Extended high-frequency partial liquid ventilation in lung injury: gas exchange, injury quantification, and vapor loss

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There is an emerging consensus that ventilator-induced lung injury is a crucial consideration in the management of patients with respiratory failure (33). Lung protective ventilatory strategies have been shown to influence the elaboration of inflammatory cytokines in alveolar lavage samples from adults with acute lung injury (27). Furthermore, adoption of lung-protective ventilatory techniques has been associated with improved outcomes in two recent clinical trials (1, 2).

Both high-frequency oscillatory ventilation (HFOV) and partial liquid breathing techniques have been advanced as offering lung protection by improving lung mechanical properties as well as limiting ongoing lung inflammation. HFOV, when utilized with an optimal lung volume strategy, has been shown to improve histopathological outcomes in animal models as well as limit the elaboration of proinflammatory cytokines (14, 18, 20, 38).

The clinical experience with high-frequency oscillation (HFO) has suggested a decreased incidence of chronic lung disease in premature newborn infants (6, 11, 28) and also in pediatric patients with acute respiratory distress syndrome (3) when an optimal lung volume strategy is utilized. Furthermore, despite early disappointment in the use of high-frequency techniques in adults (5), there is now renewed interest in the application of high-frequency ventilation in adults with lung injury (9, 10, 12, 21).

Partial liquid breathing techniques have also been shown to improve the histopathological outcomes in animal models of acute lung injury (13, 25, 31, 40). Recent investigation has suggested that high-frequency techniques may act synergistically with liquid ventilation to provide enhanced lung protection in the management of the acutely injured lung (8, 35, 37). We sought to extend these observations by examination of gas exchange, histopathological outcomes, and vapor loss characteristics in an animal model of acute lung injury managed with HFO-partial liquid ventilation (PLV).

MATERIALS AND METHODS

Animal preparation. This protocol was approved by the Animal Care and Use Committee of Children’s Hospital, and the animals were handled according to the Guidelines for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Council [DHHS publication No. (NIH) 85-23, 1985]. Nineteen healthy pigs (mean...
weight 28.9 ± 3.1 kg) were studied. Four animals did not survive the induction of lung injury; therefore, data from 15 animals were available for analysis. Animals were sedated with intramuscular ketamine 10 mg/kg, xylazine 1 mg/kg, and atropine 0.2 mg/kg. Animals were orally intubated with a cuffed 6.0-mm-ID Hi-Lo endotracheal tube (Mallinckrodt, Glendale, NY) and secured in the supine position. Muscle relaxation was produced with a 0.2 mg/kg intravenous bolus of pancuronium and maintained with a continuous infusion at 0.3 mg−kg−1·h−1; anesthesia was initially maintained with a continuous infusion of fentanyl at 25 μg·kg−1·h−1 and midazolam at 1.25 mg·kg−1·h−1, which was adjusted as needed during the experiment. The animals were mechanically ventilated with a Servo 900C ventilator (Siemens, Solna, Sweden) in a volume-cycled mode with the following settings: inspired oxygen fraction (FiO2) 1.0, tidal volume (Vt) 10 ml/kg, positive end-expiratory pressure (PEEP) 5 cmH2O, inspiratory-to-expiratory (I:E) ratio of 1:2 with rate (RR) adjusted to maintain arterial PCO2 (PaCO2) of 40 − 5 Torr (5.3 ± 0.7 kPa). A catheter was placed in the femoral artery for pressure monitoring and sampling for blood-gas analysis. The right femoral vein was cannulated with a 7.5-Fr Swan-Ganz thermodilution catheter (Baxter Healthcare, Irvine, CA) that was guided into the pulmonary artery and maneuvered into the wedge position. Hemoglobin oxygen saturation was monitored via pulse oximetry (Novametrix Oxypleth, Wallingford, CT). Monitoring also included continuous electrocardiography and continuous display of the pressure waves obtained from the catheters in the femoral artery and a branch pulmonary artery.

Lung injury. After intubation and instrumentation, repetitive saline lavage was performed with the use of isotonic saline (30 ml/kg) warmed to body temperature until a uniform degree of gas-exchange impairment was achieved: arterial oxygen saturation from pulse oximetry (SpO2) ≥ 90% on an FiO2 of 1.0. Ventilation was provided during this period and in the immediate postlavage period by using volume-cycled mechanical ventilation at preinjury settings with rate adjusted to maintain PaCO2 of 40 ± 5 Torr (5.3 ± 0.7 kPa). After surfactant depletion via lavage had been performed, the lung injury was amplified over a 4-h period by subjecting animals to cyclic alveolar distension and derecruitment, as follows: Synchronized Intermittent Mechanical Ventilation/Volume Control: Vt 15 ml/kg, PEEP 2 cmH2O, I:E 1:2, FiO2 1.0, RR adjusted to target PaCO2 40–50 Torr (5.3–6.6 kPa).

Lung recruitment using HFOV. After the induction of lung injury, all animals were converted to HFOV (Sensor Medic 3100A, Sensor Medic Critical Care, Yorba Linda, CA). During this period, an attempt was made to optimize lung volume by using an aggressive strategy designed to increase mean airway pressure until oxygenation targets were achieved: SpO2 ≥ 90% on an FiO2 of ≤0.60. HFO was instituted by using a mean airway pressure 10 cmH2O greater than that used during conventional ventilation and increased until oxygenation targets were achieved. The power setting of the oscillator was adjusted to maintain a peak-trough pressure difference (ΔP) that achieved a PaCO2 between 45 and 55 Torr (6.0–7.3 kPa) at a frequency of 7 Hz. The inspired oxygen concentration remained 0.6 for the remainder of the experiment. After lung recruitment and stabilization on HFO for 60 min, animals were then randomly assigned to one of three treatment groups: HFO-gas control, HFO-PLV compartmental perfubron (PFB) [PLV(C)], and HFO-PLV uniform PFB [PLV(U)].

Initial PFB administration. HFO-PLV was initiated with the instillation of PFB via an endotracheal tube sideport adapter at a rate of 0.5 ml·kg−1·min−1 to achieve a dose of 10 ml/kg. The administration rate was determined in pilot work and is the fastest administration rate possible without incurring reflux of PFB into the HFOV circuit. At the onset of the dosing interval, frequency was reduced in all groups from 7 to 4 Hz; no other changes were made to ventilator settings during the dosing period. In the animals assigned to the PLV(U) group, 5 ml/kg PFB were administered in the supine position over 10 min; animals were maneuvered into the prone position over 10 min, dosed with a second 5 ml/kg PFB over 10 min, and returned to the supine position after 10 min. In the animals assigned to the compartmented-PFB group, all PFB was administered in the supine position in two 5 ml/kg aliquots, as in the PLV(U) group; however, a 10-min interval was observed before and after the second aliquot. The animals in the PLV(C) group were not repositioned. Once dosing was completed, the mean airway pressure was adjusted to achieve a SpO2 of 90 ± 2% with a FiO2 of 0.6. The five control animals that were not dosed with PFB underwent exactly the same frequency change from 7 to 4 Hz at the onset of the dosing interval and were not repositioned. As in the PFB-treated animals, the mean airway pressure during this period was adjusted to achieve a SpO2 of 90 ± 2% with an FiO2 of 0.6, and no manipulation of the peak-trough ΔP was made.

Quantifying PFB loss. After the initiation of PLV, intrapulmonary PFB losses were estimated by continuous quantification of both the PFB vapor content and the exhaust flow rate from the HFOV circuit. A thermal mass flowmeter (model 4040, TSI, St. Paul, MN) was interfaced at the exhauster outlet of the HFOV circuit, yielding continuous data, and the expiratory limb of the ventilator circuit was heated to prevent any PFB vapor condensation. PFB vapor content of exhaust gas was quantified via infrared vapor (IR) analysis by using a device custom designed for this purpose (Alliance Pharmaceutical, San Diego, CA) and is described in detail elsewhere (19). Briefly, the specificity of this device for the IR detection of PFB in the presence of oxygen, carbon dioxide, nitrogen, and water vapor has been confirmed, and, additionally, IR quantification and gravimetric quantification (by weight loss) of PFB evaporative loss correlate well both in a simulator device and in swine over a large range of vapor loss rates (19). A custom couple captured all exhaust gas from the HFOV circuit and provided access for continuous sidestream sampling via a heated umbilical tube through which gas was pumped to the optical cell of the IR instrument, yielding IR absorbance data and thus vapor pressure for PFB (PFPB), converted to an absolute PFB concentration (CFPB) (mg/l) after generation of a calibration curve for PFB, according to the idealized form of the Beer-Lambert law, where absorbance (A) is defined

\[ A = -\log \frac{T}{T_0} \]  

where T/T0 is the normalized digital IR transmission data. The Beer-Lambert law also relates absorbance and concentration (C) linearly along the slope k

\[ \frac{T}{T_0} = e^{-kC} \]  

Second-order effects specific to the optical cell in the IR device were incorporated in the above formula by using a quadratic form

\[ \frac{T}{T_0} = e^{-(aC^2 + bC)} \]  

where a and b were best fit coefficients for a specific optical cell. With generation of the above data, instantaneous PFB vapor loss were assessed as follows

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PFB evaporative loss (mg/min)  
\[ = C_{PFB} \cdot V_e (l/min) \cdot \Delta t (min) \]  
where \( V_e \) is minute ventilation and \( t \) is time.

The exhaust gas stream was sampled every 30 s, generating a series of instantaneous PFB vapor loss rates and also yielding cumulative PFB vapor loss, which was tracked as a running total.

**Maintenance PFB dosing.** After the initial PFB dosing, vapor loss was monitored, and no PFB redosing was undertaken in the animals assigned to the uniform-PFB group. In the animals assigned to the compartmented-PFB group, however, intrapulmonary PFB volume was maintained by adherence to a redosing schedule: every 2 h the evaporative PFB loss quantified over the preceding 2-h interval was delivered in the supine position at a rate of 0.5 ml.kg\(^{-1}\).min\(^{-1}\). We chose to deliver the replacement dose in a bolus, rather than continuously, to limit time that PFB was present in the large airways. This minimized vapor loss of the replacement PFB itself and permitted selected monitoring of vapor loss from the alveolar PFB pool in the intervals between PFB redosing.

**HFO management during HFO-PLV.** HFO management was identical in all groups over the 15-h HFO-PLV study period. All animals were maintained with frequency set at 4 Hz and an I:E of 1:2. The power setting of the oscillator was increased in all groups over the 15-h HFO-PLV study period. The power setting of the oscillator was identical in all groups over the 15-h HFO-PLV study period.

**Data collection.** Baseline measurements were made 15 min after the induction of anesthesia and were repeated every 30 min after induction of lung injury. After the completion of PFB dosing, data were recorded every 15 min for the first hour and then hourly until the termination of the experiment.

Data collection was performed in all groups at the same time points and included arterial blood-gas tensions, hemodynamic parameters, and ventilator settings. The directly measured hemodynamic parameters included heart rate, femoral artery pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, and cardiac output by thermodilution. Pulmonary capillary wedge pressure was recorded and maintained to achieve the oxygenation targets: \( S_{paO_2} = 90\%\%\) on an \( F_{IO2} \) of \( \leq 0.60 \). 

**Lung preparation.** After termination of the 20-h experimental protocol, the anesthetic was deepened and the lungs were exposed via median sternotomy; the right lung was prepared for histopathological analysis of lung injury, and the left lung was prepared for tissue banking and myeloperoxidase assay. The main pulmonary artery trunk was exposed and isolated. A 10-Fr catheter was placed in the main pulmonary artery trunk and secured. To protect the right lung from saline perfusion, the right hilum was cross-clamped. The superior vena cava and inferior vena cava were isolated and ligated at their entry to the right atrium. Simultaneously, an incision was made in the left atrium, and the left lung was perfused with normal saline via the catheter in the main pulmonary artery trunk until the efflux from the left atrium was clear. The left hilum was then divided, the lung was removed expediently from the thoracic cavity, and multiple 2 \( \times \) 2-cm tissue blocks from all lung regions were fast frozen in liquid nitrogen before storage at \(-80 \)°C. The animal was then euthanized with a lethal dose of 15 mg/kg pentobarbital intravenously.

The trachea was then divided at its entry to the thoracic cavity, the right main pulmonary artery and veins were ligated and divided, and the lung was dissected free and removed from the thoracic cavity. The lung was placed in a 10% formalin bath and inflated with 10% formalin to a pressure of 25 cmH\(_2\)O via a catheter secured in the right mainstem bronchus and fixed over a 48-h period. Tissue blocks for sectioning were then prepared from dependent and nondependent regions of the lower, middle, and upper lobes of the right lung (6 tissue blocks per animal, 72 total blocks). The tissue was sliced, and slides were prepared from each block.

**Lung injury scoring system.** Hematoxylin and eosin-stained samples underwent review by a blinded pathologist using a semiquantitative scoring system to quantify the degree of lung injury. The following variables were scored: airway plug (mucus and cell debris within airway lumens), atelectasis (partially to completely collapsed alveoli), alveolar and interstitial inflammation (neutrophils in alveolar spaces and interstitium), edema (alveoli filled with homogeneous eosinophilic material), alveolar distension (expansion of alveoli to 1.5–2 times normal size), hemorrhage (free red blood
cells within alveoli), necrosis, and hyaline membrane formation. For each component, the percentage of injured lung was recorded. Severity of injury was then reduced to an integer from 0 (no injury) to 10 (100% injury).

**Lung myeloperoxidase activity.** Myeloperoxidase activity, indexed by protein content of lung tissue samples (16), was determined as a measure of lung neutrophil sequestration. Myeloperoxidase activity was expressed as change in optical density per minute per microgram protein.

**Statistical analysis.** Gas exchange, hemodynamic parameters and pulmonary mechanics data were compared by using repeated-measures ANOVA with PFB dose strategy as the main effect and time as the within-subject factor. The effects of time and the interaction between PFB dose strategy and time were evaluated by using two-way repeated-measures ANOVA; post hoc analysis of between-groups differences was made by use of Fisher’s paired least-significant difference test. At the time points immediately before HFOV lung recruitment and PFB dosing, all groups were compared by using a two-sample Student’s t-test. Lung injury scores were compared with Kruskall-Wallis ANOVA; post hoc analysis of between-groups differences of individual lung injury scores components was performed by using Dunn’s test for multiple comparisons. A two-tailed P value <0.05 was considered statistically significant throughout. Both linear and logarithmic curve-estimation regression models were utilized to plot and generate regression lines and the coefficient of determination (R²) for vapor loss data. Analysis was performed by using SPSS version 9.0 (SPSS, Chicago, IL) and StatView version 4.5.1 (Abacus Concepts, Berkeley, CA).

**RESULTS**

Nineteen healthy pigs were anesthetized, instrumented, and prepared as outlined above. Fifteen animals survived the induction of lung injury and completed the protocol. Baseline measurements of gas exchange, hemodynamic parameters, and pulmonary mechanics data were made after induction of anesthesia and then as described. Unless otherwise stated, all data are presented as means ± SD. The time points for data collection were the same for all groups of animals.

For all groups, saline lavage produced a significant drop in both arterial $\text{PO}_2$ ($\text{PaO}_2$) and dynamic lung compliance ($C_L < 0.001$) and an increase in the oxygenation index ($\text{PaO}_2/P_f < 0.001$) (Table 1). Lavage also led to a significant increase in cardiac index ($P = 0.005$) and pulmonary vascular resistance index ($P = 0.002$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Post-lavage</th>
<th>Post-HFO Recruitment</th>
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<tbody>
<tr>
<td></td>
<td>Gas</td>
<td>PLV</td>
<td>Gas</td>
</tr>
<tr>
<td>Paw, cmH$_2$O</td>
<td>4.4 ± 0.6</td>
<td>4.3 ± 1.3(U)</td>
<td>8.6 ± 1.7</td>
</tr>
<tr>
<td>Cdyn, ml/cmH$_2$O</td>
<td>24.2 ± 4.3</td>
<td>23.5 ± 8.2(U)</td>
<td>12.4 ± 2.6</td>
</tr>
<tr>
<td>$\text{PaO}_2$, Torr</td>
<td>524 ± 42</td>
<td>554 ± 52(U)</td>
<td>64 ± 18</td>
</tr>
<tr>
<td>OI</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.3(U)</td>
<td>14.5 ± 5.1</td>
</tr>
<tr>
<td>$\text{Paco}_2$, Torr</td>
<td>50 ± 4</td>
<td>52 ± 4(U)</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>7.45 ± 0.06</td>
<td>7.42 ± 0.07(U)</td>
<td>7.31 ± 0.08</td>
</tr>
<tr>
<td>CI, lmin$^{-1}$·kg$^{-1}$</td>
<td>0.080 ± 0.01</td>
<td>0.094 ± 0.03(U)</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td>PVRI, mmHg·min$^{-1}$·l$^{-1}$</td>
<td>110 ± 37</td>
<td>106 ± 32(U)</td>
<td>156 ± 37</td>
</tr>
</tbody>
</table>

Values are means ± SD for gas control ($n = 5$) and compartmented (C; $n = 5$) and uniform (U; $n = 5$) partial liquid ventilation (PLV) perfusion-filling groups of pigs before dosing. HFOV, high-frequency oscillation; Paw, mean airway pressure; $\text{PaCO}_2$, arterial partial pressure of carbon dioxide; Cdyn, dynamic compliance; OI, oxygenation index; $\text{PaO}_2$, arterial partial pressure of O$_2$; CI, cardiac index; PVRI, pulmonary vascular resistance index.
each of four dosing intervals. In the gas control group \((n = 5)\), we noted a modest increase in \(\text{PaO}_2\), enabling a small reduction in the Paw over the period of sham dosing; oxygenation index was thus unchanged in the control animals by the end of the sham dosing interval (Table 2). \(\text{PaCO}_2\) was stable throughout the dosing interval. The initiation of HFO-PLV in the PLV(U) group, PaO2 improved dramatically after the changes were noted in the PLV(C) group (Fig. 1). In the index within this group over time (Fig. 1). Similar cant decrease in the oxygenation over the course of the 15-h experimental period, which \(1\), permitting progressive reduction in Paw (Fig. 1) 

Gas exchange during HFO-PLV. After PFC dosing, the only experimental manipulation consisted of adjustment of Paw to achieve a \(\text{SpO}_2\) of \(90 \pm 2\%\) with \(\text{FiO}_2\) of 0.6. No manipulation of the peak-trough \(\Delta P\) or change in oscillator frequency was made. In the gas control group, we noted a steady increase in \(\text{PaO}_2\) (Fig. 1), permitting progressive reduction in Paw (Fig. 1) over the course of the 15-h experimental period, which produced a significant decrease in the oxygenation index within this group over time (Fig. 1). Similar changes were noted in the PLV(C) group (Fig. 1). In the PLV(U) group, \(\text{PaO}_2\) improved dramatically after the dosing period \((P < 0.01)\) (Fig. 1), permitting a progressive reduction in Paw. These changes were reflected in a substantially lower oxygenation index in the PLV(U) group, although it was not sustained beyond 5 h. Comparison with repeated-measures ANOVA revealed significant differences between the groups over time in \(\text{PaO}_2\) \((P < 0.001)\) and oxygenation index \((P = 0.02)\).

**Histopathology.** The eight-component lung injury scoring system was used to create an injury score for the dependent and nondependent lung. The regional scores were combined to yield a cumulative lung injury score for the entire lung. Differences in the individual components of the lung injury score were also examined for between-group differences. The cumulative injury score in the gas control group was \(-25\%\) higher than in either of the PLV groups; this trend was not significant (Fig. 4). In dependent lung, there was a 30% reduction in injury score in the PLV(U) group and a nearly 50% reduction in injury score in the PLV(C) group \((P = 0.045)\) (Fig. 4). In nondependent lung, injury scores in the gas control and the PLV(C) group were essentially the same (Fig. 4). A reduction of \(-30\%\) was noted in the PLV(U); this trend was not significant (Fig. 4). In examining components of the cumulative scores, a significant reduction in alveolar distension and atelectasis was noted in both PLV groups (Fig. 4).

When individual score components were examined separately by lung region, a significant reduction in atelectasis was noted in both PLV groups in dependent lung (Fig. 4). In nondependent lung, a significant reduction in alveolar distension was noted in both PLV groups (Fig. 4).

**Myeloperoxidase assay.** Myeloperoxidase activity indexed by weight to tissue protein content for both PLV groups was compared both to gas control for the lung as a whole and in a separate analysis for dependent and

### Table 2. Gas exchange data

<table>
<thead>
<tr>
<th>Variable</th>
<th>10 min (Gas)</th>
<th>PLV</th>
<th>20 min (Turning Prone)</th>
<th>Gas</th>
<th>PLV</th>
<th>30 min (Prone)</th>
<th>Gas</th>
<th>PLV</th>
<th>40 min (Turning Supine)</th>
<th>Gas</th>
<th>PLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paw, cmH2O</td>
<td>24.2 ± 1.48</td>
<td>27.0 ± 6.5 (C)</td>
<td>23.8 ± 1.09</td>
<td>26.0 ± 6.2 (C)</td>
<td>23.6 ± 1.14</td>
<td>26.4 ± 6.3 (C)</td>
<td>23.0 ± 1.7</td>
<td>26.2 ± 6.9 (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{PaO}_2), Torr</td>
<td>89.6 ± 40.5</td>
<td>110.2 ± 25.6 (C)</td>
<td>98.2 ± 42.1</td>
<td>126.8 ± 87.4 (C)</td>
<td>96.0 ± 36.7</td>
<td>127.2 ± 83.4 (C)</td>
<td>111.0 ± 36.7</td>
<td>110.0 ± 43.6 (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OI</td>
<td>16.2 ± 7.88</td>
<td>16.6 ± 7.03 (C)</td>
<td>15.5 ± 6.1</td>
<td>16.5 ± 8.7 (C)</td>
<td>14.7 ± 4.3</td>
<td>17.1 ± 7.9 (C)</td>
<td>16.2 ± 8.1</td>
<td>17.1 ± 8.7 (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{PaCO}_2), Torr</td>
<td>46.0 ± 3.6</td>
<td>49.3 ± 10.2 (C)</td>
<td>42.2 ± 4.4</td>
<td>44.0 ± 2.5 (C)</td>
<td>44.0 ± 1.7</td>
<td>43.6 ± 2.7 (C)</td>
<td>43.2 ± 3.9</td>
<td>41.4 ± 4.1 (C)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; \(n = 5\) pigs in each of the gas control, PLV(C), and PLV(U) groups during the 40-min dosing interval.
nondependent lung individually. For whole lung, there was a similar 65% reduction in myeloperoxidase activity in both PLV groups (P < 0.01) (Fig. 5). In dependent lung, myeloperoxidase activity was attenuated by 69% in the PLV(C) group (P < 0.02) and by 64% in the PLV(U) group (P < 0.002) (Fig. 5). In nondependent lung, myeloperoxidase activity was attenuated by 45% in the PLV(C) group (P < 0.02) and 52% in the PLV(U) group (P < 0.03) (Fig. 5).

**DISCUSSION**

In an animal model of acute lung injury, we examined gas exchange, histopathological outcomes, and myeloperoxidase activity during HFO of the perfluorocarbon-treated lung using two different dosing regimens. We also quantified perfluorocarbon vapor loss over the course of the 15-h HFO-PLV study protocol after the 40-min dosing interval. One treatment group...
received 10 ml/kg of perfluorocarbon in the supine position and replacement of evaporated perfluorocarbon, which was quantified by using an inline IR device. We chose this dose on the basis of recent data suggesting a greater incidence of barotrauma in animals treated with larger doses of perfluorocarbon (7). In a second treatment group, animals received 5 ml/kg in the supine position and a second 5 ml/kg in the prone position. After completion of dosing, these animals were returned to the supine position and received no further perfluorocarbon. This strategy was based on our previous work, which suggested improved alveolar delivery of perfluorocarbon, particularly to nondependent lung regions, when rotational dosing was utilized (4). We did not replace evaporative losses in this group, to test the hypothesis that improved alveolarization of the drug would maximize its effectiveness, particularly regarding anti-inflammatory effects (31, 39). Control animals were managed with HFO alone with sham dosing to simulate the derecruitment effects that may be produced by the manipulation of airway pressure during the instillation of perfluorocarbons.

Oxygenation, as assessed by the arterial oxygen tension and the oxygenation index, was transiently improved in the group treated with perfluorocarbon by using rotational positioning. However, 3 h after the animals were returned to the supine position, these improvements in oxygenation were not evident compared with the other two study groups. The absence of a persistent improvement in oxygenation in the rotationally dosed animals may be due to the beneficial effects of the prone position (24), which were no longer present after 3 h in the supine position. It is also possible that evaporative losses, which were not replaced in this group, interrupted the positive effects on oxygenation that were produced by the combination of rotational dosing, perfluorocarbon, and HFOV. Such a relationship was also noted by Jeng et al. (15), who, while quantifying PFB evaporative loss during conventional mechanical ventilation, demonstrated a positive correlation between \( \text{PaO}_2 \) and residual intrapulmonary PFB volume (15). Indeed, there appeared to be a drop in oxygenation below a critical volume of PFB remaining in the lungs, suggesting a role for monitoring PFB vapor loss during PLV. Furthermore, we suspect that the optimum residual PFB volume that should be maintained in the lungs (as does the need for PEEP) may both vary with the severity of lung injury between patients as well as vary over time during the course of illness; precise titration of intrapulmonary PFB volume in the clinical setting would necessitate monitoring of PFB vapor loss. There were no other differences noted in \( \text{PaCO}_2 \), arterial pH, or hemodynamic variable between groups.

Fig. 3. Plots representing body weight-normalized hourly PFB vapor loss (A), cumulative body weight normalized PFB vapor loss pressure (B), and bivariate scattergram of \( \text{PaO}_2 \) and cumulative weight-normalized vapor loss from pooled uniform PLV animals (C). Curve-filling procedure generated a logarithmic decay curve for PLV(U) group. Regression lines and the coefficients of determination (\( R^2 \)) are shown. Values are means ± SD.
We did note a significant improvement in the histopathological outcomes in the animals treated with the combination of perfluorocarbon and HFO. In the dependent lung, the degree of atelectasis was significantly lower in both perfluorocarbon-treated groups. Atelectasis and “atelectrauma,” produced by the repetitive reopening of lung units that close at end-expiration in dependent lung, are important considerations in the design of lung-protective ventilatory strategies (33). The combination of HFOV and perfluorocarbon appears to reverse atelectasis more efficiently than high-frequency ventilation alone. In the nondependent lung, the rotationally dosed animals had a significantly lower incidence of alveolar necrosis compared with the other two groups. When the lung injury data for the whole lung were analyzed, both perfluorocarbon-treated groups had less alveolar distension and less atelectasis compared with the HFO-treated controls. This is an important finding because one of the concerns when using aggressive increases in mean airway pressure to achieve an optimal lung volume during HFO is the overdistension of more compliant lung regions. This phenomenon appears to be mitigated by the combination of perfluorocarbon and HFOV. When the components of the lung injury score were combined, the improved histopathological outcomes were most evident in the dependent lung, which likely relates to the gravity-dependent distribution of the perfluorocarbons in the lung (4, 26).
Neutrophil-mediated lung injury is an important and early step in the sequence of acute lung injury (17, 18). Both HFOV and PLV have been associated with decreased pulmonary migration of neutrophils (18, 30, 36). In our study, myeloperoxidase activity, which represents the presence of the neutrophil enzyme, was also shown to be reduced in the perfluorocarbon-treated groups, and the reductions in myeloperoxidase activity were evident in both dependent and nondependent lung in both perfluorocarbon-treated groups. We also indexed myeloperoxidase activity to protein content in each sample, which may have reduced the variability of the assay (31) and enhanced our ability to detect lung protection during the combination of HFO and PLV.

We also examined vapor loss rates in both groups of perfluorocarbon-treated animals using an IR device that sampled gas in the expiratory limb of the ventilator circuit. Not surprisingly, the animals managed in the supine position had a higher cumulative vapor loss rate than the rotationally dosed animals. This likely relates directly to the fact that the supine animals were managed with regular replacement of evaporative perfluorocarbon losses and thus received a higher cumulative perfluorocarbon dose. Replacement dosing of perfluorocarbon compounds has been noted by others to produce increased perfluorochemical saturation of the expired gas and increased hourly loss rates, which may be cumulative (22). In our study, the animals managed with rotational dosing were noted to have a higher hourly vapor loss rate during the first 3 h of measurement. In fact, the vapor loss rate measured in these animals (peak 2.8 ml·kg⁻¹·h⁻¹) is significantly higher than has been reported in animals managed with conventional mechanical ventilation in the supine position (0.8–1.2 ml·kg⁻¹·h⁻¹) (23). This is most likely related to the high degree of alveolarization achieved in the rotationally dosed animals, which resulted in a large air-liquid interface and greatly increased vapor loss rate (32). The vapor loss rate measured in the animals treated with perfluorocarbon in the supine position ranged between 1.0 and 1.5 ml·kg⁻¹·h⁻¹, with the higher vapor loss rates noted in the later phases of the protocol. In these animals, the vapor loss rate may have been influenced by regular replacement of perfluorocarbon on the basis of measured losses that resulted in a stable rather than diminishing gas-liquid surface area. Because hourly vapor loss rate appears to be related to the size of the gas-liquid interface, quantification of perfluorocarbon in expired gas may provide useful information regarding the degree of alveolarization of the drug.

A number of investigators have examined the combination of high-frequency ventilation and perfluorochemical administration in animal models of lung injury. Smith and colleagues (34) described the effects on gas exchange of PFB administration in combination with a variety of high-frequency devices in neonatal piglets treated with saline lavage. In an important follow-up study, the same group of investigators examined the effect of combined high-frequency ventilation and PFB administration on lung pathology in the same model (35). There was evidence of enhanced lung protection in both dependent and nondependent lung regions.

Sukumar and coworkers (37) described the changes in hemodynamics and gas exchange during high-frequency ventilation of PFB-treated preterm lambs compared with a control group ventilated with HFO. Histopathological analysis in a small subgroup of the subjects suggested increased alveolar air spaces and preservation of alveolar morphometry in the animals managed with a combination of PFB and HFO (37). The interpretation of these data is complicated by the absence of attempts to optimize lung volume in each animal and the use of the same mean airway pressures in PFB-treated and non-PFB-treated animals.

In a recent study in larger animals, we described the effects of PFB administration during HFOV on gas exchange, hemodynamic function, and lung histopathology (8). Healthy swine underwent repetitive saline lavage and were then randomized to HFO or combined HFO-PLV. Lung volume was not optimized in either treatment group, and the HFO-PLV animals received a dose of 30 ml/kg of PFB. There were no differences in the two groups of animals regarding gas exchange or hemodynamic function. However, the animals treated with the combination of HFO and PFB manifested significantly less severe atelectasis after 2 h (8).

In the present study, we attempted to extend our preliminary findings and included a rotationally dosed group to elucidate potential benefits of maximal alveolarization of PFB (4). We ensured a uniform and profound degree of lung injury by repetitive saline lavage to a physiological end point, followed by 4 h of injury amplification using large Vt values and low-end expiratory pressures. We acknowledge that all animal models of lung injury have their shortcomings when attempting to elucidate the complex sequence of events that occur during mechanical ventilation of the acutely injured lung (29). We also chose to emulate the use of HFOV in the clinical setting by manipulation of the mean airway pressure to achieve an optimal Pao₂-to-FIO₂ ratio, the so-called “optimal lung volume strategy” (3). Our study protocol included 15 h of mechanical ventilation after the induction of lung injury and perfluorocarbon dosing to maximize our ability to detect differences in histopathological outcomes between the modes of ventilatory support examined in this study. Finally, we used a robust, eight-variable histopathological scoring system with the variable of interest scored for the entire lung section sampled to assess regional differences in lung injury by evaluation of both dependent and nondependent lung regions.

HFO of the perfluorocarbon-treated lung is an evolving story; in particular, there are insufficient data regarding histopathological findings during the combination of HFO and partial liquid breathing at varying doses of PFB. We found salutary effects in gas exchange, although unsustained, after uniform distribution of PFB and reduced indexes of lung inflammation in both PLV groups during HFO-PLV in our extended
protocol; similar benefit in gas exchange, mechanics, and indexes of inflammation were also found for PLV during conventional ventilation that did not translate into benefit during clinical trials. Our present data provide preliminary information that require further study in guiding optimal lung-protective strategies in the management of acute lung injury as well as providing insight regarding the utility of quantifying vapor loss as an estimate of the distribution of perfluorocarbon in the injured lung.

DISCLOSURES
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