Set Positive End-expiratory Pressure during Protective Ventilation Affects Lung Injury

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Background: The most appropriate method of determining positive end-expiratory pressure (PEEP) level during a lung protective ventilatory strategy has not been established.

Methods: In a lavage-injured sheep acute respiratory distress syndrome model, the authors compared the effects of three approaches to determining PEEP level after a recruitment maneuver: (1) 2 cm H2O above the lower inflection point on the inflation pressure–volume curve, (2) at the point of maximum curvature on the deflation pressure–volume curve, and (3) at the PEEP level that maintained target arterial oxygen partial pressure at a fraction of inspired oxygen of 0.5.

Results: Positive end-expiratory pressure set 2 cm H2O above the lower inflection point resulted in the least injury over the course of the study. PEEP based on adequate arterial oxygen partial pressure/fraction of inspired oxygen ratios had to be increased over time and resulted in higher mRNA levels for interleukin-8 and interleukin-1β and greater tissue inflammation when compared with the other approaches. PEEP at 2 cm H2O above the lower inflection point was most effective.

Conclusion: Although generating higher plateau pressures, PEEP levels based on pressure–volume curve analysis were more effective in maintaining gas exchange and minimizing injury than PEEP based on adequate oxygenation. PEEP at 2 cm H2O above the lower inflection point was most effective.

The use of lung protective ventilatory strategies have been well recognized for the management of patients with acute respiratory distress syndrome (ARDS).1–5 This approach to avoiding injury induced by the ventilator focuses on preventing overdistension and volutrauma, by the limitation of peak alveolar pressure and sufficient positive end-expiratory pressure (PEEP) to avoid end-expiratory derecruitment or atelectrauma and bio-trauma.1–3 In addition, many investigators have proposed the use of recruitment maneuvers (RMs) to first open collapsed lungs before setting PEEP.2,6–12 Results from both ARDSnet3 and Amato et al.2 have established small tidal volumes (approximately 6 ml/kg) in humans as an essential element of a lung protective ventilatory strategies. However, controversy still exists over the approach used to set PEEP.

There are several proposed methods to determining optimal PEEP level for lung protection. Some investigators have proposed setting PEEP above the lower inflection point (Pflex) on the inflation limb of the quasistatic inflation pressure–volume (P–V) curve.2,8,12 However, there is no clear evidence that this pressure prevents alveolar collapse over time or prevents lung injury revealed by histology and inflammatory response better than other approaches. Theoretically, the PEEP level needed to maintain the lung open at end expiration should be related to the deflation limb of the P-V curve,11,13,14 specifically, the point of maximum curvature (Pflex).15 Clinically, PEEP is usually adjusted according to oxygenation response and the fraction of inspired oxygen (FiO2) delivered.3,16–18

Using a lavage-injured sheep ARDS model, we compared three methods of setting the PEEP level after a recruitment maneuver: PEEP based on the Pflex, the PMC, and the arterial oxygen partial pressure (PaO2) at an FiO2 of 0.5. We hypothesized that PEEP at PPMC would be the most effective in minimizing derecruitment and as a result cause the least inflammatory response and histologic injury.

Methods and Measurements

This study was approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital.

Preparation

Twenty-nine fasted Dorset sheep (28.4 ± 4.5; weight, 23–38 kg) were studied. Anesthesia was induced with halothane by mask. The right jugular vein was cannulated, and a pulmonary artery catheter was inserted via an 8-French sheath introducer. An endotracheal tube (ID 9.0 mm) was then placed. Anesthesia was then administered intravenously with a loading dose of 25 mg/kg pentobarbital, 2 mg/kg ketamine, and 0.1 mg/kg pancuronium and maintained by continuous infusion of 2 mg · kg−1 · h−1 pentobarbital, 8 mg · kg−1 · h−1 ketamine, and 0.1 mg · kg−1 · h−1 pancuronium. A catheter was placed into a femoral artery for continuous monitoring of arterial blood pressure and blood gases (Paratrend; Diametrics Medical Inc., St. Paul, MN). An infusion of lactated Ringer’s solution (50 ml · kg−1 · h−1

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for the first hour, then 20 ml · kg\(^{-1} \cdot \text{h}^{-1}\) was administered. A heating blanket was used to maintain a core temperature of 39°C. A wide bore tube (ID 8.0 mm) was placed into the stomach by gastrostomy to insure complete abdominal decompression.

Mechanical ventilation was provided with a PB 7200ae (Nellcor Puritan-Bennett, Carlsbad, CA). Ventilatory settings after intubation were volume control, respiratory rate (RR) 15 breaths/min, tidal volume (VT) 12 ml/kg, I:E ratio 1:2, FIO\(_2\) 1.0, and PEEP 5 cm H\(_2\)O. RR was adjusted to achieve eucapnea (arterial carbon dioxide partial pressure [PaCO\(_2\)] = 35–45 mmHg). Arterial blood pressure and pulmonary artery pressure (PAP) were continuously monitored using calibrated pressure transducers (049924–507A; Argon, Maxsim Medical, Athens, TX). End-tidal carbon dioxide concentration was monitored by capnography. Airway pressure was monitored using a calibrated pressure transducer (model 45-32-871; Validyne, Northridge, CA). VT was monitored by the PB7200ae.

**Experimental Protocol**

Following instrumentation and a 30-min stabilization period, baseline parameters were obtained. Severe lung injury was then produced by bilateral lung lavage with 30-ml/kg instillations of isotonic saline warmed to 39°C, repeated every 15 min until PaO\(_2\) decreased to less than 50 mmHg. After initial stabilization, if PaO\(_2\) decreased to less than 50 mmHg, FIO\(_2\) was increased in the control group.

Once the maximum rate was set (about 8–10 ml/kg), and RR adjusted to keep PaCO\(_2\) 35–45 mmHg, provided no auto-PEEP (determined by end-expiratory pause) developed. After 15 min, another RM was performed in the same manner. RMs were repeated every 15 min until the PaO\(_2\) after RMs was constant (≤ 10% difference between 2 consecutive measurements of PaO\(_2\)) or PaO\(_2\) was 400 mmHg or greater. After the last RM, all measurements except the PV curve were repeated (post-RM).

After the post-RM measurements, an additional RM was performed, and animals were randomized into 3 groups based on method to set PEEP (table 1). In one group (n = 7), PEEP was set equal to P \(_{\text{MC}}\) on the deflation limb of the PV curve. If P \(_{\text{MC}}\) was greater than 26 cm H\(_2\)O, PEEP was set at 26 cm H\(_2\)O. In the second group (n = 7), PEEP was set 2 cm H\(_2\)O above P \(_{\text{MC}}\). If P \(_{\text{MC}}\) could not be identified, PEEP was set at 20 cm H\(_2\)O. In the P \(_{\text{MC}}\) and P \(_{\text{flex}}\) groups, FIO\(_2\) was initially set at 0.5 and adjusted in 0.1 increments every 15 min until PaO\(_2\) stabilized between 70 and 110 mmHg. In the third (control) group (n = 7), PEEP was set at 20 cm H\(_2\)O and was adjusted in 2-cmH\(_2\)O increments—decrements every 15 min to achieve the target PaO\(_2\) (70–110 mmHg) with FIO\(_2\) set at 0.5. After initial stabilization, if PaO\(_2\) decreased to less than 50 mmHg, FIO\(_2\) was increased in the control group.

All animals were ventilated as follows: PC, I:E ratio 1:1, plateau pressure (P \(_{\text{plat}}\)) less than 35 cm H\(_2\)O (VT about 8–10 ml/kg), and RR adjusted to keep PaCO\(_2\) 35–45 mmHg without the development of auto-PEEP. Once the maximum rate was set (≤ 30 breaths/min), permissive hypercapnia was allowed. After completion

*(fig. 1, time table of protocol). A stable lung injury was defined as a PaO\(_2\) change of less than 10% after 30 min. After establishing lung injury, another set of measurements, including a P-V curve, were performed (injury).

Following the injury measurements, high-pressure RMs were performed. Each RM was performed for 2 min in pressure control ventilation with peak inspiratory pressure at 60 cm H\(_2\)O, PEEP at 40 cm H\(_2\)O, RR at 10 breaths/min, FIO\(_2\) at 1.0, and an I:E ratio of 1:1. Immediately following one RM, the ventilator was reset to pressure control ventilation, peak inspiratory pressure at 35 cm H\(_2\)O, PEEP at 20 cm H\(_2\)O, I:E at 1:1, and FIO\(_2\) at 1.0, and RR (≤ 30 breaths/min) was adjusted to maintain PaCO\(_2\) 35–45 mmHg, providing no auto-PEEP (determined by end-expiratory pause) developed. After 15 min, another RM was performed in the same manner. RMs were repeated every 15 min until the PaO\(_2\) after RMs was constant (≤ 10% difference between 2 consecutive measurements of PaO\(_2\)) or PaO\(_2\) was 400 mmHg or greater. After the last RM, all measurements except the PV curve were repeated (post-RM).

**Table 1. Ventilator Settings after Recruitment Maneuver**

<table>
<thead>
<tr>
<th>PEEP</th>
<th>FIO(_2)</th>
<th>PIP/RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (_{\text{MC}})</td>
<td>Initially 0.5, adjusted by 0.1 every 15 min (target PaO(_2); 70–110 mmHg)</td>
<td>PIP ≤ 35 cm H(_2)O (VT, 8–10 ml/kg) RR ≤ 30 (target PaCO(_2); 35–45 mmHg; permissive hypercapnia if maximum targets meet)</td>
</tr>
<tr>
<td>P (_{\text{flex}})</td>
<td>Initially 20 cm H(_2)O, adjusted by 2 cm H(_2)O every 15 min (target PaO(_2); 70–110 mmHg)</td>
<td>Fixed at 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>Fixed at 0.5</td>
<td></td>
</tr>
</tbody>
</table>

PEEP = positive end-expiratory pressure; FIO\(_2\) = fraction of inspired oxygen; PIP = peak inspiratory pressure; RR = respiratory rate; P \(_{\text{MC}}\) = PEEP set at the point of maximum curvature; P \(_{\text{flex}}\) = PEEP set at the lower inflection point + 2 cm H\(_2\)O; PaO\(_2\) = arterial oxygen tension; VT = tidal volume; PaCO\(_2\) = arterial carbon dioxide tension.

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of the protocol, 4 sheep in each group were immediately killed for histologic and biologic analysis.

Measurements and Calculations

The following measurements were made at baseline, injury, post-RM, 50, 60, 120, and 180 min, and final baseline: total PEEP, peak inspiratory pressure, and \( P_{\text{plat}} \) were determined from the airway pressure. \( V_T \) was obtained from the PB7200. Mean arterial blood pressure, mean PAP, central venous pressure, and pulmonary capillary wedge pressure were measured. Cardiac output was measured by the thermodilution technique in triplicate and averaged. Pulmonary vascular resistance and systemic vascular resistance were calculated from cardiac output, mean blood pressure, mean PAP, central venous pressure, and pulmonary capillary wedge pressure.

Real-time data were recorded from the Paratrend continuous arterial blood gas analyzer. Arterial and mixed-venous blood samples were drawn for blood gas analysis. \( \text{PO}_2, \text{PCO}_2, \text{pH}, \) oxygen saturation, and hemoglobin content were assessed by a blood gas analyzer (model 282; Ciba Corning Diagnostics Corp., Norwood, MA) and a cooximeter (Radiometer Inc., Copenhagen, Denmark). Effective respiratory system compliance was calculated as \( V_T/(P_{\text{plat}} - \text{total PEEP}) \).

Pressure-Volume Curve

Quasistatic P-V curves were obtained using a 2-l syringe at injury. Before the P-V curve, 1 min of PC (peak inspiratory pressure 60 cm \( \text{H}_2\text{O} \), PEEP 5 cm \( \text{H}_2\text{O} \), RR 6 breaths/min, I:E ratio 1:1) was applied to establish a volume history. Then, 6 s after the last inflation, the lungs were inflated in steps of 50 ml for the first 200 ml, then steps of 100 ml with simultaneous measurement of airway pressure to a peak pressure greater than 50 cm \( \text{H}_2\text{O} \). Deflation of the lungs in a similar manner established the deflation limb of the P-V curve. Volume during P-V curve measurement was corrected for pressure, temperature, humidity, oxygen consumption, and carbon dioxide production. \( P_{\text{flex}} \) was identified by the crossing of tangents applied manually to the varying slopes of the inspiratory P-V curve. \( P_{\text{MC}} \) was identified by the mathematical fitting method that was previously described by Venegas et al.20,21

Preparation for Histologic and Cytologic Analysis and Isolation of Total RNA for Reverse-transcription Polymerase Chain Reaction Analysis

The lungs with pleural surface intact and trachea clamped were removed from 4 sheep in each group. The lungs were then separated into right for histologic analysis and left for cytological and cytokine analysis. For histologic analysis, a catheter was tied into the trachea, and the left main broncus were clamped and separated from the rest of lung. Right lobes clamped before separation were submersed in a container filled with Trump’s fixative (4% formaldehyde and 1% glutaraldehyde), unclamped, and maintained inflated to 20 cm \( \text{H}_2\text{O} \) with fixative.\(^{22} \) The tracheal catheter was then clamped, and the lungs were left floating in the container for 60 h, after which the lungs were cut horizontally into slices 1 cm thick in posterior-anterior order. In each slice, two blocks of 1 cm\(^3\), one from the apex and one from the base, were chosen at random and excised. From each block, three 5-\( \mu \)m slices were embedded in paraffin, cut on a microtome, mounted on slides, and stained with hematoxylin–eosin.

For sampling of cells infiltrated into the alveolar space, one catheter was tied into the left upper anterior bronchus for nondependent lung sampling, and another catheter was tied into the left lower posterior bronchus for dependent lung sampling. Lung lavage was then performed in each lobe separately. A 10-ml dose of saline was instilled in the lobe through the catheter and drained after 10 s. This procedure was repeated 5 times. Total leukocytes and neutrophils in sampled fluid from each bronchus were counted. Lavaged fluid from the nondependent and dependent lung were separately centrifuged in 3,000 rpm for 5 min. Isolation of total RNA was performed using RNeasy Kits (QIAGEN Inc., Valencia, CA). Isolated total RNA was immediately stored at \(-80\)°C.

Histologic Analysis

Quantitative examination by light microscopy was performed by two pathologists blinded to the experimental protocol and the region of sampling. Differences between pathologists were less than 10% on all parameters evaluated. The severity of injury was assessed based on 7 parameters: mean alveolar size, homogeneity index, inflammation index, alveolar hemorrhage index, foci of alveolar collapse per unit area, parenchymal ruptures per unit area, and edema index. Indices of mean alveolar size, inflammation index, and alveolar hemorrhage index represent mean values of 10 nonoverlapping fields for each slide. Foci of alveolar collapse and parenchymal ruptures per unit area and edema index were assessed in the entire slide. Homogeneity index was calculated as a SD of alveolar size of 10 nonoverlapping fields for each slide divided by mean alveolar size. Mean alveolar size or mean linear intercept was assessed at a magnification of 400\( \times \). Using a 100-point grid consisting of 50 lines of known length, mean linear intercept was calculated as the relation between the total length and the number of alveolar intercepts. Inflammation index was determined at magnification of 400\( \times \) as the relation between the...
total number of neutrophils (present in the alveolar space or septum) per field, divided by the number of alveoli intercepts in each field. Hemorrhage index was determined by the number of erythrocytes divided by the total number of alveoli containing erythrocytes per field. Foci of alveolar collapse were defined as round-shaped or plate-like areas of collapse (usually containing 50–100 identifiable air spaces at a higher magnification) and scored as the number of collapsed areas per area of tissue at a magnification of 100X. Parenchymal rupture was defined as foci of septal disruption adjacent to alveolar collapse forming intraparenchymal bubbles and scored as total number of well-defined foci of parenchymal rupture per unit area of the lung at a magnification of 100X. Edema index was scored as the average total number of vessels presenting with perivascular edema divided by the total number of vessels per slide at a magnification of 100X.

**Quantitative Reverse-transcription Polymerase Chain Reaction Analysis**

To access inflammatory response, the proinflammatory cytokines interleukin-1β and -8 were analyzed at mRNA level by reverse-transcription polymerase chain reaction (PCR). Quantitative examination was performed by one of the authors (M. S.), who was blinded to the experimental protocol and the region of sampling. Isolated mRNA aliquots were used to determine mRNA levels specific for sheep: interleukin-1β and -8. Reverse transcription PCR enzyme-linked immunosorbent assay (ELISA) was conducted as previously described with minor modification. First-strand cDNA was synthesized, and PCR amplification of cDNA and quantification of PCR product was performed using PCR ELISA (Boehringer Mannheim Corp., Indianapolis, IN) according to manufacturer’s instruction, with a target specific sense primer and a target specific biotinated antisense primer in the presence of digoxigenin-labeled dUTP. The reaction protocol used a programmed thermal cycler (GeneAmp 9600; Perkin Elmer Corp., Emeryville, CA) with a sequence of 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. After purification by PCR purification kits (QIAGEN Inc.), the PCR products were immobilized onto streptavidin-coated 96-well ELISA plates and detected by antidigoxigenin antibody conjugated with peroxidase and the substrate ABTS (Boehringer Mannheim Corp.). The colorimetric signal at 450 nm was recorded by a microplate reader. The absorbance values of mRNA for interleukin-1β and -8 were normalized by those for GAPDH, a house-keeping gene product, as a control.

**Statistical Analysis**

Data are expressed as mean ± SD unless otherwise specified. Basic data for the 3 groups were compared by one-way analysis of variance (ANOVA), and ventilatory and circulatory parameters between the 3 groups were compared using two-way ANOVA (for time stage and groups). If statistical significance was reached, a post hoc analysis (Tukey honest significance difference HSD test) was performed. Ventilatory and circulatory parameters over time (from baseline to post-RM, and from post-RM to 180 min, separately) within each group were compared by repeated-measures ANOVA, and when statistical significance was reached it was followed by Duncan post hoc analysis (pairwise comparisons were considered significant at P < 0.05). Analysis of biologic data for 6 combination of 3 groups and 2 regions were performed by two-way ANOVA followed by Tukey honest significance difference HSD test. Analysis of histopathologic data for 6 combination of 3 groups and 2 regions were performed by Kruskal-Wallis nonparametric ANOVA followed by Mann–Whitney test. A statistics software package (STATISTICA 5.1; StatSoft Inc., Tulsa, OK) was used, and P < 0.05 was considered statistically significant.

**Table 2. Characteristics of Sheep in Each Group (n = 7)**

<table>
<thead>
<tr>
<th></th>
<th>P_{MC} Group</th>
<th>P_{Max} Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>28.6 ± 4.7</td>
<td>25.9 ± 3.8</td>
<td>30.6 ± 4.2</td>
</tr>
<tr>
<td>Lavages (n)</td>
<td>2.4 ± 0.5</td>
<td>1.7 ± 0.8*</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>Recruitment maneuvers (n)</td>
<td>2.9 ± 0.9</td>
<td>3.3 ± 0.8</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>20.0 ± 2.7</td>
<td>20.5 ± 2.5</td>
<td>20.3 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>30.6 ± 6.5</td>
<td>28.6 ± 4.5</td>
<td>30.5 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>518 ± 44</td>
<td>524 ± 56</td>
<td>539 ± 68</td>
</tr>
<tr>
<td></td>
<td>60 ± 14*</td>
<td>69 ± 17*</td>
<td>72 ± 24*</td>
</tr>
<tr>
<td></td>
<td>477 ± 61†</td>
<td>464 ± 54†</td>
<td>512 ± 76†</td>
</tr>
<tr>
<td></td>
<td>36.1 ± 3.9</td>
<td>31.0 ± 4.2</td>
<td>32.5 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>14.0 ± 1.6*</td>
<td>12.8 ± 0.8*</td>
<td>14.8 ± 3.1*</td>
</tr>
<tr>
<td></td>
<td>20.1 ± 6.7*</td>
<td>18.7 ± 4.7†</td>
<td>26.4 ± 8.3†</td>
</tr>
<tr>
<td>Minimum PEEP after RM (cm H_2O)</td>
<td>25.7 ± 1.0‡</td>
<td>22.0 ± 2.6§</td>
<td>12.9 ± 1.6</td>
</tr>
<tr>
<td>PEEP at 180 min (cm H_2O)</td>
<td>25.9 ± 1.1‡</td>
<td>22.4 ± 2.6§</td>
<td>16.9 ± 4.7</td>
</tr>
</tbody>
</table>

* P < 0.01 versus baseline. † P < 0.01 versus injury. ‡ P < 0.01 versus control. § P < 0.05 versus control.

P_{Max} = PEEP set at the point of maximum curvature; P_{Max} = PEEP set at the lower infection point + 2 cm H_2O; PAO_2 = arterial oxygen tension; RM = recruitment maneuver; Cis = respiratory system compliance; PEEP = positive end-expiratory pressure.
Results

Eight sheep died or were killed before randomization: 3 were excluded before baseline because of low PaO2, 1 died during lavage, and 4 were excluded during RM because of no or little response to RM (PaO2 after RM was < 200 mmHg). Profiles of the 21 sheep included in the protocol are listed in table 2. PMC was greater than Pflex in all sheep. Because only 1 sheep had PMC lower than 26 cm H2O in the PMC group, and 3 sheep had PMC higher than 30 cm H2O, the average set PEEP of this group was about 5 cm H2O lower than average PMC of the group. The number of saline lung lavages was fewer in the Pflex group than in the control group (P < 0.05). There were no other differences between groups at baseline.

Ventilatory Parameters

Set Fio2 had significant group-versus-time interactions (P < 0.0005; fig. 2) and was lower in the PMC and the Pflex groups over time compared to the control group. PaCO2 (P < 0.0001), tidal volume (P < 0.02), and respiratory system compliance (P < 0.0001) had significant group-versus-time interactions: P < 0.05 versus control, #RM, and †30 min.
groups than in the control group after RM (P < 0.05). Total PEEP had significant group-versus-time interactions (P < 0.0001; fig. 2) and was higher in the P_MC and the P_flex groups than in the control groups after RM (P < 0.05). Pao2/Fio2 had significant group-versus-time interactions (P < 0.0001) and was lower in the P_MC and the P_flex group at 180 min than in the control group (P < 0.05). Shunt fraction had significant group-versus-time interactions (P < 0.0001) and was lower in the P_MC and the P_flex groups than in the control group (P < 0.05).

Respiratory rate did not differ over time but was higher in the P_MC group than in the control group (P < 0.05; fig. 3). Vt had significant group-versus-time interactions (P < 0.02) and was smaller in the P_MC group than in the control group (P < 0.05). Paco2 and pH (fig. 3 and table 3) had significant group-versus-time interactions (P < 0.0001 and P < 0.001, respectively). Paco2 in the P_MC group at 180 min was higher than that in the P_flex and control groups. Effective respiratory system compliance (fig. 3) decreased over time only in the control group (P < 0.0001). P_plat had significant group-versus-time interactions (P < 0.006; table 3) and were higher in the P_MC and the P_flex groups at 60 min than in the control group (P < 0.05).

### Table 3. Ventilatory Parameters over the Course of the Experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Injury</th>
<th>Post-RM</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pplat* (cm H2O)</td>
<td>P_MC</td>
<td>14.7 ± 1.6</td>
<td>30.1 ± 5.5</td>
<td>34.8 ± 0.5</td>
<td>35.3 ± 0.5</td>
<td>35.3 ± 0.5</td>
<td>35.3 ± 0.5</td>
</tr>
<tr>
<td>Pflex</td>
<td>15.4 ± 2.1</td>
<td>29.6 ± 3.7</td>
<td>34.6 ± 1.7</td>
<td>33.4 ± 2.6</td>
<td>33.7 ± 2.0</td>
<td>33.7 ± 2.3</td>
<td>34.3 ± 1.4</td>
</tr>
<tr>
<td>Control</td>
<td>16.0 ± 1.9</td>
<td>30.7 ± 4.7</td>
<td>33.7 ± 2.2</td>
<td>30.4 ± 1.6</td>
<td>28.0 ± 3.3</td>
<td>31.6 ± 3.4</td>
<td>32.3 ± 4.1</td>
</tr>
<tr>
<td>pH*</td>
<td>P_MC</td>
<td>7.46 ± 0.06</td>
<td>7.40 ± 0.06</td>
<td>7.44 ± 0.08</td>
<td>7.30 ± 0.11</td>
<td>7.28 ± 0.12</td>
<td>7.22 ± 0.13</td>
</tr>
<tr>
<td>Pflex</td>
<td>7.45 ± 0.03</td>
<td>7.40 ± 0.04</td>
<td>7.43 ± 0.06</td>
<td>7.34 ± 0.09</td>
<td>7.35 ± 0.08</td>
<td>7.31 ± 0.07</td>
<td>7.33 ± 0.09</td>
</tr>
<tr>
<td>Control</td>
<td>7.47 ± 0.05</td>
<td>7.42 ± 0.06</td>
<td>7.46 ± 0.04</td>
<td>7.43 ± 0.05</td>
<td>7.40 ± 0.04</td>
<td>7.40 ± 0.04</td>
<td>7.33 ± 0.09</td>
</tr>
<tr>
<td>PaO2/FIO2 (mmHg)</td>
<td>P_MC</td>
<td>518 ± 44</td>
<td>60 ± 14</td>
<td>477 ± 61</td>
<td>157 ± 51</td>
<td>114 ± 47</td>
<td>95 ± 30</td>
</tr>
<tr>
<td>Pflex</td>
<td>524 ± 56</td>
<td>69 ± 17</td>
<td>464 ± 54</td>
<td>146 ± 33</td>
<td>108 ± 16</td>
<td>98 ± 23</td>
<td>98 ± 15</td>
</tr>
<tr>
<td>Control</td>
<td>539 ± 68</td>
<td>72 ± 24</td>
<td>512 ± 76</td>
<td>150 ± 60</td>
<td>99 ± 12</td>
<td>77 ± 17</td>
<td>69 ± 27</td>
</tr>
<tr>
<td>Vt/Vt*</td>
<td>P_MC</td>
<td>0.44 ± 0.07</td>
<td>0.59 ± 0.08</td>
<td>0.57 ± 0.13</td>
<td>0.67 ± 0.11</td>
<td>0.65 ± 0.12</td>
<td>0.69 ± 0.14</td>
</tr>
<tr>
<td>Pflex</td>
<td>0.51 ± 0.04</td>
<td>0.54 ± 0.07</td>
<td>0.54 ± 0.13</td>
<td>0.58 ± 0.17</td>
<td>0.57 ± 0.17</td>
<td>0.59 ± 0.12</td>
<td>0.59 ± 0.11</td>
</tr>
<tr>
<td>Control</td>
<td>0.47 ± 0.08</td>
<td>0.51 ± 0.11</td>
<td>0.47 ± 0.12</td>
<td>0.45 ± 0.13</td>
<td>0.44 ± 0.08</td>
<td>0.51 ± 0.11</td>
<td>0.58 ± 0.18</td>
</tr>
</tbody>
</table>

* Pplat (P < 0.0006), pH (P < 0.001), and Vt/Vt (P < 0.0008) had a significant group versus time interactions. † P < 0.05 versus control. ‡ P < 0.05 versus post-RM. § P < 0.05 versus 60 min.

### Table 4. Hemodynamic Parameters over the Course of the Experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Injury</th>
<th>Post-RM</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PAP (mmHg)</td>
<td>P_MC</td>
<td>15 ± 4</td>
<td>17 ± 3</td>
<td>19 ± 4</td>
<td>22 ± 3</td>
<td>25 ± 4</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>Pflex</td>
<td>17 ± 4</td>
<td>20 ± 4</td>
<td>21 ± 3</td>
<td>22 ± 3</td>
<td>23 ± 3</td>
<td>24 ± 2</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Control</td>
<td>17 ± 3</td>
<td>18 ± 4</td>
<td>18 ± 3</td>
<td>18 ± 2</td>
<td>20 ± 2</td>
<td>21 ± 2</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>SVR (dyn · s · cm^-2)</td>
<td>P_MC</td>
<td>2,235 ± 633</td>
<td>2,016 ± 888</td>
<td>2,479 ± 1,230</td>
<td>2,481 ± 1,326</td>
<td>2,103 ± 992</td>
<td>1,636 ± 723</td>
</tr>
<tr>
<td>Pflex</td>
<td>2,703 ± 853</td>
<td>2,203 ± 294</td>
<td>2,936 ± 512</td>
<td>2,315 ± 514</td>
<td>2,290 ± 562</td>
<td>1,903 ± 586</td>
<td>2,042 ± 711</td>
</tr>
<tr>
<td>Control</td>
<td>1,984 ± 546</td>
<td>1,554 ± 593</td>
<td>2,029 ± 720</td>
<td>1,764 ± 659</td>
<td>1,528 ± 581</td>
<td>1,542 ± 692</td>
<td>1,325 ± 540</td>
</tr>
<tr>
<td>PVR* (dyn · s · cm^-2)</td>
<td>P_MC</td>
<td>133 ± 34</td>
<td>193 ± 67</td>
<td>239 ± 83</td>
<td>331 ± 110</td>
<td>363 ± 103</td>
<td>368 ± 127</td>
</tr>
<tr>
<td>Pflex</td>
<td>207 ± 93</td>
<td>269 ± 67</td>
<td>285 ± 71</td>
<td>319 ± 94</td>
<td>308 ± 66</td>
<td>304 ± 69</td>
<td>321 ± 86</td>
</tr>
<tr>
<td>Control</td>
<td>162 ± 43</td>
<td>180 ± 48</td>
<td>186 ± 31</td>
<td>180 ± 41</td>
<td>201 ± 63</td>
<td>214 ± 68</td>
<td>225 ± 94</td>
</tr>
</tbody>
</table>

* Mean PAP (P < 0.002) and PVR (P < 0.04) had significant group versus time interactions. † P < 0.05 versus post-RM. ‡ P < 0.05 versus 30 min. § P < 0.05 versus control.

RM = recruitment maneuver; PAP = pulmonary artery pressure; SVR = systemic vascular resistance; PVR = pulmonary vascular resistance; P_MC = positive end-expiratory pressure (PEEP) set at the point of maximum curvature; P_flex = PEEP set at the lower inflection point + 2 cm H2O.

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Microscopic photographs are shown in the dependent zone in the control group. Representative light photographs for homogeneity index between dependent and nondependent zone in the Pmc, Pflex, and control groups, respectively. Neutrophil counts in dependent regions were 261 ± 218, 151 ± 5, and 124 ± 210/μl and those in nondependent regions were 108 ± 65, 105 ± 87, and 91 ± 69/μl in the Pmc, Pflex, and control groups, respectively. There was no significant difference in leukocyte or neutrophil counts.

**Reverse-transcription Polymerase Chain Reaction**

Expression of mRNA for both interleukin-1β and -8 was significantly increased in the control group compared with the other groups (P < 0.05). In the control group the expressions in dependent region were significantly greater than those in nondependent regions (P < 0.01; fig. 6).

### Discussion

The primary findings of this study can be summarized as follows: (1) The method used to set PEEP following an RM affects gas exchange, lung mechanics, inflammatory mediator production, and histology; (2) PEEP set 2 cm H2O above Pflex attenuated changes in gas exchange and lung mechanics over time and resulted in minimal lung inflammation and mRNA expression for interleukin-1β and -8; (3) PEEP set at Pmc showed no benefit over PEEP at Pflex + 2 cmH2O but resulted in marked hypercarbia; and (4) inflammatory response and lung injury were greater in dependent lung than nondependent lung.

Since the first use of PEEP in ARDS patients by Ashbaugh et al. in 1967, there has been considerable discussion on how much PEEP will most improve patient outcome. Numerous approaches to setting PEEP based on various physiologic parameters have been described. However, most of these have been based only on gas exchange and cardiovascular response. Few have considered pulmonary mechanics when determining the set PEEP. Today, most clinicians set PEEP based on oxygenation response, adjusting PEEP and FiO2 to establish a PaO2 based on an algorithm (defined or conceptual) relating these variables.

### Table 5. Histologic Examination

<table>
<thead>
<tr>
<th>Group Lung Region</th>
<th>Pmc Dependent</th>
<th>Pmc Nondependent</th>
<th>Pflex Dependent</th>
<th>Pflex Nondependent</th>
<th>Control Dependent</th>
<th>Control Nondependent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhagic index</td>
<td>0.08 (0.06,0.13)</td>
<td>0.07 (0.04, 0.11)</td>
<td>0.08 (0.05, 0.15)*</td>
<td>0.03 (0.03,0.06)</td>
<td>0.10 (0.08, 0.15)*</td>
<td>0.05 (0.04, 0.09)</td>
</tr>
<tr>
<td>Homogeneity index</td>
<td>0.17 (0.15,0.21)</td>
<td>0.26 (0.17, 0.31)</td>
<td>0.15 (0.14, 0.21)</td>
<td>0.20 (0.19,0.29)</td>
<td>0.17 (0.14, 0.22)*</td>
<td>0.24 (0.21, 0.32)</td>
</tr>
<tr>
<td>Foci alveolar collapse</td>
<td>2.29 (0.39,3.13)</td>
<td>3.09 (0.48, 4.35)</td>
<td>1.17 (0.00, 2.41)</td>
<td>2.13 (1.68,2.19)</td>
<td>1.13 (0.00, 1.36)</td>
<td>2.93 (1.82, 3.71)</td>
</tr>
<tr>
<td>Ruptures/cm²</td>
<td>1.20 (0.38,3.39)</td>
<td>3.53 (0.48, 6.32)</td>
<td>1.17 (0.00,2.00)</td>
<td>2.64 (1.59,3.20)</td>
<td>0.87 (0.00,1.26)</td>
<td>2.93 (1.82, 3.99)</td>
</tr>
<tr>
<td>Vessels with edema (%)</td>
<td>0.37 (0.25,0.61)</td>
<td>0.36 (0.28,0.50)</td>
<td>0.38 (0.26,0.63)</td>
<td>0.52 (0.34,0.76)</td>
<td>0.58 (0.30,0.75)</td>
<td>0.70 (0.38,0.85)</td>
</tr>
</tbody>
</table>

Values are expressed as median (25th percentile, 75th percentile).

* P < 0.05 versus nondependent lung in the same group.

Pmc = positive end-expiratory pressure (PEEP) set at the point of maximum curvature; Pflex = PEEP set at the lower inflection point + 2 cm H2O.

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Positive End-expiratory Pressure Above the Lower Inflection Point

Matamis et al. proposed setting PEEP based on the results of the P-V curve, observing the elimination of Pflex as PEEP exceeded this level. Amato et al. demonstrated increased survival in ARDS when PEEP was set at Pflex + 2 cmH2O and VT at 6 ml/kg compared with a high VT (12 ml/kg) and low PEEP (based on oxygenation) strategy.

The level of end-expiratory pressure required to maintain the lung open in ARDS is dependent on regional transpulmonary pressure and the vertical gradient established. The factors affecting this gradient include: (1) the closing pressure of individual lung units, (2) superimposed pressure, and (3) pressure transmitted from outside the lung. As discussed by Hickling, Pflex represents the minimal opening pressure of select lung units. BothGattinioni et al., using computer tomography, and Kunst et al., using impedance tomography, have suggested that Pflex is representative of the opening pressure of nondependent lung. Closing pressure, especially in this surfactant washout model, is lower than opening pressure, but in a large animal model the vertical gradient superimposing pressure on dependent lung (lung height times density) requires high PEEP levels to keep dependent lung open. In addition, pressure transmitted from outside the lung, especially the abdomen, can also be large. As a result, our data differ considerably from that of Rimensberger et al., who used a small animal model (rabbits). They could maintain PaO2 following an RM with PEEP less than Pflex, but their regional transpulmonary pressure differences were low compared with our model. As a result, in our model and presumably in humans, pressures at or above Pflex may be needed to maintain the oxygenation benefit of a RM. The greater the regional transpulmonary pressure as a result of higher surface active forces, greater superimposed pressure, and greater transmitted pressure, the greater the required PEEP.

An RM was applied before PEEP was set. This insured that the recruited lung maintained by PEEP in all groups was on the expiratory limb of the P-V curve, insuring maximum end-expiratory lung volume at the set PEEP level. This approach is consistent with the approach recently mathematically described by Hickling, setting PEEP after the lung was fully opened. PEEP at Pflex following a recruitment maneuver results in a much greater lung volume than the same PEEP level without an RM. The key to the beneficial effects of PEEP at Pflex + 2 cmH2O in this experiment may have been the previous use of an RM. The beneficial effects of PEEP at Pflex + 2 cmH2O occurred despite a significantly higher Pplat in the Pflex group compared with the control group. However, it should be noted that a greater number of lavages (3.1 vs. 1.7; table 2) were required in the control than the Pflex group. We cannot be sure this did not

Fig. 5. Neutrophil accumulation in dependent lung, magnification 400×. Positive end-expiratory pressure (PEEP) at (A) above the lower inflection point (Pflex), (B) at the point of maximum curvature (Pmax), and (C) at set fraction of inspired oxygen (Fio2).
affect outcome, but all measured variables after injury were the same across all groups, indicating a consistent level of injury.

**Positive End-expiratory Pressure at the Point of Maximum Curvature**

Although our intent was to set PEEP at the measured P\textsubscript{MC}, we were only able to set PEEP at this level in one sheep; in all others the set PEEP was lower than P\textsubscript{MC} (26 cm H\textsubscript{2}O). At higher PEEP levels we could not ventilate adequately and still maintain P\textsubscript{plat} less than 35 cm H\textsubscript{2}O. However, this still resulted in a PEEP level (P\textsubscript{MC} group) 4–6 cm H\textsubscript{2}O higher than the P\textsubscript{flex} group, whereas the PEEP level in the control group was about 6–8 cm H\textsubscript{2}O lower than P\textsubscript{flex} + 2 cmH\textsubscript{2}O, establishing a clear and large difference in PEEP levels among the three groups. In addition, V\textsubscript{T} did vary among groups, P\textsubscript{MC} < P\textsubscript{flex} < control.

Arterial P\textsubscript{aO}_2/FIO\textsubscript{2} in the P\textsubscript{MC} group was equal to that at P\textsubscript{flex}, but normal ventilation could not be maintained, and there was a nonsignificant trend toward greater lung injury than at P\textsubscript{flex}. The higher RR as well as the greater mean airway pressure and mean PAP in this group may account for this trend. As recently demonstrated by Broccard et al.\textsuperscript{36} and Hotchkiss et al.,\textsuperscript{37} more rapid RRs and higher mean airway pressures, respectively, in canine oleic acid injury models induce greater lung injury than lower rates and pressures. Fu et al.\textsuperscript{38} also demonstrated that, at high lung volumes, stress failure of capillary endothelial and alveolar epithelial cells increases. The minimal difference in histology between groups in this study may simply be a result of the short duration of the experiment. Because peak alveolar pressure was limited in all groups to 35 cm H\textsubscript{2}O, the short (3-h) course of the study may have been insufficient to fully demonstrate the injury potentially induced by a specific approach. In addition, we only evaluated interleukin-1\textbeta{} and -8 mRNA levels in lavage fluid. Pathways unaffected by these mediators may have been responsible for the observed inflammatory response.

**Positive End-expiratory Pressure at Set Fraction of Inspired Oxygen**

This approach, although commonly used, may result in setting PEEP below the level that prevents end-expiratory collapse. Over time, high superimposed and transmitted pressures were sufficient to allow lung to close. The increase in inflammatory response revealed by the increase in neutrophil infiltration and mRNA expression for interleukin-1\textbeta{} and -8 in this group is similar to that demonstrated by numerous others in both animal models\textsuperscript{39–41} and humans\textsuperscript{42} when inadequate PEEP was set. The inflammatory response induced by this approach was great. Based on this response, we had expected the histologic injury to have been greater. However, because peak alveolar pressure in this group was low (32 cm H\textsubscript{2}O), the recruitment-collapse injury along with the biotrauma, presumed to be the responsible factor for injury, may require a longer period of ventilation (> 3 h) to be fully manifested. However, it should also be stressed that V\textsubscript{T} was higher in this group than in the P\textsubscript{flex} or P\textsubscript{MC} groups, but end inspiratory stretch tended to be least in this group. We thus cannot rule out the effect of V\textsubscript{T} on our results, but the group that was optimal, P\textsubscript{flex}, had a V\textsubscript{T} between that of the control and P\textsubscript{MC} groups. It should be stressed that the PEEP level in this group was 15–16 cm H\textsubscript{2}O, a level not considered modest.\textsuperscript{2,3}

**Dependent versus Nondependent Injury**

Greater and more severe inflammatory response and lung injury in the control group was observed in the dependent region of the lung. This is consistent with results by Broccard et al.\textsuperscript{15} in dogs and was most probably a result of recruitment-collapse injury in the control group. It would appear that PEEP set by oxygenation in this model may have been adequate to limit nondependent but not dependent lung injury.

**Limitations**

The primary limitation of this study was the fact that it was performed in a large-animal model of ARDS, not humans. In addition, the injury was induced by saline...
lavage and may not be representative of the specific pathophysiology observed in human ARDS. Furthermore, because we did not test PEEP set at $P_{f_{\text{PEEP}}}$ or $P_{f_{\text{PEEP}}}$ minus a few centimeters of water, we cannot specifically state that $P_{f_{\text{PEEP}}} + 2 \text{ cm H}_2\text{O}$ is the only method of setting PEEP. The short time course of the study may have resulted in a limited histologic response compared with the marked inflammatory mediator response. The lung in all groups were fixed for histologic examination at 20 cm H$_2$O. This was lower than the PEEP level in the $P_{f_{\text{PEEP}}}$ and $P_{f_{\text{PEEP}}}$ groups; as a result, the level of foci of alveolar collapse may have been overestimated in these two groups. The low peak alveolar pressure in all groups may require a very lengthy study period for more marked histologic manifestations of the effects of the different end-expiratory lung volumes to be observed.

In conclusion, the approach used to set PEEP after RM does have an effect on gas exchange, inflammatory mediator response, and histology. PEEP set above $P_{f_{\text{PEEP}}}$ in this model resulted in the best gas exchange, and PEEP set at $P_{f_{\text{PEEP}}} + 2 \text{ cm H}_2\text{O}$ and $P_{f_{\text{PEEP}}}$ resulted in less lung injury as evaluated by histologic and inflammatory mediator insult when compared with inadequate PEEP (control group). PEEP based solely on oxygenation response, despite a lower plateau pressure, resulted in progressive deterioration in gas exchange, marked inflammatory mediator response, and lung injury.

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