Open lung approach vs acute respiratory distress syndrome network ventilation in experimental acute lung injury

P. M. Spieth 1,2,3*, A. Güldner 1, A. R. Carvalho 4, M. Kasper 5, P. Pelosi 6, S. Uhlig 7, T. Koch 1 and M. Gama de Abreu 1

1 Department of Anesthesia and Intensive Care Therapy, University Hospital Dresden, Dresden, Germany
2 Departments of Anesthesia, Medicine, Physiology and Interdepartmental Division of Critical Care Medicine, University of Toronto, Toronto, ON, Canada
3 Keenan Research Center in the Li Ka Shing Knowledge Institute of St. Michael’s Hospital, Toronto, ON, Canada
4 Laboratory of Respiration Physiology, Biophysics Institute Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
5 Institute of Anatomy, Medical Faculty of the Technical University Dresden, Dresden, Germany
6 Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy
7 Institute of Pharmacology and Toxicology, University of Aachen, Aachen, Germany

* Corresponding author. E-mail: peter.spieth@uniklinikum-dresden.de

Editor's key points

- The contribution of positive end-expiratory pressure (PEEP) to the outcome in acute respiratory distress syndrome (ARDS) is unclear.
- The ARDSnet approach uses low tidal volume but the benefit of high PEEP is controversial.
- Using a pig model, these authors showed that the open lung approach resulted in better oxygenation and redistribution of pulmonary blood flow than the ARDSnet protocol.
- There was little evidence of an inflammatory response using either approach.

Background. Setting and strategies of mechanical ventilation with positive end-expiratory pressure (PEEP) in acute lung injury (ALI) remains controversial. This study compares the effects between lung-protective mechanical ventilation according to the Acute Respiratory Distress Syndrome Network recommendations (ARDSnet) and the open lung approach (OLA) on pulmonary function and inflammatory response.

Methods. Eighteen juvenile pigs were anaesthetized, mechanically ventilated, and instrumented. ALI was induced by surfactant washout. Animals were randomly assigned to mechanical ventilation according to the ARDSnet protocol or the OLA (n=9 per group). Gas exchange, haemodynamics, pulmonary blood flow (PBF) distribution, and respiratory mechanics were measured at intervals and the lungs were removed after 6 h of mechanical ventilation for further analysis.

Results. PEEP and mean airway pressure were higher in the OLA than in the ARDSnet group [15 cmH2O, range 14–18 cmH2O, compared with 12 cmH2O; 20.5 (sd 2.3) compared with 18 (1.4) cmH2O by the end of the experiment, respectively], and OLA was associated with improved oxygenation compared with the ARDSnet group after 6 h. OLA showed more alveolar overdistension, especially in gravitationally non-dependent regions, while the ARDSnet group was associated with more intra-alveolar haemorrhage. Inflammatory mediators and markers of lung parenchymal stress did not differ significantly between groups. The PBF shifted from ventral to dorsal during OLA compared with ARDSnet protocol [−0.02 (−0.09 to −0.01) compared with −0.08 (−0.12 to −0.06), dorsal–ventral gradients after 6 h, respectively].

Conclusions. According to the OLA, mechanical ventilation improved oxygenation and redistributed pulmonary perfusion when compared with the ARDSnet protocol, without differences in lung inflammatory response.

Keywords: lung, blood flow; lung, respiratory distress syndrome; model, respiratory failure; ventilation, mechanical; ventilation, positive end-expiratory pressure

Accepted for publication: 10 March 2011

It has been demonstrated that the mortality associated with acute respiratory distress syndrome (ARDS) may be reduced by the use of low tidal volumes (Vₜ) during mechanical ventilation. However, the role of positive end-expiratory pressure (PEEP) in the context of protective mechanical ventilation remains controversial. PEEP may recruit and stabilize previously collapsed lung areas, thus reducing shear stress through cyclic alveolar collapse and re-opening. Different studies reported a reduction in mortality when patients were ventilated with high PEEP levels. However, the beneficial effects of high PEEP on the outcome of patients with ARDS have been challenged. Currently, there are two main approaches for lung-protective mechanical ventilation used in clinical practice. The first one, which has been suggested by the ARDS Network is aimed to minimize lung strain while...
maintaining a minimal acceptable oxygenation. According to this strategy, \( V_t \) should be equal or lower than 6 ml kg\(^{-1}\) and airway plateau pressures lower than 30 cmH\(_2\)O. To achieve these goals, fixed combinations of \( FIO_2 \) and PEEP are used and adjusted periodically to achieve certain oxygenation goals. The recommendations of the ARDSnet protocol have also been described in combination with higher PEEP levels.\(^7\) Although a high PEEP strategy showed no improvement in the outcome of acute lung injury (ALI)/ARDS patients in general,\(^7\) it has been associated with improved lung function\(^7\) and better outcomes in severe hypoxaemic patients\(^9\) when compared with a low PEEP strategy.

The second strategy is known as open lung approach (OLA), and is aimed mainly at reducing dynamic strain. In OLA, recruitment manoeuvres are used to open up the lungs and PEEP is titrated to gas exchange\(^10\) or respiratory variables\(^6\)\(^1\)\(^1\) to keep the lungs open. The OLA may therefore provide further beneficial effects on the lung tissue by avoiding cyclic collapse/re-opening\(^12\)\(^13\) and reduced bacterial translocation\(^14\)\(^15\) when the PEEP is titrated in a decremental PEEP trial.\(^16\)

In this study, we compared the effects of mechanical ventilation according to the ARDSnet and the OLA on functional variables of the respiratory system and inflammatory response. We hypothesized that, under controlled conditions, the OLA would be associated with improved respiratory function and lung protection as compared with the ventilation according to the ARDSnet.

**Methods**

All animal procedures were approved by the Institutional Animal Care Committee and the Government of the State of Saxony, Germany, and were conducted according to the National Institute of Health guidelines for the care and use of laboratory animals. The animals were ordered via the Experimental Center of the Medical Faculty of the Technical University of Dresden from a local farm accredited by the governmental animal care committee. In a recently published study, we compared variable ventilation in combination with the ARDSnet protocol and OLA.\(^17\) As that study was designed for evaluating the effects of variable ventilation, we did not compare the conventional ARDSnet and OLA groups directly.\(^17\) Because of the high clinical relevance, we decided to perform a comparative analysis of the findings in the ARDSnet and OLA animals that were not treated with variable tidal volumes, and expand the analysis of the ARDSnet and OLA groups in the present paper in order to provide highly standardized experimental information for both ventilation strategies.

**Experimental protocol**

Eighteen female pigs (24.4–37.0 kg) were anaesthetized with ketamine (10 mg kg\(^{-1}\) induction; 5–30 mg kg\(^{-1}\) h\(^{-1}\) maintenance) and midazolame (1 mg kg\(^{-1}\) induction; 1.5–6 mg kg\(^{-1}\) h\(^{-1}\) maintenance), paralysed with pancuronium (bolus of 4 mg every hour), mechanically ventilated and instrumented. The depth of anaesthesia was monitored continually by observing arterial blood pressure and heart rate. After instrumentation, a period of 15 min was allowed for animals to stabilize. Baseline measurements were then obtained and ALI was induced by means of surfactant depletion. After injury, animals were randomly assigned to the ARDSnet or OLA groups (n=9 per group). Mechanical ventilator settings are reported in Table 1. Gas exchange, lung mechanics, haemodynamic parameters, and plasma cytokines were assessed after instrumentation (Baseline), established lung injury (Injury), and thereafter hourly from the beginning of the therapy for 6 h (Times 1–6). Samples from bronchoalveolar lavage fluid (BAL) were obtained from the first lavage used to induce injury and before killing the animal. Post-mortem, lungs were extracted to determine pulmonary blood flow (PBF) distribution, histopathological lung damage, lung mechanical stress, and inflammatory mediators.

**Instrumentation**

Animals were kept in supine position throughout the whole study. After oro-tracheal intubation with a 8.0 ID tracheal tube, right internal carotid artery, and external jugular vein were surgically exposed and a 5 Fr sheath was inserted in the artery and an 8.5 Fr sheath in the vein, respectively. A 7.5 Fr pulmonary artery catheter was advanced throughout the central venous access and positioned in the pulmonary artery. Correct position was confirmed by typical waveforms of the pressure tracings. Respiratory gases were measured by means of a respiratory mass spectrometer. Airway pressure and flow signals were recorded using differential pressure transducers and a heated pneumotachograph as previously described.\(^18\) After instrumentation, the lungs

**Table 1 Ventilator settings. ARDSnet, lung-protective ventilation according the ARDS Network protocol; OLA, lung-protective mechanical ventilation according to the open lung approach; VCV, volume-controlled ventilation; \( FIO_2 \), fraction of inspired oxygen; RR, ventilatory frequency; \( V_t \), tidal volume; PEEP, positive end-expiratory pressure; Ers, elastance of the respiratory system; I:E, ratio of total inspiratory to expiratory time; flow, inspiratory gas flow.**

<table>
<thead>
<tr>
<th>Mode</th>
<th>ARDSnet</th>
<th>OLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>( FIO_2 )</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>RR (min(^{-1}))</td>
<td>20–40 to achieve pH &gt; 7.30</td>
<td>20–40 to achieve pH &gt; 7.30</td>
</tr>
<tr>
<td>( V_t ) (ml kg(^{-1}))</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>PEEP (cmH(_2)O)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>I:E</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Flow (litre min(^{-1}))</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>
were gently recruited by applying an airway pressure of 30 cmH$_2$O for 30 s.

**Measurements of functional parameters**

Gas exchange was measured using conventional blood gas analysis (Radiometer ABL 505; Radiometer OSM3) and derived parameters were calculated according to the standard formulae. Mean arterial and pulmonary arterial blood pressures (MAP, MPAP), and heart rate (HR) were measured continuously using the haemodynamic monitor system (CM5, Agilent, Böblingen, Germany). Cardiac output (CO) measurements were performed by means of the conventional thermodilution method. Respiratory mechanics were calculated on a breath-by-breath basis during a period of 3 min at each measurement point as previously described. Spatial distribution of PBF was determined from fluorescent microspheres (FluoSpheres, Molecular Probes, 15 µm diameter) at baseline, injury and after 6 h of mechanical ventilation as previously described.

**Induction of lung injury**

After baseline measurements, lung injury was induced by repetitive lung lavages (30 ml kg$^{-1}$ 0.9% NaCl at 37–39°C). Injury was considered stable if $P_{O_2}/F_{O_2} < 200$ mmHg for at least 30 min. After confirmation of lung injury, animals were randomly assigned to the treatment groups using closed envelopes. In order to ensure a systematic comparison between OLA and ARDSnet during the observational period of 6 h, the study protocol did not allow changing ventilator settings in response to lung mechanics (OLA) or blood gas analysis (ARDSnet) after the initial setting as may be performed in the clinical setting.

**Mechanical ventilation according to the ARDSNet protocol**

In the ARDSnet group, $F_{O_2}$ was adjusted to 0.7, $V_I$ was reduced to 6 ml kg$^{-1}$, and respiratory frequency was adjusted in order to maintain pH $>7.30$. According to the ARDSnet protocol,$^1$ PEEP levels of 10, 12, or 14 cmH$_2$O could have been used in combination with the selected $F_{O_2}$ of 0.7. We decided to use a PEEP of 12 cmH$_2$O in the ARDSnet group based on previous experimental experience to allow stability of lung function during the observation period while avoiding excessive lung distension at end-inspiration. There were no recruitment manoeuvres performed in this group. Mechanical ventilation settings are summarized in Table 1.

**Mechanical ventilation according to the OLA**

$F_{O_2}$, $V_I$, and respiratory frequency were set in the same way as for the ARDSnet group, but PEEP was selected according to a decremental PEEP trial. Briefly, PEEP was set at 20 cmH$_2$O and a recruitment manoeuvre with a continuous airway pressure of 50 cmH$_2$O for 30 s was performed. After that, PEEP was stepwise reduced to 10 cmH$_2$O in decrements of 2 cmH$_2$O. The elastance of the respiratory system (Ers) was measured continuously at each PEEP step and values were taken after a period of 3 min. After the decremental PEEP trial, a second recruitment manoeuvre was performed and the PEEP was set according to the minimal Ers. Mechanical ventilation settings are summarized in Table 1.

**Post mortems and tissue sampling**

After the end of the experimental protocol, animals were killed by injection of 2 g thiopental and 50 ml 1 M KCl. During the removal of heart and lungs en bloc, a continuous positive airway pressure identical to the PEEP was maintained. The right upper lobe was fixed by perfusion with 4% buffered formaldehyde solution at constant airway and perfusion pressure (equivalent to PEEP and MPAP levels at Time 6, respectively). Samples of lung tissue (~8 cm$^3$) were obtained from gravitational-dependent (dorsal–segmentum 2–posterius) and gravitational-non-dependent zones (ventral–segmentum 3–anterius) of the right upper lobe and immersed in 4% buffered formaldehyde solution. Later, tissue samples were embedded in paraffin, cut into slices of 5 µm thickness, and stained with haematoxylin–eosin for histological analysis.$^2$ Samples of lung tissue from gravitationally dependent and non-dependent regions of the right lower lobe were snap-frozen in liquid nitrogen, kept at $-80^\circ$C until processing for further biochemical and molecular biological analysis.

To detect early inflammatory response, concentrations of tumour necrosis factor-alpha (TNF-$\alpha$) and interleukin-6 (IL-6) were measured in blood plasma at baseline, injury, and thereafter every hour (Times 1–6). IL-8 in blood plasma served as marker for systemic leucocyte recruitment. In lung homogenates, mRNA expression and protein concentrations of IL-6 and IL-8 were measured to detect early inflammatory response and leucocyte recruitment. The mRNA expression of transforming growth factor-beta (TGF-β) served as a marker for early pro-fibrotic activity. The mRNA expression of the mechanosensitive proteins amphiheguin and tenascin-c (TNC) was used as the surrogate of mechanical stress in lung tissue. IL-6 and IL-8 concentrations were measured in BAL. Cytokine levels in lung homogenate, blood plasma, and BAL were measured using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer’s instructions. Primer specification and PCR amplification programmes were previously published.$^1$ For the analysis of PBF, lungs were prepared as previously described.$^2$ Briefly, left lungs were flushed with hydroxyethyl starch (Voluven, Fresenius Kabi, Bad Homburg, Germany) and air-dried with continuous tracheal airflow for 7 days (continuous pressure of 25 cmH$_2$O). Thereafter, lungs were cut, processed, and fluorescent intensity was measured with a spectrophotometer.$^1$ Three-dimensional reconstructions were performed using the TecPlot software package (Release 1 2006; TecPlot Inc., Bellevue, WA, USA).
Statistical analysis

Functional variables are presented as mean (SD). One-way ANOVA was used to test the comparability between groups at baseline and injury. Paired t-tests were used to test the effects of lung injury on variables (baseline vs injury). Differences of means and time course of effects from Time 1 to Time 6 between groups (group and time × group effects) were tested by repeated-measures ANOVA. Non-normal distributed variables are presented as median with first and third quartiles (Q1–Q3). Statistical analysis was performed by means of the Kruskal–Wallis or Wilcoxon test as appropriate. All tests were performed using SPSS (SPSS version 15.0, Chicago, IL, USA). Corrections for multiple measurements were performed by means of the Sidak (ANOV A) or the Bonferroni–Holms (Wilcoxon) procedure, respectively. Statistical significance was accepted if P<0.05.

Results

The PEEP adjusted in the OLA group according to minimal Ers [15 cmH2O (14–18 cmH2O)] was significantly higher than in the ARDSnet group (12 cmH2O; P<0.05). Groups did not differ significantly with regard to body weight and number of lavages needed to achieve acute lung injury. Before randomization, groups were comparable in all measured variables despite a slight increase in MAP in the OLA group at baseline.

Respiratory parameters

The induction of acute lung injury led to a significant increase in mean, peak, and transpulmonary airway pressures, and elastance and resistance in both groups (Table 2). Mean airway pressure was significantly lower in the ARDSnet group (Table 2). Other respiratory variables did not differ significantly between groups (Table 2). Intrinsic PEEP was negligible in both groups (Table 2) and evolution of respiratory variables over time (time × group effect) did not yield significant differences between groups (Table 2).

Gas exchange

Induction of lung injury resulted in a significant deterioration of gas exchange (Table 3 and Fig. 1, respectively). \( P_{CO_2} \), venous admixture (\( Q_{va}/Q_t \)), and oxygen extraction rate increased after induction of acute lung injury (Supplementary material, Table S1 and Fig. S1, respectively). \( P_{aO_2}/FiO_2 \) was significantly higher and \( Q_{va}/Q_t \) lower in the OLA compared with the ARDSnet group (Fig. 1).

Haemodynamics

Heart rate decreased and MPAP increased significantly after injury (Table 4), but values did not differ between groups. Further haemodynamic variables did not differ significantly between groups and are listed in Supplementary material, Table S2.

Distribution of PBF

Spatial PBF distribution in one representative animal per group is illustrated in Figure 2. After injury, a significant redistribution of blood flow from dorsal to ventral, from caudal to cranial, and from central to peripheral lung regions occurred. After 6 h of therapy, we found a progressive redistribution of PBF towards pre-injury conditions. As compared with the ARDSnet group, OLA was associated with a significant redistribution of PBF towards caudal lung regions (Table 3).

Histological analysis

Results of quantitative histological analysis are summarized in Table 4. Cumulative diffuse alveolar damage (DAD) scores did not differ significantly between groups and lung regions. However, OLA was associated with significantly less intra-alveolar haemorrhage in the whole lungs and in gravitationally dependent and non-dependent regions compared with ARDSnet. On the other hand, significantly more alveolar overdistension in the whole lungs and in non-dependent regions could be observed in the OLA when compared with the ARDSnet group. The analysis of regional distribution of histopathological correlates revealed significantly increased inflammatory infiltration in the dependent and significantly increased overdistension in the non-dependent lung regions within the OLA group.

Inflammatory response and mechanical stress

The mRNA expression of IL-6 did not differ significantly in lung tissue overall. In gravitationally dependent lung regions, IL-6 mRNA expression did not differ significantly between groups. Also in gravitationally non-dependent lung regions, IL-6 mRNA expression was not significantly different between groups. Within the OLA group, mRNA expression of TNC and TGF-β was significantly increased in non-dependent compared with dependent lung regions (both P=0.018) (see Supplementary material, Table S3).

Concentrations of IL-6 and IL-8 in lung tissue did not differ significantly between groups overall or in gravitationally dependent and non-dependent lung regions. Within the ARDSnet and OLA groups, IL-8 concentrations were significantly higher in gravitationally dependent compared with non-dependent lung regions (P=0.028 and P=0.008, respectively) (see Supplementary material, Table S4). Levels of TNF-α, IL-6, and IL-8 in plasma did not differ significantly between groups (Supplementary material, Table S5) and concentrations of IL-6 in BAL fluid did not differ significantly between groups (Supplementary material, Table S6).

Discussion

Compared with the ARDSnet protocol, the major findings of this study were that: OLA (i) resulted in higher PEEP and mean airway pressure, but comparable peak and transpulmonary airway pressures; (ii) led to an improvement in the oxygenation and redistribution of PBF towards caudal lung zones; (iii) resulted in comparable global histological lung injury scores, but was associated with increased alveolar
overdistension mainly in non-dependent zones and less haemorrhage mainly in dependent areas; (iv) did not differ significantly in terms of markers of inflammation or mechanical stress.

The PEEP adjustment in the ARDS Network protocol is focused on the maintenance of an acceptable oxygenation with reduced lung stretch. As in this strategy, the setting of PEEP is based on the relationship between $P_{A}O_2$ and $FIO_2$, it can be easily accomplished. However, the physiological rationale and the lack of individuality of this approach have been questioned. Furthermore, the ARDSnet protocol may be associated with increased atelectasis owing to relatively low PEEP levels and the lack of recruitment manoeuvres (permissive atelectasis). The use of a high PEEP strategy without lung recruitment could partially overcome these limitations. Higher PEEP values may allow gradual recruitment of lung units, without the need for excessive increase in airway pressures. However, this strategy

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Respiratory variables. Values are given as mean (so). ARDSnet, ventilation according to the ARDS Network protocol; OLA, ventilation according to the open lung approach. $V_t$, tidal volume; $RR$, ventilatory frequency; $MV$, minute ventilation; $P_{mean}$, mean airway pressure; $P_{peak}$, peak airway pressure; $P_{trans}$, transpulmonary pressure; $Ers$, elastance of the respiratory system; $Rrs$, resistance of the respiratory system; PEEPi, intrinsic positive end-expiratory pressure</th>
<th>Group</th>
<th>Baseline</th>
<th>Injury</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
<th>Time 4</th>
<th>Time 5</th>
<th>Time 6</th>
<th>Baseline vs Injury</th>
<th>Group effect</th>
<th>Time × group effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_t$/kg (ml kg$^{-1}$)</td>
<td>ARDSnet</td>
<td>9.3 (0.8)</td>
<td>9.2 (0.7)</td>
<td>6.1 (0.6)</td>
<td>6.2 (0.7)</td>
<td>6.2 (0.6)</td>
<td>6.0 (0.5)</td>
<td>6.0 (0.3)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>9.5 (0.6)</td>
<td>9.7 (0.7)</td>
<td>6.0 (0.5)</td>
<td>6.1 (0.5)</td>
<td>6.0 (0.4)</td>
<td>5.9 (0.2)</td>
<td>6.1 (0.7)</td>
<td>6.0 (0.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$RR$ (min$^{-1}$)</td>
<td>ARDSnet</td>
<td>17.9 (4.6)</td>
<td>17.4 (4.9)</td>
<td>26.7 (5.2)</td>
<td>27.2 (6.0)</td>
<td>27.2 (6.0)</td>
<td>27.6 (5.9)</td>
<td>27.6 (5.9)</td>
<td>27.6 (5.9)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>17.1 (5.1)</td>
<td>16.4 (3.2)</td>
<td>28.6 (9.1)</td>
<td>28.6 (9.1)</td>
<td>28.6 (9.1)</td>
<td>28.6 (9.1)</td>
<td>28.6 (9.1)</td>
<td>28.6 (9.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$MV$ (litre min$^{-1}$)</td>
<td>ARDSnet</td>
<td>5.2 (1.1)</td>
<td>5.0 (1.0)</td>
<td>5.1 (1.0)</td>
<td>5.3 (1.0)</td>
<td>5.3 (1.0)</td>
<td>5.4 (0.8)</td>
<td>5.2 (0.8)</td>
<td>5.2 (0.9)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>4.7 (1.1)</td>
<td>4.6 (1.0)</td>
<td>5.0 (1.0)</td>
<td>5.1 (1.0)</td>
<td>5.0 (1.0)</td>
<td>4.9 (1.0)</td>
<td>5.1 (1.0)</td>
<td>5.0 (1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{mean}$ (cmH$_2$O)</td>
<td>ARDSnet</td>
<td>10.2 (0.9)</td>
<td>14.2 (0.8)</td>
<td>17.8 (0.7)</td>
<td>17.9 (0.9)</td>
<td>17.9 (1.0)</td>
<td>18.0 (1.0)</td>
<td>18.0 (1.0)</td>
<td>P&lt;0.001</td>
<td>P=0.011</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>10.0 (1.3)</td>
<td>14.7 (4.2)</td>
<td>20.0 (2.7)</td>
<td>20.4 (2.6)</td>
<td>20.6 (2.5)</td>
<td>20.5 (2.4)</td>
<td>20.5 (2.3)</td>
<td>20.5 (2.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{peak}$ (cmH$_2$O)</td>
<td>ARDSnet</td>
<td>17.9 (2.0)</td>
<td>28.8 (2.0)</td>
<td>25.3 (1.9)</td>
<td>25.5 (1.5)</td>
<td>25.3 (1.8)</td>
<td>25.3 (2.0)</td>
<td>25.6 (2.0)</td>
<td>26.0 (2.0)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>17.7 (2.0)</td>
<td>31.0 (8.8)</td>
<td>26.2 (5.2)</td>
<td>27.2 (4.8)</td>
<td>27.5 (4.3)</td>
<td>27.2 (4.2)</td>
<td>27.2 (4.1)</td>
<td>27.4 (4.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{trans}$ (cmH$_2$O)</td>
<td>ARDSnet</td>
<td>2.2 (1.3)</td>
<td>5.3 (1.2)</td>
<td>7.7 (2.4)</td>
<td>7.9 (4.7)</td>
<td>7.0 (4.7)</td>
<td>7.0 (4.2)</td>
<td>7.0 (4.1)</td>
<td>5.9 (4.0)</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>1.7 (1.1)</td>
<td>5.0 (2.6)</td>
<td>7.4 (1.4)</td>
<td>7.3 (1.6)</td>
<td>7.7 (2.0)</td>
<td>7.7 (2.0)</td>
<td>6.8 (2.0)</td>
<td>6.6 (1.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ers$ (cmH$_2$O litre$^{-1}$)</td>
<td>ARDSnet</td>
<td>36.9 (9.1)</td>
<td>75.8 (20.1)</td>
<td>64.0 (13.6)</td>
<td>63.0 (14.5)</td>
<td>61.6 (14.5)</td>
<td>61.1 (13.3)</td>
<td>64.0 (14.4)</td>
<td>66.1 (17.6)</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>37.8 (5.5)</td>
<td>78.6 (22.2)</td>
<td>51.7 (21.3)</td>
<td>57.8 (18.3)</td>
<td>60.9 (15.0)</td>
<td>59.6 (14.9)</td>
<td>58.4 (15.4)</td>
<td>60.0 (14.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Rrs$ (cmH$_2$O litre$^{-1}$)</td>
<td>ARDSnet</td>
<td>4.9 (0.9)</td>
<td>5.8 (1.7)</td>
<td>3.5 (1.6)</td>
<td>3.9 (0.7)</td>
<td>3.8 (0.9)</td>
<td>3.6 (1.0)</td>
<td>3.9 (1.1)</td>
<td>3.9 (1.6)</td>
<td>P=0.036</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>5.2 (1.0)</td>
<td>8.4 (5.4)</td>
<td>4.1 (1.7)</td>
<td>3.8 (1.6)</td>
<td>3.8 (1.5)</td>
<td>3.6 (1.5)</td>
<td>3.7 (1.5)</td>
<td>3.7 (1.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEEPi (cmH$_2$O)</td>
<td>ARDSnet</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.2)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.2 (0.2)</td>
<td>0.2 (0.2)</td>
<td>0.1 (0.2)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OLA</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.3)</td>
<td>0.1 (0.0)</td>
<td>0.2 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
does not reset the so-called lung volume history, possibly resulting in higher driving pressures for the same \( V_T \) when compared with the OLA.\(^\text{11}\) Furthermore, an individualized titration of PEEP is not used. In the OLA, the adjustment of PEEP follows a physiological rationale, such as the optimization of the elastic properties of the respiratory system, gas exchange, or both. Therefore, this approach results in more individual PEEP adjustment. Moreover, the OLA requires that a recruitment manoeuvre be performed to open up the lungs, which may influence lung history and the PEEP as well. This approach, used in combination with a decremental PEEP trial, has been proved beneficial in different studies,\(^\text{25–27}\) but its value in the improvement of patient outcome has not been established.\(^\text{8, 28}\) The PEEP settings used in this study were not chosen to yield maximal differences between groups rather to reproduce common clinical practice.\(^\text{7}\) We therefore also waived the option of frequent adjustments of PEEP and \( F_{O_2} \) levels in the ARDSnet group or the performance of further recruitment manoeuvres in the OLA group in order to keep the groups comparable. Although individualized PEEP settings based on changes in oxygenation (ARDSnet) or respiratory mechanics (OLA) are in general desirable, frequent adjustments within a 6 h time period does not mimic clinical reality. The PEEP differences in both arms of this study were relatively low (\( \sim 3 \text{ cmH}_2\text{O} \)) but were associated with differences in functional and lung injury variables. Although lower PEEP levels in the ARDSnet group would have likely resulted in different absolute values of functional variables, trends would have been probably comparable.

In our study, both protective ventilation strategies were able to improve the respiratory function after the onset of acute lung injury, but OLA was found superior to ARDSnet with respect to oxygenation. As the surfactant depletion model responds very well to recruitment manoeuvres,\(^\text{29}\) the improvement in oxygenation is not surprising. Certainly, a PEEP of 12 cmH\(_2\)O, as used in the ARDSnet group, may have resulted in gradual lung recruitment, explaining in part the small difference between both the groups. However, neither the pattern of PBF distribution, nor the lack of improvement in Ers or the development of intrinsic PEEP over the time provided evidence for significant alveolar recruitment in the ARDSnet group.

Our data suggest that the redistribution of PBF from gravitationally dependent to non-dependent lung regions may contribute to better ventilation-perfusion matching and therefore improved oxygenation in the OLA group. The improved ventilation-perfusion matching is most likely caused by increased alveolar recruitment in the caudal (juxtadiaphragmatic) lung regions owing to the application of higher PEEP levels. Although we did not measure regional ventilation in this study, the redistribution of PBF probably reflects a redistribution of ventilation towards dependent lung zones. Despite different PEEP levels in the groups, \( P_{\text{peak}} \) and \( P_{\text{trans}} \) did not differ significantly, most likely because of a more homogenous distribution of ventilation in the OLA group owing to a more pronounced alveolar recruitment. However, \( P_{\text{mean}} \) was significantly higher in the OLA group. This discrepancy in the results of the analysis of airway pressures may also be explained by the fact that the application of PEEP has a more direct effect on \( P_{\text{mean}} \) and less on \( P_{\text{peak}} \) and \( P_{\text{trans}} \) owing to the distribution of airway pressure in the respiratory tract.

We could not confirm our initial hypothesis that OLA is associated with less histological damage and reduced inflammatory response compared with the ARDSnet group. Reduced recruitment in the non-dependent lung regions in the ARDSnet group should contribute to increased inhomogeneity of mechanical stress and strain.\(^\text{30}\) However, OLA led to an increase in alveolar overdistension, mainly in non-dependent lung regions. Although we could not detect differences between groups, mRNA expression of TNC and TGF-\( \beta \) was higher in non-dependent compared with dependent zones only in the OLA group, suggesting mechanical stress

---

### Table 3 Pulmonary blood flow distribution.

<table>
<thead>
<tr>
<th>Ventilation mode</th>
<th>Gradient</th>
<th>Baseline</th>
<th>Injury</th>
<th>Time 6 (6 h of therapy)</th>
<th>Baseline vs Injury</th>
<th>Injury vs Time 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARDSnet</td>
<td>Dorsal–ventral</td>
<td>−0.05 [−0.09 to 0]</td>
<td>0.08 [0.03–0.14]</td>
<td>−0.02 [−0.09 to −0.01]</td>
<td>P=0.012</td>
<td>P=0.011</td>
</tr>
<tr>
<td>OLA</td>
<td>Dorsal–ventral</td>
<td>−0.03 [−0.05 to 0.03]</td>
<td>0.07 [0.04–0.09]</td>
<td>−0.08 [−0.12 to −0.06]</td>
<td>P=0.012</td>
<td>P=0.008</td>
</tr>
<tr>
<td>ARDSnet</td>
<td>Caudal–cranial</td>
<td>0.01 [0.01–0.05]</td>
<td>0.09 [0.06–0.1]</td>
<td>0.04 [0.04–0.11]</td>
<td>P=0.008</td>
<td>NS</td>
</tr>
<tr>
<td>OLA</td>
<td>Caudal–cranial</td>
<td>0.01 [0.01–0.02]</td>
<td>0.05 [0.05–0.07]</td>
<td>0 [−0.01 to 0.02]</td>
<td>P=0.014</td>
<td>P=0.008</td>
</tr>
<tr>
<td>ARDSnet</td>
<td>Central–peripheral</td>
<td>−0.11 [−0.15 to −0.05]</td>
<td>−0.05 [−0.11 to −0.03]</td>
<td>−0.14 [−0.2 to −0.1]</td>
<td>NS</td>
<td>P=0.011</td>
</tr>
<tr>
<td>OLA</td>
<td>Central–peripheral</td>
<td>−0.11 [−0.15 to −0.08]</td>
<td>−0.04 [−0.05 to −0.03]</td>
<td>−0.14 [−0.17 to −0.13]</td>
<td>P=0.007</td>
<td>P=0.011</td>
</tr>
</tbody>
</table>

---
and upregulation of pro-inflammatory cytokines in these lung regions. These findings are in accordance with computer tomography studies showing increased hyperaeration in non-dependent regions when higher PEEP levels are applied in ALI/ARDS.31–33 Our findings support the hypothesis that higher levels of PEEP may result in improved alveolar recruitment of dependent zones, but are associated with simultaneous overdistension of non-dependent areas. With the ARDSnet strategy, increased pulmonary haemorrhage was observed, which is in accordance with previous reports on patients with ALI/ARDS.12,34 On the other hand, less haemorrhage in dorsal lung regions in OLA could also be explained by a physical ‘tamponade effect’, i.e. higher alveolar gas pressures counteracting intra-alveolar bleeding. The contradictory effects of PEEP on the histopathological appearance of both protective ventilation strategies as indicated by haemorrhage and alveolar overdistension may contribute to explain the non-conclusive results of clinical trials comparing higher vs lower levels of PEEP in combination with low tidal volumes,6–8,28 but cannot be extrapolated to focal or less recruitable lung injury.29,35

Our study has several limitations. First, when interpreting our data, it should be considered that the surfactant depletion is an extremely well ‘recruitable’ model of lung injury, which is associated with only moderate tissue injury.19,36 Thus, the relationship between gas exchange response and lung recruitment may be stronger than that observed in other experimental models or in clinical ARDS.

Fig 1 Oxygenation. Data are shown as mean (sd) (error bars). Ratio of arterial partial pressure of oxygen to inspired oxygen fraction (P$_{aO2}$/F$_{IO2}$) (A) and venous admixture (Q$_{VA}$/Q$_{t}$) (B) in animals ventilated according to the ARDS Network protocol (ARDSnet) or the open lung approach (OLA).
of different aetiologies. Computer tomography studies suggest that the morphology of lung injury such as focal vs non-focal injury distribution may effect the response to recruitment and PEEP setting. However, surfactant depletion is the model of choice when studying effects of mechanical ventilation alone on the lung parenchyma as secondary inflammatory stimuli owing to chemical or biological hazards are avoided.

Secondly, the PEEP level used in the ARDSnet group may have been comparatively high. The resulting $P_{aO_2}$ levels in that group suggest that gradual recruitment likely occurred, reducing the differences between groups. However, 12 cmH$_2$O corresponds to the lowest PEEP used in the ALVEOLI study and we did not have evidence for the development of intrinsic PEEP. A recent meta-analysis suggested that higher PEEP levels such as used in the ALVEOLI study may be beneficial when compared with lower PEEP levels used in the ARMA trial with respect to ventilator-free days and even mortality. Thirdly the apparent stability of the effects of OLA on gas exchange and respiratory system mechanics must be interpreted with caution. Our study period was limited to 6 h and extrapolation of the results to prolonged periods of time is not warranted. Despite the lack of adjustment of PEEP/FIO$_2$ combinations, our study showed important differences in dynamic lung strain between the groups investigated as suggested by the time-course of Ers. Thus, the most important difference between the ARDSnet and the OLA, namely the reduction of static lung strain vs reduction of dynamic lung strain, respectively, was fairly reproduced.

Fourthly we paralysed the animals throughout the whole study period by means of continuous infusion of pancuronium. A recent clinical trial suggested that mechanical ventilation and neuromuscular block led to rapid disuse atrophy of the diaphragm bearing the risk of muscle weakness of the main respiratory muscle. However, a study recently published by Papazian and colleagues found improved 90-day survival, increased ventilator-free days, and reduced incidence of barotrauma without increased muscle weakness in patients suffering from severe ARDS who received neuromuscular block in the early phase of their disease. The effects of neuromuscular block on histopathology and inflammatory response in our study with a relatively short period of mechanical ventilation remain speculative, but as both groups received the same dosage of pancuronium, putative effects may be affecting both groups equally.

**Conclusions**

In this surfactant-depletion model of ALI, mechanical ventilation according to the OLA improved arterial oxygenation, but neither respiratory mechanics nor pulmonary inflammatory response compared with mechanical ventilation with...
lower PEEP according to the ARDSnet protocol. Further studies are warranted to determine if OLA is detrimental in less recruitable lung injury models or in the clinical setting.

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

Acknowledgements
We are indebted to the staff of the Experimental Center and the Institute of Anatomy of the Medical Faculty ‘Carl Gustav Carus’, Technical University of Dresden, Germany, and the students of the Pulmonary Engineering Group for their support. The mechanical ventilator used in this study was kindly provided by Dräger Medical, Lübeck, Germany. We are indebted to Mr Eberhard Kuhlisch, MSc for his statistical advice. The authors also thank Mrs Sabine Müller and Dr Lilla Knels for their valuable support.

Conflict of interest
None declared.

Funding
This work was supported by grant no. AB 135/3-1 by the German Research Foundation (DFG). P.M. Spieth is the recipient of the University of Toronto Eli Lilly Critical Care Fellowship Award.

References


16. Suarez-Sippmann F, Bohm SH. Recruit the lung before titrating the right positive end-expiratory pressure to protect it. *Crit Care* 2009; 13: 134


26. Hickling KG. Best compliance during a decremental, but not incremental, positive end-expiratory pressure trial is related to open-lung positive end-expiratory pressure: a mathematical model of acute respiratory distress syndrome lungs. *Am J Respir Crit Care Med* 2001; 163: 69–78

27. Kacmarek RM, Kollet RH. Should recruitment maneuvers be used in the management of ALI and ARDS? *Respir Care* 2007; 52: 622–31


34. Rouby JJ, Constantin JM, Roberto De AG, Zhang M, Lu Q. Mechanical ventilation in patients with acute respiratory distress syndrome. *Anesthesiology* 2004; 101: 228–34


