aeration of atelectatic lung regions involved a smaller fraction of the lung. Significant redistribution of ventilation to normally aerated lung regions, which can promote the local concentration of mechanical stresses, was the predominant functional behavior in surfactant-depleted lung. Potential tidal recruitment of atelectatic lung regions involved a smaller fraction of the imaged lung. Significant ventilation redistribution to aerated lung regions places these at risk of increased stretch injury.

Conclusions: Ventilation of poorly aerated dependent lung regions, which can promote the local concentration of mechanical stresses, was the predominant functional behavior in surfactant-depleted lung. Potential tidal recruitment of atelectatic lung regions involved a smaller fraction of the imaged lung. Significant ventilation redistribution to aerated lung regions places these at risk of increased stretch injury.

What We Already Know about This Topic

- Ventilation-induced lung injury occurs when collapsed lung is repeatedly recruited or when normal lung is overdistended

What This Article Tells Us That Is New

- Using a novel lung-imaging technique that uses synchrotron radiation, the administration of positive end-expiratory pressure was shown to improve aeration in collapsed lung but not to eliminate overventilation of normal lung units in experimental lung injury

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Received from the Pediatric Anesthesia Unit, Geneva Children's Hospital, University Hospitals of Geneva and the University of Geneva, Geneva, Switzerland. Submitted for publication August 23, 2011. Accepted for publication March 12, 2013. This work was supported by the Tampere Tuberculosis Foundation (Helsinki, Finland), the European Synchrotron Radiation Facility (Grenoble, France), the Swiss National Science Foundation grant 3200B0-118231 (Bern, Switzerland), the Academy of Finland (Helsinki, Finland, grant 126747), Hungarian Basic Scientific Research Grant OTKA K81179 (Budapest, Hungary), and the Department of Anesthesiology Pharmacology and Intensive Care, University Hospitals of Geneva (Geneva, Switzerland). Supported by the Conseil Régional de Picardie, France (Amiens, France, REG08009; to Dr. Bayat), and by a Bolyai Janos Research Fellowship (Budapest, Hungary; to Dr. Petak).

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tissues. Both clinical\(^2\)\(^-\)\(^5\) and experimental studies\(^6\)\(^-\)\(^10\) have shown that excessive stretch due to ventilation of the lung with large tidal volumes and increased plateau pressures can cause mechanical injury. Another mechanism of lung injury in this condition is the cyclic opening and closure of airways and atelectatic alveoli, or “atelectrauma.”\(^1\)\(^1\) Alternatively, the local concentration of mechanical stress and strain at the boundary between normally aerated and collapsed lung units can produce much larger local pressures than those applied to the airways, potentially leading to mechanical injury even in the absence of cyclic recruitment.\(^1\)\(^2\) So far, the protective effect of positive end-expiratory pressure (PEEP) has been attributed to the prevention of repeated collapse and opening of atelectatic terminal airways and alveoli.\(^1\)\(^3\) However, direct evidence of this phenomenon remains scarce. Previous experiments suggest that lung inflammation produced by prolonged mechanical ventilation in surfactant-depleted lung is most likely to occur in poorly aerated (PA) dependent lung regions,\(^1\)\(^4\) and have attributed lung injury mainly to cyclic recruitment in this condition.\(^1\)\(^5\) A better understanding of the regional functional behavior of the lung units in the presence of surfactant depletion and poor lung aeration and the changes induced by altering PEEP is crucial for devising protective mechanical ventilation strategies.

Previously, measurements of respiratory mechanical parameters\(^1\)\(^6\)\(^,\)\(^1\)\(^7\) and lung volume,\(^1\)\(^8\) have been used to characterize lung peripheral changes in the presence of ALI.\(^1\)\(^9\),\(^2\)\(^0\) Indices related to ventilation heterogeneity, such as lung clearance index (LCI), may give further insight into the overall ventilation heterogeneity; however, none of the abovementioned measurements or their combination allow assessment of the regional behavior of terminal air space. Computed tomography has previously been used to assess the regional distribution of lung volume, hyperinflation, and underaeration in patients with ALI.\(^1\)\(^7\),\(^2\)\(^1\) However, static computed tomography images do not allow measuring the distribution of regional lung ventilation and its changes with mechanical ventilation settings. Recently, we developed a lung-imaging technique; the K-edge subtraction (KES) method, which uses synchrotron radiation to quantify the regional concentration of inhaled stable xenon.\(^2\)\(^2\),\(^2\)\(^3\) This technique allows simultaneous measurement of parenchymal density and aeration and regional lung ventilation. The combination of these two parameters can provide further insight into the functional behavior of peripheral lung units during mechanical ventilation.\(^2\)\(^4\)

The current study was designed to quantitatively assess the functional behavior of peripheral lung units by determining regional lung aeration and ventilation distribution in order to describe how increases of PEEP level alter this behavior in a whole-lung lavage model of surfactant depletion. We further assessed whether changes in the regional ventilation distribution measured by KES imaging can be detected at the airway opening by relating these regional changes to overall measurements of lung volume, inert gas wash-out, and respiratory mechanics with PEEP-level increase and surfactant depletion.

**Materials and Methods**

**Animal Preparation**

Animal care and experimental procedures were in accordance with the Guidelines for the Care and Use of Animals published by the American Physiological Society and approved by the Internal Evaluation Committee for Animal Welfare in Research of the European Synchrotron Radiation Facility, Grenoble, France. The experiments were performed on six male New Zealand rabbits (2.6 ± 0.04 kg). A catheter (22 gauge) was inserted into the marginal ear vein under local anesthesia, using 5% topical lidocaine. Anesthesia was induced by the intravenous injection of 25 mg/kg of thiopental sodium. The animal was tracheotomized with a no. 3, Portex tube (Smiths Medical, Kent, United Kingdom). The left carotid artery and jugular vein were catheterized for blood gas measurements and for drug delivery. Anesthesia was then maintained with IV administration of 0.2 mg·kg\(^{-1}\)·h\(^{-1}\) of midazolam. After ensuring adequate anesthesia from the hemodynamic parameters, continuous IV infusion of atracurium (1.0 mg·kg\(^{-1}\)·h\(^{-1}\)) was started. The animal was immobilized in the vertical position in a cylindrical polyvinyl chloride custom-made holder.

Pressure-controlled mechanical ventilation was delivered using a custom-made apparatus, described in detail previously.\(^2\)\(^5\) The ventilator allowed synchronizing mechanical ventilation with the image acquisition. The pressure was set in order to obtain a tidal volume of 7.0 ml/kg at baseline, with a maximum threshold pressure of 30 cm H\(_2\)O, a baseline-inspired fraction of oxygen of 21%, and an initial PEEP of 3 cm H\(_2\)O, with the respiratory rate set (at 50–60/min) to achieve an arterial PCO\(_2\) close to 40 Torr. During imaging the animal breathed a mixture of xenon (70%) and oxygen (30%). The respiratory gas flow was monitored by using a heated pneumotachometer (Hans Rudolph, Kansas City, MO). The endotracheal pressure was monitored continuously. All monitored signals were amplified, digitized at 400 Hz (Powerlab, ADI Instruments, Oxfordshire, United Kingdom), and recorded on a computer.

**Synchrotron Radiation Computed Tomography Imaging**

The KES imaging technique allows quantitative measurements of regional specific ventilation (s\(V\)) as well as lung tissue density. A detailed description of the methodology and instrumental setup has been extensively discussed in previous studies.\(^2\)\(^2\),\(^2\)\(^3\),\(^2\)\(^6\),\(^2\)\(^7\) This imaging technique uses dual x-ray beams at slightly different energies. X-rays from a synchrotron radiation source are required because, as opposed to standard x-ray sources, they allow the selection of monochromatic beams from the full x-ray spectrum while conserving enough intensity for imaging with sufficient temporal resolution. Two computed tomography images
are thus simultaneously acquired and subtracted after the inhalation of the xenon–oxygen gas mixture. Visualization and quantitative measurement of xenon within the airways is based on the property that the attenuation coefficient of xenon increases by a factor of 5.4 when the energy of the incident x-ray beam crosses the energy threshold of 34.56 keV, which is the xenon K-edge. Using the dual-energy KES imaging method, the densities due to tissue and xenon can be calculated separately in each voxel in the image, using a specifically developed computer algorithm explained in detail elsewhere. A “xenon-density” image allows the direct quantitative measurement of this gas within the airways, and that of the regional gas volume. Dynamic KES imaging during xenon wash-in allows the measurement of regional sV. A “tissue-density” image obtained from the same data allows quantitative measurement of the regional tissue density.

The experiments were performed at the Biomedical Beamline of the European Synchrotron Radiation Facility (ESRF, Grenoble, France). The monoenergetic beams with an energy difference of 250 eV were produced from the continuous synchrotron radiation spectrum by a bent silicon crystal. The beams focused and crossed at the animal position, beyond which they diverged and were recorded by a liquid nitrogen–cooled, high-purity germanium, dual-line detector (Eurisys Measure, Lingolsheim, France). The horizontal pixel size of the detector was 0.35 mm, and the vertical beam height was 0.7 mm. Image reconstruction was performed using the filtered-back-projection algorithm, using the Interactive Data Language (IDL; RSI, Boulogne-Billancourt, France).

Image Analysis
Images were processed by using the MatLab programming package (Mathworks Inc., Natick, MA). Lung tissue was selected within the tissue-density computed tomography images, by region growing segmentation. The local specific ventilation or ventilation normalized to the gas volume within the voxel (sV) was calculated from the time constant of the xenon wash-in using a single compartment model fit of xenon concentration versus time. A 5 × 5 pixel moving average window was applied to the xenon-density images before the model fit. The mean value of sV over each horizontal slice was determined (sVm). The heterogeneity of ventilation was calculated as the coefficient of variation of sV within the lung area contained in the image slice. In each sV image, the histogram of sV was calculated, and fit with a log-normal function. The median (μ) and SD (σ) of the distribution were extracted from the fit. Normal, high, and low sV were defined with reference to the median value of each slice at baseline, as described later in the article. The ventilated alveolar area was defined as the area of the ventilation image where sV was greater than the median of the distribution minus 2 SDs: μ − 2σ.

The lung-tissue density (D) in mg/ml was converted to Hounsfield units. The area of lung comprised within the images was computed and totaled over the three axial image slices to calculate the total lung area. A density below −900 Hounsfield units was used to determine the area of lung zones where hyperinflation was most likely to occur. Lung regions with a density of −900 to −500 Hounsfield units were qualified as normally aerated, regions with density of −500 to −100 as PA, and atelectasis was defined as lung regions with a density from −100 to 0 Hounsfield units, as in previous studies in the literature. In order to characterize the functional behavior of normally aerated, PA, and hyperinflated lung regions, the area of lung within each category was further divided into subcategories as follows: no ventilation, defined as: sV < 0.2 l/min; low sV: 0.2 l/min < sV < (μ − 2σ); normal sV: sV = μ ± 2σ; high sV: (μ + 2σ) < sV. Trapping was defined as aerated areas with no sV. Comparison of lung aeration and sV was performed pixel by pixel, where ventilation images were based on a 5 × 5 pixel filtered xenon-density images, whereas tissue-density images were left unfiltered. Each subcategory was expressed as percentage of the total lung area within the image slice.

Measurement of End-expiratory Lung Volume and LCI
End-expiratory lung volume (EELV) was measured with a multiple-breath wash-in/wash-out technique, using an ultrasonic flow meter and helium as tracer gas, as described previously. Helium was administered into the ventilatory circuit until it reached a steady-state concentration of 4–5%, and the EELV was calculated from the recorded wash-out curve. LCI was calculated as the number of lung volume turnovers required to clear the lungs of the inert marker gas to 1/40th of the initial concentration.

Measurement of Respiratory Mechanics
The forced-oscillation technique was used to measure the airway and respiratory tissue parameters separately. For this purpose, a small-amplitude (1 cm H2O peak-to-peak) pressure forcing signal was delivered into the trachea via a polyethylene tube (100-cm length, 0.375 cm ID) while the mechanical ventilation was paused at end-expiration. The pseudorandom forcing signal ranging from 0.5 to 21 Hz was generated by loudspeaker-in-box system. The pressure inside the box was set to the level of PEEP in order to maintain constant pressure during the forced-oscillatory recordings. Lateral pressures were measured at the loudspeaker end (Pp) and the distal end (Pd) of the wave-tube with miniature sidearm transducers (ICS 33NA00D). These pressure signals were low-pass filtered (<25 Hz) and digitized at a sampling frequency of 128 Hz. The pressure transfer function (Pp/Pd) was created by fast Fourier transformation from the 8-s recordings, and the input impedance of the respiratory system (Zrs) was computed from this pressure transfer function as the load impedance of the wave-tube. Three to five Zrs spectra were ensemble-averaged under each PEEP level. A model that includes airway resistance (Raw), inertances (Iaw) in series with constant-phase tissue
compartments incorporating tissue damping (G) and elastance (H) was fitted to the averaged Zrs data.\textsuperscript{39}

**Study Protocol**

After standardizing the volume history by inflating the lungs to the 30 cm H\textsubscript{2}O, 3 to 4 Zrs recordings were collected during end-expiratory pauses of the mechanical ventilation with PEEP of 3 cm H\textsubscript{2}O (fig. 1). EELV was measured by performing two to three reproducible (i.e., values within 10%) helium wash-in/wash-outs. The measurements were completed by acquiring 10 subsequent KES subtraction images during xenon wash-in at three different axial positions selected approximately at the fourth (apical, nondependent), sixth (middle), and eighth (caudal, dependent) thoracic vertebral levels, based on a thoracic projection image. The PEEP level was then increased to 9 cm H\textsubscript{2}O, and the low-frequency forced oscillation, EELV, and KES imaging sequence were repeated. The PEEP level was decreased to 3 cm H\textsubscript{2}O, the rabbits were positioned to supine body posture, and whole-lung lavage was next performed by three consecutive intratracheal instillation and immediate withdrawal of 20 ml/kg normal saline. To ensure surfactant depletion, the retrieved fluid was reinstalled and aspirated two more times. Low-frequency forced oscillation, EELV, and KES image acquisition were repeated on PEEP 3, PEEP 9, and again on PEEP 3 cm H\textsubscript{2}O.

**Statistical Analysis**

The scatters in the parameters were expressed by the SEM values, except for blood gas data, for which scatter was expressed as interquartile range. The Shapiro–Wilk test was used to test data for normality. Both the mechanical and imaging parameters were normally distributed. Accordingly, one-way repeated-measures ANOVA was used to evaluate the changes in the mechanical and functional imaging parameters. When another additional factor was taken into account in the analyses (e.g., lung-imaging level), another two-way repeated-measures ANOVA was applied to evaluate the effects of these variables on the mechanical and imaging parameters. Pairwise comparisons were performed by using Holm–Sidak multiple comparison procedures. The Spearman correlation test was used to assess the strength of associations between the parameters. The statistical analyses were conducted by SigmaPlot (version 11.0; Systat Software, Inc., Chicago, IL). Statistical tests were carried out with the significance level set at \( P \) value less than 0.05.

**Results**

The changes in the respiratory mechanics, EELV and LCI in control condition and after lavage are shown in figure 2. The increase of PEEP level led to significant decreases in Raw \( (P = 0.03) \) in normal lung, without significant changes in \( G \), \( G \), and \( H \). After lavage, \( G \) and \( H \) significantly increased \( (P = 0.021 \text{ and } P = 0.029 \text{ vs. control, respectively}) \). In this condition, increasing the PEEP level from 3 to 9 cm H\textsubscript{2}O led to significant lowering in Raw \( (P = 0.002) \), which was associated with decreases in \( G \) \( (P = 0.027) \) and \( H \) \( (P = 0.02) \). The increase of PEEP level resulted in significant increases in EELV both under control conditions \( (P < 0.001) \) and after lung lavage \( (P < 0.001) \). The LCI did not change significantly with PEEP in control condition. After lavage, this parameter increased significantly \( (P < 0.001) \) and exhibited significant decreases with PEEP-level increase \( (P = 0.001) \).

Gas-exchange data are summarized in table 1. After lavage, the inspired fraction of oxygen was raised in order to maintain viable oxygenation, based on the arterial blood gases \( (\text{PaO}_2 > 80 \text{ mmHg}) \). The alveolar–arterial \( \text{PO}_2 \) gradient significantly increased \( (P < 0.001 \text{ vs. control}) \), and metabolic acidosis progressively developed after lavage.

In control condition, PEEP-level increase significantly decreased the mean arterial pressure \( (103.8 \pm 5.2 \text{ at PEEP3 vs. } 83.9 \pm 9.3 \text{ mmHg at PEEP9}; P = 0.004) \). Although the mean arterial pressure was significantly lowered by lavage \( (78.9 \pm 10.4 \text{ mmHg at PEEP 3}; P = 0.010 \text{ vs. control}) \), increase of PEEP level did not produce further significant reductions in this condition \( (70.8 \pm 22.2 \text{ mmHg at PEEP9}; P = 0.159 \text{ vs. PEEP3}) \).

Figure 3 demonstrates representative sample composite images showing the distribution of \( sV \) and lung-tissue density in a dependent image slice. In control condition, increase of PEEP level reduced both lung-tissue density and \( sV_m \), due to the increase in regional lung aeration. After lavage-induced surfactant depletion, PA regions appeared, along with patchy regions of atelectasis and trapping, with redistribution of ventilation to the remaining normally aerated

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**Fig. 1.** Study protocol. EELV = end-expiratory lung volume measurement using the He wash-out technique; KES = K-edge subtraction imaging; FOT = forced-oscillatory lung mechanical measurements; PEEP = positive end-expiratory pressure.
lung regions. Notably, a substantial portion of PA regions were ventilated, with high, normal, and low $sV$. After lavage, increasing PEEP level to 9 cm H$_2$O caused a large reduction in PA and atelectatic regions and in ventilation heterogeneity. Return to PEEP 3 caused partial reappearance of PA regions and a significant increase in gas trapping, and ventilation distribution remained significantly more heterogeneous than in control condition.

Figure 4 depicts the changes in the parameters computed from the KES images with the different PEEP levels before and after lavage. Significant decreases in $sVm$ ($P < 0.001$ for both control and lavage) and lung-tissue density ($P = 0.003$; $P = 0.014$, control and lavage, respectively) were found after increase in PEEP level, whereas the total lung area increased, due to the increase in lung volume. Lung lavage increased ventilation heterogeneity, manifested in significant increases in coefficient of variation of $sV$ and reduced the ventilated alveolar area. These changes were even more prominent after the reduction of PEEP back to 3 cm H$_2$O. The combined area of poorly and nonaerated regions significantly increased after lung lavage. Increase of PEEP level in lavaged lung significantly decreased the poorly and nonaerated zones ($P < 0.001$ vs. control), reduced ventilation heterogeneity, and increased ventilated alveolar area. A marked increase in the area of the hyperinflated lung units was observed with PEEP-level increase in control condition ($P < 0.001$), whereas this area was much smaller on both PEEP levels after lavage ($P = 0.004$; $P < 0.001$, lavage PEEP 3 and 9, respectively).

The relative area of lung regions in each of the categories defined based on aeration and $sV$ are summarized in figure 5.
for the entire lungs and in figure 6 for the different axial image levels. In control condition, PEEP-level increase to 9 cm H₂O increased the area of lung regions that were hyper-inflated but had a normal sV/P (P < 0.001 vs. control PEEP3). After lavage, at PEEP 3, a spectrum of different functional behaviors was observed. The amount of atelectasis was small but significant, and larger in the dependent lung image (P = 0.046; fig. 6). A substantial amount of lung regions were PA in this condition. The PA regions included areas with high (P < 0.001 lavage vs. control), normal (P < 0.001 lavage vs. control), or decreased (P < 0.05 lavage vs. control) sV, and were more abundant in the middle and dependent lung images (fig. 6A). A significant proportion of lung regions with normal aeration showed high (P = 0.003) or low sV (P = 0.011 vs. control PEEP3). Normally aerated high-sV regions significantly increased from the dependent toward the nondependent image slices after whole-lung lavage (fig. 6B).

Increase of PEEP level after surfactant depletion significantly reduced poor aeration and atelectasis (P = 0.049 vs. lavage PEEP 3; figs. 5 and 6), increased the amount of lung

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**Table 1.** Gas-exchange Parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td>PEEP (cm H₂O)</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>FiO₂</td>
<td>0.21</td>
<td>0.21</td>
<td>0.69 (0.62–0.79)</td>
<td>0.47 (0.44–0.71)</td>
<td>0.63 (0.49–0.72)</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>108 (105–111)</td>
<td>113.5 (99–127)</td>
<td>163 (92–309)</td>
<td>176 (145–283)</td>
<td>204 (87–350)</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>30.5 (25–32)</td>
<td>32.5 (26–35)</td>
<td>32.0 (29–34)</td>
<td>31.5 (30–39)</td>
<td>33.0 (31 to 35)</td>
</tr>
<tr>
<td>pH</td>
<td>7.47 (7.45–7.49)</td>
<td>7.33* (7.31–7.34)</td>
<td>7.37* (7.34–7.38)</td>
<td>7.32* (7.22–7.36)</td>
<td>7.31* (7.21–7.36)</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/L)</td>
<td>22.4 (21.9–24.3)</td>
<td>18.6 (15.6–21.5)</td>
<td>18.9 (18.8–20.4)</td>
<td>18.4* (16.7–19.6)</td>
<td>18.3* (14.4–19.9)</td>
</tr>
<tr>
<td>(A – a) PO₂ (mmHg)</td>
<td>10.0 (5.9–19.9)</td>
<td>12.4 (5.0–19.9)</td>
<td>250.4* (123.4–430.1)</td>
<td>80.8 (33.7–284.1)</td>
<td>170.7 (129.3–231.1)</td>
</tr>
</tbody>
</table>

Values are median (interquartile range).

*P < 0.05 vs. control on PEEP 3 cm H₂O.

(A – a) PO₂ = estimated alveolar–arterial PO₂ gradient; FiO₂ = inspired fraction of oxygen; HCO₃⁻ = bicarbonate concentration; PaO₂ = arterial partial pressure of oxygen; PaCO₂ = arterial partial pressure of CO₂; pH = arterial pH; PEEP = positive end-expiratory pressure.
Fig. 4. Parameters computed based on K-edge subtraction images (mean ± SD). \( s\dot{V}_m \) = mean specific ventilation, averaged over the three imaged lung slices; CV = coefficient of variation; VAA = ventilated alveolar area; total lung area = cumulated lung area within the three image slices; PEEP = positive end-expiratory pressure. * \( P < 0.005 \) PEEP 3 versus PEEP 9 within a condition; # \( P < 0.05 \) control versus lavage within a PEEP; § \( P < 0.05 \) lavage at PEEP 3.
with both normal aeration and sV (P = 0.006 vs. lavage PEEP 3), but a significant fraction of normally aerated lung still showed high sV at PEEP 9 (P < 0.001 vs. lavage PEEP 3). With the reduction of PEEP back to 3 cm H2O, gas trapping significantly increased (P = 0.03 vs. lavage PEEP 9), as did the amount of hyperinflated regions with low sV (P = 0.021 vs. lavage PEEP 9), although these regions represented only 2.0% of the total lung area. Regions with poor aeration and atelectasis also tended to reappear upon reduction of PEEP in the lavaged lungs.

Correlations between the imaging and the overall lung function parameters dependent upon ventilation heterogeneity are demonstrated in figure 7. The coefficient of variation of sV measured by KES imaging, was significantly correlated to LCI measured by helium wash-out, and to both G and H measured by forced-oscillation technique.

EELV showed significant correlation with (A–a) PO2 gradient (R = −0.652; P < 0.001), as did the sVm (R = 0.52; P < 0.001) and the area of PA (R = 0.60; P < 0.001). The (A–a) PO2 gradient was also significantly correlated to the respiratory mechanical parameters Raw (R = 0.57; P = 0.002), G (R = 0.51; P = 0.007), and H (R = 0.43; P = 0.03), and to the ventilation heterogeneity index obtained by helium wash-out (LCI; R = 0.50; P = 0.008).

Figure 8 depicts the relationships between the concomitant changes in H and EELV after increasing PEEP level under control condition and after lavage. PEEP-level increase led to systematic increases in EELV, independent of the lung condition. These changes were associated with opposite alterations in H with increases in this parameter under the control condition (R = 0.78; P < 0.001) and decreases after lung lavage (R = −0.86; P < 0.001).

Discussion

Functional behavior of peripheral lung units and the effect of PEEP were characterized by confronting simultaneous measurements of regional lung aeration and ventilation distribution in a whole-lung lavage model of ALI. Our data show that: (1) after surfactant depletion, significant atelectasis and poor aeration occurred predominantly in dependent lung regions, and a larger share of normally aerated lung regions were ventilated and showed low, normal, or high sV values; (3) increase of PEEP level to 9 cm H2O in surfactant-depleted lung reduced poor aeration, however, a substantial amount of normally aerated lung regions still showed a high sV; (4) with the reduction of PEEP back to 3 cm H2O, ventilation heterogeneity worsened, with significant trapping and redistribution of ventilation to normally aerated nondependent lung regions.

After lavage, increased surface tension due to surfactant depletion and locally, the presence of fluid in the air spaces, led to poor aeration. Increase in PEEP level in this condition decreased the PA lung regions (fig. 5). A remarkable finding in this study is that a large part of the imaged lung area was ventilated despite being PA after lavage (fig. 5). Although a large fraction of these regions had high sV, substantial proportions had either normal or decreased sV. Increased sV can be explained by reduced fraction of gas within the image voxel. Normal or reduced sV in a PA region can be explained by concomitant peripheral airway narrowing, due to increased surface tension, the presence of fluid, and the loss of tethering forces due to poor aeration in the surrounding tissues, but also by intermittent airway closure, a mechanism that has been shown to result in lung injury during prolonged mechanical...
ventilation. Reduced aeration without complete atelectasis can result from the collapse of a fraction of the alveoli contained within the image voxel. This hypothesis is supported by data from intravital microscopy in injured lung demonstrating clusters of closed alveoli surrounded by neighboring aerated alveoli. The loss of compliance in small alveolar clusters redistributes tidal volume to larger alveoli, promoting their ventilation and increasing spatial heterogeneity.

Theoretically, the expansion of aerated alveoli surrounding collapsed regions leads to heterogeneous distribution of stress and strain during lung inflation within PA regions. This mechanism has been implicated in the promotion of ventilator-induced lung injury after surfactant depletion. Heterogeneous alveolar closure and small airway narrowing can lead to heterogeneity of $sV$ below the effective voxel resolution.

![Graphs showing quantitative distributions of atelectatic, trapped, and poorly aerated (PA) and normally aerated (NA) regions between dependent (triangles), middle (squares), and nondependent (diamonds) image slices; PA and NA regions are subdivided into regions with low, normal, or high $sV$, and expressed as percentage (mean ± SD) of imaged lung area within each region of interest (ROI; dependent, middle, nondependent); $sV$ = specific ventilation; PEEP = positive end-expiratory pressure. * $P < 0.05$ versus control PEEP3; # $P < 0.05$ versus the nondependent image slice.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/930992/)

![Graphs showing correlations between parameters reflecting regional ventilation heterogeneities from the K-edge subtraction imaging (CV of $sV$) and overall parameters measured at the airway opening by He-washout (LCI) and forced-oscillation technique (G: tissue damping; H: elastance). CV = coefficient of variation; LCI = lung clearance index; $sV$ = specific ventilation.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/930992/)
After increase of PEEP level to 9 cm H$_2$O, atelectasis virtually disappeared, suggesting that pressure increments similar to the respiratory driving pressure can tidally recruit these regions. This observation is important because tidal recruitment of alveoli is another potential mechanism shown to produce lung injury although its contribution has been challenged.

Another consequence of lavage was that a significant fraction of normally aerated regions had high $s\dot{V}$, suggesting that these regions received a larger fraction of the tidal ventilation through redistribution from the PA regions (fig. 5; dark green). This phenomenon persisted after increase of PEEP level. This observation is consistent with an increased elastance of the lung regions that were PA at PEEP 3. The redistribution of ventilation in favor of the aerated non-dependent regions places them at risk of increased stretch, depending on the tidal volume. Experimental evidence suggests that excessive stretch of the lung tissues has injurious consequences and can lead to ventilator-induced lung injury. Using an injurious ventilation strategy in surfactant-depleted rabbits, Otto et al. found that cyclic recruitment in dependent lung regions produced more injury than stretch in the nondependent regions. However, the expression of chemokines progressed more rapidly in nondependent lung regions, suggesting the role of increased stretch in promoting lung inflammation. In rats, Tsuchida et al. found that high tidal volumes of 25 ml/kg associated with a low PEEP of 4 to 7 cm H$_2$O led to stretch-induced lung injury in nondependent lung regions as opposed to the atelectatic dependent lung regions, whereas a protective ventilation strategy with a tidal volume of 8 ml/kg and a PEEP of 14 cm H$_2$O did not. However, there was no demonstration of cyclic recruitment of atelectatic dependent regions in that study. Neither of the abovementioned studies characterized regional lung function. Using positron emission tomography to measure regional perfusion, ventilation, and inflammation in a one-lung lavage-induced surfactant depletion model in sheep, de Prost et al. found that 4 h of mechanical ventilation produced a metabolic activity indicative of lung inflammation that was maximal in PA dependent lung regions. A rather noninjurious mechanical ventilation strategy was used in the current study, with tidal volumes of 7 ml/kg and a plateau pressure limited to 30 cm H$_2$O. Our data show that ventilation of PA lung regions was likely to produce stress concentration in lung tissues, and redistribution of ventilation to normally aerated regions occurred to a similar extent, with a smaller degree of recruitment of atelectatic regions. Further study is needed to determine which of these phenomena are topographically associated with the development of lung injury with prolonged mechanical ventilation. However, our data are in agreement with previous studies suggesting that mechanical ventilation even with small tidal volumes and limited plateau pressures can promote the local concentration of mechanical stress primarily in PA dependent lung regions. With more injurious ventilation strategies, the increased injury in nondependent lung regions may become more probable due to the redistribution of ventilation in favor of these zones.

When decreasing PEEP level back to 3 cm H$_2$O, the area of PA and atelectatic lung zones increased particularly in the middle and dependent image slices (fig. 6). Regional ventilation heterogeneity (coefficient of variation of $s\dot{V}$) was worse than the initial measurements at PEEP 3, and ventilation redistribution to normally aerated regions was still present. Concomitant hyperinflation and low $s\dot{V}$ in this condition may have been due not only to airway narrowing, but also to intermittent airway closure. Gas trapping was significantly worse than the measurements before increase in level of PEEP (figs. 4, 5, and 6), which can be explained by the closure of unstable peripheral airways that were held open at PEEP 9. It is possible that extended periods of mechanical ventilation (~3 h) after lavage may have triggered lung inflammation and injury due to the local concentration of mechanical stresses.

Although LCI allows the detection of increased ventilation heterogeneities (fig. 7), this parameter is not sensitive to overdistension. The increase in EELV with PEEP-level increase led to opposite changes in H in lavaged versus normal lung (fig. 8), suggesting that combined measurements of EELV and H may allow overall characterization of recruitment and overdistension with increase in PEEP level. However, our findings suggest that a spectrum of regional functional behaviors occur after lavage-induced surfactant depletion, which cannot be detected solely on the basis of elastance, EELV, and LCI measurements.

The whole-lung lavage model of ALI has been extensively used to investigate the development of alveolar flooding and surfactant dysfunction, leading to a heterogeneous regional collapse of peripheral air spaces. However,
this model does not produce increased permeability edema formation, which is a hallmark of acute respiratory distress syndrome. The extent to which PA lung zones are tidally recruited under mechanical ventilation may be lower in high-permeability edema. The application of a single moderate PEEP level of 9 cm H₂O was chosen in the current study that reestablished the basal elastance value. Although stepwise increases in PEEP would have been preferable to determine the optimal level of PEEP in the presence of ALL, this was not possible because of the complexity of the measurements. The technical aspects of analyzing structural and functional changes using KES imaging have been extensively discussed previously. The horizontal x-ray beam used for functional imaging in the current study required that the animal be positioned vertically. This posture may slightly improve the respiratory mechanics in rabbit. Nevertheless, it did allow us to assess the regional differences in lung function with respect to gravity.

In summary, simultaneous measurements of regional lung aeration and ventilation in a lavage-induced model of ALL provided a quantitative assessment of the changes in regional lung function with increase in PEEP level. In surfactant-depleted lung, PA dependent lung regions were ventilated, a phenomenon likely to promote the local concentration of mechanical stress and strain. Although we found evidence of tidal recruitment of atelectatic dependent lung regions, this phenomenon occurred in a small fraction of the lung. Specific ventilation was redistributed to nondependent aerated lung, which can potentially place these regions at risk of increased stretch injury. The beneficial effect of a PEEP level associated with minimal respiratory elastance, could primarily be due to the prevention of exaggerated mechanical stress and strain due to ventilation of PA dependent lung, and to a lesser extent, the cyclic recruitment of atelectatic lung regions, but did not prevent ventilation redistribution to normally aerated lung. Further study is needed to determine which of the following functional behaviors: increased stretch due to redistribution of tidal ventilation, cyclical recruitment of atelectatic lung regions, or heterogeneous distribution of mechanical stress and strain due to expansion of PA lung regions, are topographically associated with the development of ventilator-induced lung injury.

References


