Effects of surfactant depletion on regional pulmonary metabolic activity during mechanical ventilation

Nicolas de Prost,1 Eduardo L. Costa,1,3 Tyler Wellman,1,4 Guido Musch,1 Tilo Winkler,1
Mauro R. Tucci,1,3 R. Scott Harris,2 Jose G. Venegas,1 and Marcos F. Vidal Melo1

1Department of Anesthesia, Critical Care and Pain Medicine and 2Department of Medicine (Pulmonary and Critical Care Unit), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; 3Respiratory Intensive Care Unit, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; and 4Department of Biomedical Engineering, Boston University, Boston, Massachusetts

Submitted 14 March 2011; accepted in final form 23 July 2011

VENTILATOR-INDUCED LUNG INJURY (VILI) is a major entity leading to increased morbidity and mortality in mechanically ventilated patients (1). Inflammation represents a fundamental process in the generation and progression of VILI (59), and neutrophils are key cells participating in the inflammatory response to injurious mechanical ventilation (8, 29, 39). [18F]fluorodeoxyglucose ([18F]-FDG) is a positron emission tomography (PET) glucose-analog tracer, which is taken up predominantly by metabolically active cells. In the inflamed nontumoral lung, [18F]-FDG has been shown to be a marker of neutrophilic inflammation (3, 13, 27, 28, 39, 47, 53).

Surfactant depletion is an important mechanism of lung dysfunction in several diseases (31, 33). During mechanical ventilation, surfactant depletion can facilitate atelectrauma, a potential factor promoting VILI (4, 18, 59, 60). Two distinct injury mechanisms are proposed in this setting. One, based on theoretical considerations, leads to local pressures much greater than pressures applied to the airway, and is due to inhomogeneous stress distribution occurring in lungs composed of atelectatic regions surrounded by expanded lung (34). The other, the cyclic opening/closing of airways and alveoli, shown experimentally to produce lung injury (37, 41, 50).

Based on such considerations, in the heterogeneously expanding lung, local lung inflammation should occur predominantly in regions likely to undergo either concentration of stresses or cyclic recruitment. In mechanically unstable supine lungs, such as surfactant-depleted lungs, these areas of large mechanical forces would correspond to regions of low aeration in the dependent lung (41). However, little is known on the in vivo topographical association between regional alveolar aeration and inflammatory changes (9), particularly in lungs of size comparable to those of humans. Indeed, even small animal studies are conflicting. For example, whereas studies in surfactant-depleted rabbits showed inflammatory signs to predominately in regions of size comparable to those of humans (61). The clinical relevance of this topic has been emphasized by the suggestion that the mass of opening and closing lung tissue would be an independent predictor of death in patients with the acute respiratory distress syndrome (ARDS) (7).

We developed PET methods to quantify regional [18F]-FDG uptake in sheep lungs during acute lung injury (ALI), which are topographically coregistered with previously presented PET techniques to quantify regional lung aeration, ventilation, and perfusion (38–40, 64). In the current study, we use those imaging methods to test the hypothesis that regions of low aeration colocalize with increased regional [18F]-FDG uptake in surfactant-depleted lungs, suggestive of in vivo increased regional metabolic activity and inflammatory response.

To address this question, we took advantage of a unilateral saline lavage model. This model provides an internal comparison of lung regions positioned equivalently along the vertical axis and submitted to equal mechanical ventilation pressures, while presenting different (lavaged vs. nonlavaged) mechan-
METHODS

Experimental preparation. The experimental procedures were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital (Boston, MA). Six sheep (22.0 ± 1.8 kg) were anesthetized, intubated, and mechanically ventilated. All procedures were performed under strict aseptic conditions. A femoral artery catheter was percutaneously cannulated for arterial blood samples and blood pressure monitoring. A 9-French introducer and a pulmonary artery catheter were inserted using the right internal jugular vein. A tracheotomy was performed and a 35- or 37-French left-sided double-lumen endobronchial tube was inserted. While ventilating the right lung with inspired oxygen fraction ($F_{\text{O}_2}$) = 1, left lung surfactant depletion was produced by alveolar saline lavage, as previously described (5, 49, 62). Starting from the supine position, warm saline (~400 ml) was instilled in the left bronchus (pressure ~30 cmH$_2$O), followed by draining to gravity. After three aliquots, animals were turned prone for another three aliquots to homogenize lavage of ventral and dorsal regions. An average of 400 ml (range 300–600) remained in the lungs, which is consistent with previous studies (30). The double-lumen endobronchial tube was then replaced by a regular endotracheal tube and double lung ventilation was resumed.

Experimental protocol. Animals were supine in the PET scanner (Scanditronix PC4096; General Electric, Milwaukee, WI) with the field of view just above the dome of the diaphragm. Mechanical ventilation aimed at producing wide regional pressure changes within the limits employed in clinical practice. It was applied for 4 h using positive end-expiratory pressure (PEEP) of 10 cmH$_2$O, $F_{\text{O}_2}$ = 0.6, inspiratory-to-expiratory ratio 1:2, tidal volume adjusted to a plateau pressure ($P_{\text{plat}}$) of 30 cmH$_2$O, and respiratory rate adjusted to normocapnia. A PEEP of 10 cmH$_2$O was needed to maintain viable oxygenation throughout the experiment, based on preliminary experiments. A $P_{\text{plat}}$ of 30 cmH$_2$O was used to provide a large tidal volume while limiting hyperinflation (24). Transmission and $^{13}$NN emission PET scans were performed following lung lavage once the animal was positioned in the PET camera and at the end of the 4-h period of mechanical ventilation. $^{18}$F-FDG-PET scans were acquired only after the last set of $^{13}$NN scans. Physiological measurements were performed within 10 min of PET scans.

PET imaging protocol and processing. The imaging methods and analysis were previously described in detail (38–40, 64). Briefly, the PET camera acquired 15 transverse cross-sectional slices of 6.5-mm thickness providing three-dimensional information over a 9.7-cm-long field of view corresponding to ~70% of the total lung volume (64). Resulting reconstructed PET images consisted of an interpolated matrix of 128×128×15 voxels (2×2 mm in-plane) with a spatial resolution of ~6.5 mm defined as full width at half maximum. Three different types of scans were performed, as follows.

1) Transmission scans were obtained over 10 min prior to each emission scan to correct for attenuation in emission scans and to calculate the fraction of gas ($F_{\text{gas}}$) of different regions of interest (ROIs) from regional tissue density ($F_{\text{tissue}}$) as $F_{\text{gas}} = 1 - F_{\text{tissue}}$. Because the transmission scan cannot differentiate tissue components with similar density, $F_{\text{tissue}}$ in the lungs represents the fractional content of all components with unit density, it therefore includes not only the contribution of parenchyma but also of blood, inflammatory infiltrates, and edema.

2) $^{13}$NN emission scans: i) Emission scans with $^{13}$NN-saline. These were performed for assessment of regional perfusion and shunt. The tracer $^{13}$NN-gas (~10-min half-life) was generated by a cyclotron and dissolved in degassed normal saline. The imaging protocol started with a tracer-free lung. The ventilator was turned off at the beginning of the exhalation, and the airway pressure was maintained at a value equal to the mean airway pressure during ventilation. A 20–30 ml bolus of $^{13}$NN-saline solution was then injected at a rate of 10 ml/s into the right internal jugular vein. Simultaneously, collection of a series of consecutive images was started. After an apnea period of 60 s, mechanical ventilation was restarted. The total imaging sequence lasted 4 min and consisted of eight images of 2.5 s and four images of 10 s during apnea and six images of 10 s and four images of 30 s during the washout phase.

Because of the low solubility of nitrogen in blood and tissues (partition coefficient water-to-air is 0.015 at 37°C), the pulmonary kinetics of infused $^{13}$NN shows distinct characteristics in regions that are perfused and aerated and regions that are perfused but not aerated (i.e., shunting units). In perfused and aerated regions, virtually all $^{13}$NN diffuses into the alveolar airspace at first pass, and during apnea it accumulates in proportion to regional perfusion. In regions with shunting alveolar units, $^{13}$NN kinetics during apnea show a peak of tracer concentration in the early PET frames, corresponding to arrival of the bolus of tracer with pulmonary blood flow, followed by a decrease toward a plateau. This decrease of activity reflects lack of retention of $^{13}$NN in nonaerated units, and its magnitude is related to regional shunt. Perfusion and shunt fraction of the test and control lungs were calculated with a tracer kinetic model (22, 40, 64).

ii) Emission scans with inhaled $^{13}$NN-gas. The lungs were ventilated with a previously described (63, 67) closed breathing circuit containing $^{13}$NN-gas until equilibration. A period of 6–10 min was allowed to ensure complete tracer equilibration in regions of low ventilation (because of its low solubility in tissues, inhaled $^{13}$NN remains confined to the airspace). A washout scan of the inhaled $^{13}$NN was then acquired. Starting from tracer equilibration in the lungs, an imaging sequence consisting of 16 frames (12×10 s and 4×30 s) was started. The washout was initiated at 60 s in the sequence by switching the breathing system to a tracer-free gas. Regional specific ventilation ($svr$, i.e., ventilation per unit of lung gas volume) was calculated as the inverse of the time constant derived from the tracer washout curve obtained after mechanical ventilation was resumed (63, 67). Regional absolute ventilation was computed from the product of $svr$ and regional lung volume as ventilation = $svr$$F_{\text{gas}}$ $V_{\text{ROI}}$, where the ROI volume ($V_{\text{ROI}}$) was computed as the product of the number of voxels in the ROI and the single voxel volume. $^{18}$F-FDG-PET emission scans were obtained for quantification of regional $^{18}$F-FDG kinetics. After $^{13}$NN clearance, $^{18}$F-FDG (5–10 mCi) was infused at a constant rate through the jugular catheter over 60 s and, simultaneously, with the beginning of $^{18}$F-FDG infusion, sequential PET frames (6×30 s, 7×60 s, 15×120 s, 1×300 s, 3×600 s) were acquired over 75 min. Blood samples were collected from pulmonary arterial blood at: 5′30′′, 9′30′′, 25′, 37′, and 42′30′′ to calibrate the input function (51). $^{18}$F-FDG-PET scans were acquired only after injury because of the 110-min half-life of $^{18}$F-FDG.

Quantification of $^{18}$F-FDG kinetics. After being transported into the cell by the same mechanism as glucose, $^{18}$F-FDG is phosphorylated by hexokinase to $^{18}$F-FDG-6-phosphate, which accumulates in proportion to the metabolic rate of the cell. $^{18}$F-FDG uptake rate ($Ki$), a measure of cellular metabolic activity, was computed by fitting the measured lung $^{18}$F-FDG kinetics with Sokoloff’s three-compartment model (54).

Regional $Ki$ normalized for tissue fraction, blood fraction, and wet-to-dry ratio (w/d) ($Ki_T$) was calculated to account for the effects of regional changes in $J$ lung deerecruitment and blood volume, by dividing $Ki$ by the tissue fraction, after excluding the blood volume ($F_{\text{blood}} - F_{\text{blood}} = F_{\text{gas}} - F_{\text{blood}}$, where $F_{\text{blood}}$ is the fractional volume of the blood compartment calculated with Sokoloff’s model (46) and 2) wet-to-dry ratio, to account for increased regional density due to elevated lung water rather than lung tissue, for both lavaged and nonlavaged lungs. Using a reference value for normal sheep lung wet-to-dry ratio ($w/d$) ~3.7) (43, 48) we calculated:

$$Ki_T = Ki_{\text{w/d}} = Ki_{\text{w/d}}(1 - F_{\text{blood}} - F_{\text{blood}})/(F_{\text{tissue}} - F_{\text{blood}}) = Ki_{\text{w/d}}(1 - F_{\text{gas}} - F_{\text{blood}})/(F_{\text{blood}})$$.
ANOVA with repeated measures (ROIs). Bonferroni corrected post hoc comparisons were used to compare physiological values before and after mechanical ventilation.

Values are means ± SD or median (25%–75% percentiles) when appropriate; Vt, tidal volume; Crs, compliance of the respiratory system; Qs/Qt, global shunt fraction and low respiratory system compliance (Table 1). Hemodynamics and respiratory variables were stable during the study period (Table 1). The nonsignificant increase in Vt after 4 h was due to Vt adjustment in some animals to keep the plateau pressure at 30 cmH2O, according to the protocol.

Regional 18F-FDG kinetics. Lavaged lungs exhibited higher whole lung Ki, i.e., higher cellular metabolic activity, than nonlavaged lungs (2.9 ± 0.6 vs. 1.5 ± 0.3 10−3/min; P = 0.018; Fig. 1D). Tracer kinetics in lavaged lungs was clearly different from that in nonlavaged lungs (Fig. 2). Lavaged lungs retained a larger fraction of their injected activity than nonlavaged lungs.

Regional analysis of 18F-FDG kinetics revealed that the high whole lung Ki in lavaged lungs was predominantly due to the increase in Ki of the dependent low aeration regions (4.2 ± 0.6 vs. 1.9 ± 0.5 10−3/min, P < 0.001; Fig. 3A). In contrast, nondependent areas exhibited similar Ki values to those of the similarly expanded (i.e., similar Fgas) nonlavaged lung. Such regional differences in Ki occurred despite the fact that the animals were lavaged similarly in the supine and prone positions.

The heterogeneity of Ki distribution was significant within the lavaged lungs. Ki in dependent regions was 2.6 times higher than Ki in the nondependent regions (Fig. 3A). Importantly, the difference in 18FDG uptake between dependent and nondependent regions was maintained when values were normalized by regional tissue fraction and wet-to-dry ratios (KiT; Fig. 3B), indicating that the effect was not merely due to different regional tissue densities. In contrast, minimal heterogeneity was observed in the nonlavaged lungs when this normalization was applied (Fig. 3B), indicating that within the nonlavaged lung measured regional heterogeneity was predominantly a result of heterogeneous tissue density.

Differences in regional Ki and KiT were further explored through their association with Fgas. Ki (Fig. 4A) and KiT (Fig. 4B) displayed in a hot scale with black = low and white = high values in the image. There is higher aeration, specific ventilation, and perfusion with vertical dependence in the nonlavaged lung, whereas the lavaged lung shows higher 18F-FDG uptake.

### Table 1. Global physiological variables at baseline and after 4 h of mechanical ventilation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (n = 6)</th>
<th>4 h (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vt, ml/kg</td>
<td>15.7 ± 5.6</td>
<td>16.6 ± 4.7</td>
<td>0.28</td>
</tr>
<tr>
<td>PEEP, cmH2O</td>
<td>10 (10–11)</td>
<td>10 (9–11)</td>
<td>1.00</td>
</tr>
<tr>
<td>Respiratory rate, /min</td>
<td>23 ± 4</td>
<td>23 ± 4</td>
<td>1.00</td>
</tr>
<tr>
<td>PaCO2/FIO2, torr</td>
<td>149 (102–320)</td>
<td>161 (123–173)</td>
<td>0.28</td>
</tr>
<tr>
<td>Crs, ml/cmH2O</td>
<td>17.4 ± 6.3</td>
<td>18.6 ± 4.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Qs/Qt (0.18–0.48)</td>
<td>0.32 (0.11–0.56)</td>
<td>0.35 (0.11–0.56)</td>
<td>0.77</td>
</tr>
<tr>
<td>PVR, dynes/cm²</td>
<td>393 ± 99</td>
<td>314 ± 84</td>
<td>0.52</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>42 ± 3</td>
<td>42 ± 3</td>
<td>0.53</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>102 ± 12</td>
<td>98 ± 18</td>
<td>0.73</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>155 ± 43</td>
<td>157 ± 41</td>
<td>0.94</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>4.1 ± 1.3</td>
<td>3.3 ± 0.5</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values are means ± SD or median (25%–75% percentiles) when appropriate; Vt, tidal volume; Crs, compliance of the respiratory system; Qs/Qt, global shunt fraction calculated with the Berggren equation; PVR, pulmonary vascular resistances; MPAP, mean pulmonary arterial pressure; MAP, mean arterial pressure.

Selection of voxels for analysis. The lung field was delineated using perfusion and gas fraction images. It was divided for analysis along the vertical axis into three adjacent regions of interest (ROIs) of equal size (i.e., stained by eosin), alveolar hemorrhage, septal thickening, and capillary congestion were evaluated semi-quantitatively with a modified four-grade scale (absent = 0, mild = 1, moderate = 2, and marked = 3) (35). The degree of lung injury was assessed by sum of averaged scores of items from 0 to 12 in 10 fields.

Lung neutrophil counts and histological examination. Lung tissue samples from nondependent (ventral) and dependent (dorsal) regions of each lung were embedded in paraffin. Five-micrometer-thick sections were cut, mounted, and stained with hematoxylin and eosin for light microscopy. Neutrophils were counted in 10 randomly selected high-power (×400) fields per lung region (2 slides of each lung per animal). In addition, perivascular and alveolar protein-rich edema (i.e., stained by eosin), alveolar hemorrhage, septal thickening, and capillary congestion were evaluated semi-quantitatively with a modified four-grade scale (absent = 0, mild = 1, moderate = 2, and marked = 3) (35). The degree of lung injury was assessed by sum of averaged scores of items from 0 to 12 in 10 fields.

Data are expressed as means ± SD if normally distributed or median (interquartile range 25%–75%) otherwise. We compared physiological values before and after mechanical ventilation using paired t-test or Wilcoxon’s signed-rank test when appropriate. PET-acquired data were compared immediately after lavage vs. after 4 h of mechanical ventilation and between lungs by two-way ANOVA with repeated measures (ROIs). Bonferroni corrected post hoc tests were performed when overall P value <0.05.

### RESULTS

**Global physiological variables.** As a result of unilateral lung lavage, animals were hypoxemic at baseline, with high shunt fraction and low respiratory system compliance (Table 1). Hemodynamics and respiratory variables were stable during the study period (Table 1). The nonsignificant increase in Vt after 4 h was due to Vt adjustment in some animals to keep the plateau pressure at 30 cmH2O, according to the protocol.

Regional 18F-FDG kinetics. Lavaged lungs exhibited higher whole lung Ki, i.e., higher cellular metabolic activity, than nonlavaged lungs (2.9 ± 0.6 vs. 1.5 ± 0.3 10−3/min; P = 0.018; Fig. 1D). Tracer kinetics in lavaged lungs was clearly different from that in nonlavaged lungs (Fig. 2). Lavaged lungs retained a larger fraction of their injected activity than nonlavaged lungs.

Regional analysis of 18F-FDG kinetics revealed that the high whole lung Ki in lavaged lungs was predominantly due to the increase in Ki of the dependent low aeration regions (4.2 ± 0.6 vs. 1.9 ± 0.5 10−3/min, P < 0.001; Fig. 3A). In contrast, nondependent areas exhibited similar Ki values to those of the similarly expanded (i.e., similar Fgas) nonlavaged lung. Such regional differences in Ki occurred despite the fact that the animals were lavaged similarly in the supine and prone positions.

The heterogeneity of Ki distribution was significant within the lavaged lungs. Ki in dependent regions was 2.6 times higher than Ki in the nondependent regions (Fig. 3A). Importantly, the difference in 18F-FDG uptake between dependent and nondependent regions was maintained when values were normalized by regional tissue fraction and wet-to-dry ratios (KiT; Fig. 3B), indicating that the effect was not merely due to different regional tissue densities. In contrast, minimal heterogeneity was observed in the nonlavaged lungs when this normalization was applied (Fig. 3B), indicating that within the nonlavaged lung measured regional heterogeneity was predominantly a result of heterogeneous tissue density.

Differences in regional Ki and KiT were further explored through their association with Fgas. Ki (Fig. 4A) and KiT (Fig. 4B) displayed in a hot scale with black = low and white = high values in the image. There is higher aeration, specific ventilation, and perfusion with vertical dependence in the nonlavaged lung, whereas the lavaged lung shows higher 18F-FDG uptake.

---

**Fig. 1.** Single slice images of regional gas fraction (Fgas; A), specific ventilation (B), mean-normalized perfusion (C), and 18F-fluorodeoxyglucose (18F-FDG; D) uptake rate (Ki, computed voxel-by-voxel with Patlak’s method (42)) in one experiment. Left of the image corresponds to the left in the animal. Each image is displayed in a hot scale with black = low and white = high values in the image. There is higher aeration, specific ventilation, and perfusion with vertical dependence in the nonlavaged lung, whereas the lavaged lung shows higher 18F-FDG uptake.
increased with decreasing $F_{\text{gas}}$ values ($P < 0.01$) and with surfactant depletion ($P < 0.01$), with significant interaction ($P < 0.01$). Consistent with heterogeneity of $K_i$ in horizontal ROIs of lavaged lungs, $K_i$ was significantly higher in poorly aerated regions ($F_{\text{gas}}$ ranges 0.1–0.3 and 0.3–0.5) of lavaged lungs than in normally aerated regions ($F_{\text{gas}}$ ranges 0.5–0.7 and 0.7–0.9) of the same lungs ($P < 0.01$; Fig. 4A). When lavaged and nonlavaged lungs were compared according to $F_{\text{gas}}$ ranges (Fig. 4B), $K_{iT}$ in $F_{\text{gas}}$ ranges between 0.1–0.3 ($P < 0.01$) and 0.3–0.5 ($P < 0.05$) was larger for the lavaged than the nonlavaged lung, while no significant difference in $K_{iT}$ between lavaged and nonlavaged lungs was found for $F_{\text{gas}}$ ranges 0.0–0.1, 0.5–0.7, and 0.7–0.9.

Regional lung aeration and ventilation. Mean gas fraction ($F_{\text{gas}}$) was lower in lavaged than in nonlavaged lungs (0.44 ± 0.16 vs. 0.69 ± 0.02; $P < 0.05$). Regional $F_{\text{gas}}$ was also lower in the lavaged than in the nonlavaged lungs for the middle and dependent regions (Fig. 1A and Fig. 5A). Accordingly, when classified by aeration and compared with nonlavaged lungs, lavaged lungs displayed a significantly higher fraction of nonaerated [$i.e., F_{\text{gas}} < 0.1: 13.7\% (7.3–27.8)$ vs. 0.4% (0.0–4.1); $P < 0.05$] and poorly aerated [$i.e., 0.1 \leq F_{\text{gas}} < 0.5: 36.8\% (24.4–46.0)$ vs. 8.4% (7.1–19.0); $P < 0.01$] voxels, which predominated in dependent regions.

Ventilation to the lavaged lung was lower than that to the nonlavaged lungs [1.00 l/min (0.61–1.67) vs. 3.59 l/min (2.03–5.74); $P < 0.05$], i.e., total ventilation was distributed 23% to the lavaged and 77% to the nonlavaged lung. Dependent, middle, and nondependent regions of lavaged lungs were all significantly less ventilated than those of nonlavaged lungs (Fig. 1B and 5B). Regional ventilation was also heteroge-

---

**Fig. 2.** Time activity curves of decay-corrected tracer activity in the lavaged (●) and the nonlavaged (○) lungs of the experiment presented in Fig. 1. $^{18}$F-FDG kinetics was clearly distinct in lavaged and nonlavaged lungs. Tracer kinetics showed a fast peak, corresponding to the inflow of the $^{18}$F-FDG bolus in the lung, followed by an exponential decrease toward an approximate plateau in both lungs. This plateau corresponded to 45% of the peak in the nonlavaged and 83% of the peak in the lavaged lung, evidencing the larger accumulation of tracer in the lavaged lung. Modeling of $^{18}$F-FDG kinetics showed that this increased accumulation of tracer was due to an increase in $^{18}$F-FDG uptake in the lavaged compared with the nonlavaged lung ($K_i = 0.0043$ vs. 0.0011/min, respectively).

**Fig. 3.** $^{18}$F-FDG net uptake rate ($K_i$) in isogravitational regions of interest of lavaged (●) and nonlavaged (○) lungs without (A) or with (B) normalization for tissue density and wet-to-dry ratios [i.e., $K_{iT} = K_i(w/d)/(1 - F_{\text{gas}} - F_{\text{blood}})(w/d))]$. Note the significant regional heterogeneity in the distribution of $K_i$. Horizontal lines represent median values. *$P < 0.05$; **$P < 0.01$. $P$ values come from 2-way ANOVA with repeated measures.

**Fig. 4.** $^{18}$F-FDG net uptake rate ($K_i$) in regions of interest defined by $F_{\text{gas}}$ ranges of lavaged (●) and nonlavaged (○) lungs without (A) or with (B) normalization for tissue density and wet-to-dry ratios [i.e., $K_{iT} = K_i(w/d)/(1 - F_{\text{gas}} - F_{\text{blood}})(w/d))]$. In nonlavaged lungs, $K_{iT}$ varies minimally between $F_{\text{gas}}$ ranges, suggesting that differences in $K_i$ are mainly dependent on lung tissue density. In contrast, in lavaged lungs $K_{iT}$ is significantly affected by $F_{\text{gas}}$ ($P < 0.01$). Horizontal lines represent median values. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. $P$ values come from 2-way ANOVA with repeated measures.
neously distributed along the vertical axis for lavaged and nonlavaged lungs (P < 0.001; Fig. 5B). There were minimal changes in mean and regional gas fraction and ventilation from baseline (data not shown) to end of the study.

Regional perfusion and shunt fraction. On average, the lavaged lung received 47±8% and the nonlavaged lung 53±8% of the total perfusion at baseline (P = 0.44). Distribution of fractional perfusion was distinct in each lung, with dependent regions of lavaged lungs exhibiting markedly lower perfusion than those of nonlavaged lungs (24±7 vs. 38±5% at baseline; P < 0.01; Figs. 1C and 5C). Within the same lung, dependent lung areas showed larger perfusion than nondependent regions (P < 0.001; Fig. 5C). Analysis of the perfusion in the different Fgas ranges (data not shown) further confirmed that perfusion was significantly lower in lavaged than in nonlavaged lungs (P < 0.001). Regional perfusion remained remarkably stable between baseline and after 4 h of mechanical ventilation. Mean shunt fraction was higher in lavaged than in non-lavaged lungs [0.30 (0.17–0.36) vs. 0.16 (0.05–0.34); P < 0.05]. Dependent regions of the lavaged lungs exhibited a higher shunt fraction than those of nonlavaged lungs (Fig. 5D).

Neutrophil counts and histological examination. Peripheral blood neutrophils increased from 1,450/mm³ (1,200–2,600) to 4,750/mm³ (2,850–5,450) from baseline to 4 h of mechanical ventilation (Fig. 6A). Lung neutrophil counts were significantly higher in lavaged than in nonlavaged lungs [8.5 (6.8–12.8) vs. 5.5 (3.3–10.9) per field; P < 0.001], reaching significance in dependent regions (P < 0.01; Figs. 6B and 7).

Lung injury scores of lavaged and nonlavaged lungs were all ≤1/12, without statistically significant difference (Table 2).

Wet-to-dry lung ratios. Lung wet-to-dry ratios were markedly higher in lavaged than in nonlavaged lungs (P < 0.001). This effect was significant in dependent (7.3 ± 1.2 vs. 4.9 ± 0.5; P < 0.01), middle (7.7 ± 2.1 vs. 4.8 ± 0.4; P < 0.001), and nondependent (6.5 ± 1.3 vs. 4.9 ± 0.5; P < 0.05) regions on post hoc tests.

DISCUSSION

The main findings of this study were 1) pulmonary cellular metabolic activity as assessed by 18F-FDG uptake was increased in surfactant-depleted lung regions after 4 h of mechanical ventilation in sheep and was detectable noninvasively with PET; 2) the distribution of 18F-FDG uptake was clearly heterogeneous within the lavaged lungs of these supine ventilated sheep, with larger 18F-FDG uptake in dependent poorly

Fig. 5. Fgas (A), ventilation (B), perfusion fraction (C), and shunt fraction (D) for dependent, middle, and nondependent regions of interest of lavaged (●) and nonlavaged (○) lungs after 4 h of mechanical ventilation. Horizontal lines represent median values. *P < 0.05; **P < 0.01; ***P < 0.001. P values come from 2-way ANOVA with repeated measures.

Fig. 6. A: blood neutrophil counts at baseline and after 4 h of mechanical ventilation (MV). B: lung neutrophil counts in dependent and nondependent regions of lavaged (●) and nonlavaged (○) lungs. There was an overall increase in lung neutrophil counts after alveolar lavage (P < 0.001), but higher neutrophil counts only in the lung tissue of the dependent lavaged lung compared with the nonlavaged lung. Horizontal lines represent median values. **P < 0.01.
aerated regions than in nondependent, normally aerated lung regions; and 3) increased 18F-FDG uptake occurred despite significantly lower perfusion and ventilation to surfactant-depleted areas.

In the acutely inflamed lung, 18F-FDG-PET is understood as a measure of neutrophilic inflammation (13, 27, 28, 39). Recent studies in humans and animals indicate that it may be a valuable tool to study the mechanisms of ALI/ARDS (3, 14, 39), predict severe respiratory failure (47), and evaluate the effect of therapeutic interventions (11). Regional variation of mechanical forces leading to cellular injury and inflammation is a key mechanism of VILI (4, 34). Thus 18F-FDG-PET could provide valuable noninvasive and whole lung information about the disease process in vivo. However, there is no knowledge on changes in 18F-FDG uptake promoted by regional mechanical forces, how early they occur, and how they relate to other regional physiological variables. Answering these questions is imperative if the technique is to be used to study VILI in humans.

**Model of unilateral surfactant depletion and mechanical ventilation with 10 cmH2O PEEP and 30 cmH2O plateau pressure.** Surfactant depletion with saline lavage is a well-established model of alveolar and small airway collapse, which promotes mechanical lung injury during mechanical ventilation (4, 31, 33, 36, 66). The unilateral lavage model brought several important advantages to the study (5, 49). It allowed for the performance of a well controlled and standardized supine-prone surfactant depletion. It also provided an internal comparison of lung regions with distinct mechanical properties (lavaged vs. nonlavaged), yet positioned similarly along the vertical axis, and submitted to similar transpulmonary pressures (comparison of ROIs along the vertical axis). The minor inflammation produced by lung lavage (31, 33, 36, 61, 66) is another important characteristic of the chosen model, which facilitates the detection of small changes in regional metabolic activity. Additionally, the model yielded typical functional changes observed in derecruited lung regions, i.e., lower perfusion, aeration, and ventilation, and higher shunt than those in normal lung areas (20, 31, 33, 40). These features mirror regional changes expected to occur in heterogeneously diseased lungs, reflecting redistribution of perfusion toward normally aerated lung areas (38).

The used ventilatory pattern aimed at producing wide regional pressure changes within limits employed in clinical practice and not at studying a clinically recommended mode of ventilation. Gas fraction analysis confirmed achievement of that aim with minimal overinflation, consistent with Pplat constrained at 30 cmH2O (19, 26) and a significant amount of non- and poorly aerated regions in the lavaged lungs, while still maintaining a viable oxygenation with PEEP = 10 cmH2O.

### Table 2. Lung histological analysis

<table>
<thead>
<tr>
<th></th>
<th>Lavaged Lungs (n = 6)</th>
<th>Nonlavaged Lungs (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ventral</td>
<td>Dorsal</td>
</tr>
<tr>
<td>Alveolar edema (/3)</td>
<td>0.0 (0.0–0.6)</td>
<td>0.3 (0.2–0.6)</td>
</tr>
<tr>
<td>Septal edema (/3)</td>
<td>0.5 (0.2–0.8)</td>
<td>0.1 (0.0–0.3)</td>
</tr>
<tr>
<td>Alveolar hemorrhage (/3)</td>
<td>0.1 (0.0–0.1)</td>
<td>0.2 (0.0–0.4)</td>
</tr>
<tr>
<td>Septal congestion (/3)</td>
<td>0.2 (0.0–0.8)</td>
<td>0.0 (0.0–0.1)</td>
</tr>
<tr>
<td>Total score (/12)</td>
<td>1.0 (0.4–2.7)</td>
<td>0.8 (0.4–1.3)</td>
</tr>
</tbody>
</table>

Values are median (25%–75% percentiles)

![Fig. 7. Lung tissue sections of dependent (dorsal) regions of the lavaged (A) and of the nonlavaged (B) lungs from one animal (the same as in Figs. 1 and 2) stained with hematoxylin and eosin (magnification ×400). The lavaged lung exhibited a remarkable neutrophilic infiltration (arrows) and a mild thickening of alveolar walls, but none of the other features assessed in the lung injury score (35) (i.e., capillary congestion, alveolar edema, and alveolar hemorrhage). Note that neutrophils are located within the septa (i.e., in the interstitium or the capillaries) and not in the alveoli, suggesting an early phase of their migration into the lungs.](image-url)
additional contribution to loss of aeration was lung water, as attested by higher wet-to-dry ratios in lavaged than in nonlavaged lungs. In nonlavaged lungs, aeration and wet-to-dry ratios were compatible with previous observations in normal lungs (43, 44, 48).

The discrete degree of histological lung injury (but not of cellular metabolic activity as discussed below) observed in lavaged lungs is compatible with the known mild inflammatory infiltrate produced by lung lavage (36, 66) likely associated with the pressure limited ventilatory strategy and short duration of the study (36, 66). The low histological injury scores in the nonlavag ed lungs are consistent with achievement of limited overinflation and injury as targeted with the experimental design. Interestingly, neutrophil counts in peripheral blood increased dramatically during the study, probably due to release of neutrophils from the marginated pool with mechanical ventilation and lung lavage (14, 56). As a result, areas of increased neutrophil chemoattraction had a larger neutrophil supply to call on. The results of lung tissue neutrophil counts suggest that this occurred in dependent lavaged lungs.

Regional $^{18}$F-FDG kinetics during unilateral alveolar lavage. Cellular metabolic activity measured with $^{18}$F-FDG uptake was markedly higher in dependent regions of the surfactant-depleted compared with those of the nondepleted lung after only 4 h of mechanical ventilation with plateau pressures bounded by an accepted clinical limit and PEEP = 10 cmH$_2$O. Importantly, higher $^{18}$F-FDG uptake in those regions persisted after accounting for differences in regional wet-to-dry ratios and tissue density (Fig. 3B), emphasizing that the higher $^{18}$F-FDG uptake was not merely due to edema or alveolar collapse. In contrast, the nondependent regions of the lavaged lung had $^{18}$F-FDG uptake similar to those of the contralateral nonlavaged lung. This occurred despite the fact that lung lavage was carried out equivalently in the supine and prone positions and thus expected to promote a relatively homogeneous surfactant depletion from dorsal and ventral regions, suggesting that heterogeneity in $^{18}$F-FDG uptake could not purely be ascribed to surfactant depletion.

Further insight on this finding was provided by the $^{18}$F-FDG analysis based on $F_{\text{gas}}$ ranges. Within lavaged lungs, $^{18}$F-FDG uptake rate ($K_i$) was larger in regions of poor vs. normal aeration ($F_{\text{gas}}$ ranges 0.1–0.5 vs. 0.5–0.9 in Fig. 4A). Comparing both lungs for similar regions of poor aeration, $^{18}$F-FDG uptake ($K_i$ and $K_{iR}$) was larger in the lavaged than in the nonlavaged lungs (Fig. 4, A and B). In contrast, in normally aerated regions $^{18}$F-FDG uptake rate ($K_i$ and $K_{iR}$) was similar in lavaged and nonlavaged lungs. Such finding suggests that surfactant depletion by itself cannot explain the regional changes in $^{18}$F-FDG kinetics and that regional phenomena within the lavaged lung likely contributed to the development of the observed heterogeneity in $^{18}$F-FDG uptake.

Several mechanical and nonmechanical factors could account for this finding of increased and heterogeneous $^{18}$F-FDG uptake in lavaged lungs. The literature is limited on the topic since no study to date addressed changes in regional lung metabolic activity caused by surfactant depletion. Considering the model used, a critical factor is the presence of elevated regional mechanical forces due to surfactant depletion (4, 34) potentially yielding corresponding increases in regional metabolic activity. Indeed, we showed that exaggerated peak pressures applied to a homogeneously inflated normal sheep lung led to increased regional $^{18}$F-FDG uptake, particularly when repetitive alveolar collapse was imposed (39). Increased concentration of stresses (34) are likely to occur in dependent, poorly aerated regions given the alveolar instability induced in the surfactant depletion model with the presence of collapsed areas neighboring expanded alveoli. Cyclic recruitment could also have occurred, although in smaller magnitude, due to application of a PEEP = 10 cmH$_2$O and mainly due to a limited driving pressure (10, 17). In fact, dependent regions with high $^{18}$F-FDG uptake had poor aeration (mean gas fraction~20%), whereas nondependent normally aerated (mean gas fraction~70%) surfactant-depleted regions showed low $^{18}$F-FDG uptakes. Another reported mechanism is the steep pressure gradient near the air-fluid front during reopening of collapsed or fluid-occluded Airways generating large mechanical forces injurious to epithelial cells (4). Previous in vitro (65) and small animal (41, 61) studies showed the association between application of mechanical forces to the lung and the triggering of inflammation. Taken together, these mechanisms could account for our observation of increased regional metabolic activity and neutrophilic infiltrate. Additionally, the increased mechanical forces could lead to increased metabolism of parenchymal cells that usually have lower metabolic rates (21), further augmenting total $^{18}$F-FDG uptake (39).

Mechanisms independent of mechanical forces could also contribute to increased $^{18}$F-FDG uptake during surfactant depletion. Impairment of alveolar host defenses due to surfactant depletion could have facilitated increased inflammation in the lavaged lung (68). It is conceivable that such factor could have interacted with surfactant dysfunction and airspace collapse and reopening for a final increase in regional metabolic activity of dependent regions. Irrespective of the mechanism, our findings indicate that early changes in regional cellular metabolism occur in poorly aerated regions during mechanical ventilation following surfactant depletion with saline lung lavage and are detectable with $^{18}$F-FDG-PET.

Similar $^{18}$F-FDG uptake was found in surfactant-depleted nondependent regions and nonsurfactant-depleted lungs with no significant difference in neutrophil counts. This is consistent with reduced mechanical forces to those normally aerated areas. Such a result is compatible with the absence of significant histological lung injury in a rabbit model of surfactant inactivation when lungs were kept normally aerated with PEEP (57).

A recent study in a mixed population of ARDS patients (predominantly with pneumonia and sepsis) found similar $^{18}$F-FDG uptake in lung regions with gas fractions between 10 and 50% ($-500 \leq \text{Hounsfield units} < -100$) and 0 and 10% ($-100 \leq \text{Hounsfield units}$). The authors inferred that regions undergoing cyclic recruitment showed the same metabolic activity of continuously collapsed regions and implied a smaller role of atelectrauma to metabolic activity (2). It is difficult to ascertain the relevance of those results to provide insight to our findings. This is because 1) lung metabolic activity is markedly changed by infection (27) and even mild endotoxemia (14); 2) infection can also produce changes in regional perfusion, which could alter regional metabolic activity (14); 3) it is impossible to exclude entirely the presence and grade the magnitude of cyclic recruitment in regions with 0–10% gas fraction; 4) ALL in humans is usually multifactorial, not allowing for independent identification of the role of
18F-FDG uptake and surfactant depletion; 5) the duration and severity of lung injury were much larger in the ARDS patients than in our experiments, potentially resulting in more lung inflammation with a different distribution, making it difficult to isolate the contribution of cyclic recruitment to lung inflammation in that study; and 6) cyclic recruitment was estimated using end-inspiratory and end-expiratory breath-holds rather than dynamically, and might, thus, have been overestimated. This emphasizes the need to better understand the determinants of 18F-FDG uptake in the lungs and the relevance of the mild inflammation model and assessment of regional perfusion we used to allow for investigation of effects specifically due to surfactant depletion.

Regional 18F-FDG uptake, perfusion, and ventilation. There is minimal data on the effect of surfactant depletion on regional perfusion. We found regional perfusion to be significantly smaller in the dependent regions of lavaged than nonlavaged lungs. This shift of perfusion to the contralateral well aerated lung is likely due to hypoxic pulmonary vasoconstriction (20).

Remarkably, higher 18F-FDG uptake occurred only in surfactant-depleted dependent regions, despite lower perfusion and ventilation to these areas. A consequence of the reduction in regional perfusion in dependent regions of surfactant-depleted lungs is the expected decrease in delivery of circulating inflammatory cells and mediators to those areas. Yet 18F-FDG uptake was increased in those regions despite their reduced perfusion. There has been recent controversy on the effect of regional perfusion on 18F-FDG uptake in ARDS patients (46) due to the heterogeneous distribution of regional perfusion in acutely injured lungs (38, 55). It is important to note that the variable Ki, the net 18F-FDG uptake rate, measures the amount of tracer transferred from blood to tissue and not simply the activity of the tracer within a ROI. We recently found a relationship between regional lung 18F-FDG uptake and regional perfusion in a sheep model of endotoxemia (14), likely associated with regional delivery of endotoxin. Our regional 18F-FDG-uptake results, consistent with our histological findings, suggest that changes in neutrophil trafficking due to surfactant depletion and mechanical ventilation bound by accepted clinical limits can produce an increase in local neutrophilic inflammation even when perfusion to surfactant-depleted regions is reduced.

Ventilation to the lavaged lungs was markedly lower (23%) than to the nonlavaged lungs, consistent with the lower compliance resulting from surfactant depletion (31). Consequently, tidal volume was unevenly distributed, with a lower fraction directed to lavaged lungs. Thus, if volutrauma were present, those regions would be less exposed to it. This suggests that low-volume mechanisms were likely more relevant in the production of increased 18F-FDG uptake in lavaged regions.

18F-FDG uptake in nonlavaged regions. Interestingly, there was no obvious increase of KiT nor histological lung injury after 4 h of mechanical ventilation in dependent regions of the nonlavaged lungs (Fig. 3B), although those were also poorly aerated and, thus, potentially subject to concentration of stresses (34). The finding was corroborated by the similarity of mean Ki in poorly and normally aerated regions of the nonlavaged lung (Fig. 4A). Ki values in nonlavaged regions were also similar to measurements in normal areas of previous studies (~0.002/min), suggesting no significant metabolic activation (14, 39, 53). Of note, those dependent nonlavaged regions were exposed to higher perfusion of blood with increased neutrophil counts (Fig. 6A) and to higher ventilation than the low compliance and less perfused lavaged lung.

On the basis of the discussion above, those results could be explained by the mechanical and nonmechanical effects of normal surfactant, reducing regional forces and inflammation (4, 58). The finding also indicates a significantly different metabolic response of normal vs. surfactant-depleted lung areas within the same animal to equal levels of mechanical ventilation pressures, even when normal areas are in unfavorable perfusion and ventilation conditions.

Limitations. Limitations of the used imaging techniques have been discussed previously in detail (6, 16). Given that the 18F-FDG signal is based on uptake of a glucose analog, any other cellular and noncellular factors leading to accumulation of the tracer within the field of view could compromise the specificity of the measurement. In conditions of ALI, previous studies suggested that the majority of the 18F-FDG signal is associated with neutrophilic inflammation (12, 13, 28, 39, 69). Nevertheless, a number of other cells are known to show increased 18F-FDG uptake such as malignant cells, endothelial cells, macrophages, and lymphocytes (16). Increased tissue edema would also increase the volume of distribution of 18F-FDG within the field of view, a factor that can be accounted for through appropriate modeling (52). Finally, the Na+/glucose transporter at the apical membrane of alveolar epithelial cells could influence 18F-FDG uptake due to its increased activity when plasma glucose enters the alveoli (15). Future studies will be required to accurately partition the contribution of these factors to the 18F-FDG signal.

There is no study assessing the quantitative relationship between degrees of cyclic recruitment and their respective effects on the functional measurements using PET. We believe that this factor did not play a major role in the current results because 1) previous studies including lavaged lungs showed good correlation between PET-derived methods and global gas exchange measurements (40, 64) and 2) cyclic recruitment was likely not a predominant effect in this study, as discussed above.

On the histological methods, quantification of neutrophils was performed by counting in tissue sections, a method prone to inaccuracies in identifying interstitial and capillary neutrophils. Although these methods were enough to provide confirmatory data in the current work, immunohistochemical techniques should be used if accurate quantification would be required. Additionally, neutrophil counts were not corrected for regional lung inflation, implying that more collapsed regions could have higher neutrophil counts. This potential bias was minimized by homogenization of lung expansion during fixation when lungs were recruited, positioned vertically, and filled with fixative to a pressure of 27 cmH2O.

The residual saline from lung lavage [estimated volume = 400 ml (300–600)] could have contributed to the observed increases in gas exchange in addition to surfactant depletion. There is considerable resorption of alveolar fluid in the lungs, which is magnified by mechanical ventilation with PEEP (23, 32). Furthermore, previous work in saline lavaged supine and prone sheep showed that a sizable amount of the regional gas exchange dysfunction is resolved by the prone position, irrespective of its application before or after the supine position (45). Such findings suggest that surfactant depletion and not
residual saline is the predominant contributor to the observed dysfunction (40, 45).

In conclusion, PET-measured 18F-FDG uptake was heterogeneously increased in surfactant-depleted lung regions after only 4 h of supine mechanical ventilation bounded by clinical pressure limits. This increase was predominantly located in poorly aerated dependent regions of the lavaged lung, although lavage was conducted to create homogeneous surfactant deple
tion throughout that lung and despite reduced relative perfusion and ventilation to the dependent lavaged areas. Increased regional cellular metabolic activity including neutrophilic inflammation due to magnified regional mechanical forces, potentially interacting with reduction of alveolar host response due to surfactant depletion, likely explain the increase in 18F-FDG uptake. In contrast, normally aerated nondependent regions exhibited similar 18F-FDG uptake in both lungs. Such findings support the use of 18F-FDG uptake measurements for the early noninvasive assessment of regional metabolic/inflam-
matory changes due to surfactant depletion during mechanical ventilation in the heterogeneously perfused and aerated lung.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grant HL-5R01HL086827. G. Musch received salary support from 5K08HL076464, N. de Prost received a scholarship from the Fondation Recherche Médicale (FRM) and from the College des Enseignants de Pneumologie (CEP) with the support of AstraZeneca, and M. R. Tucci received a scholarship from CAPES (Brazilian Ministry of Education).

DISCLOSURES

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

REFERENCES


5. Blanch L, Van der Kloot TE, Youngblood AM, Murias G, Naveira A, Adams AB, Romero PY, Nahum A. Application of tracheal gas infla-


12. Chen DL, Schuster DP. Positron emission tomography with [18F]fluoro-


30. Lachmann B, Robertson B, Vogel J. In vivo lung lavage as an experi-


