Biological Impact of Transpulmonary Driving Pressure in Experimental Acute Respiratory Distress Syndrome

Cynthia S. Samary, Ph.D., Raquel S. Santos, M.Sc., Ph.D., Cíntia L. Santos, Ph.D., Nathane S. Felix, M.S., Maira Bentes, R.T., Thiago Barboza, M.D., Vera L. Capelozzi, M.D., Ph.D., Marcelo M. Morales, M.D., Ph.D., Cristiano S. N. B. Garcia, Ph.D., Sergio A. L. Souza, Ph.D., John J. Marini, M.D., Marcelo Gama de Abreu, M.D., Ph.D., Pedro L. Silva, Ph.D., Paolo Pelosi, M.D., F.E.R.S., Patrícia R. M. Rocco, M.D., Ph.D.

ABSTRACT

Background: Ventilator-induced lung injury has been attributed to the interaction of several factors: tidal volume (V_T), positive end-expiratory pressure (PEEP), transpulmonary driving pressure (difference between transpulmonary pressure at end-inspiration and end-expiration, ΔP_L), and respiratory system plateau pressure (Pplat,rs).

Methods: Forty-eight Wistar rats received Escherichia coli lipopolysaccharide intratracheally. After 24 h, animals were randomized into combinations of V_T and PEEP, yielding three different ΔP_L levels: ΔP_LLOW (V_T = 6 ml/kg, PEEP = 3 cm H_2O); ΔP_LMEAN (V_T = 13 ml/kg, PEEP = 3 cm H_2O or V_T = 6 ml/kg, PEEP = 9.5 cm H_2O); and ΔP_LHIGH (V_T = 22 ml/kg, PEEP = 3 cm H_2O or V_T = 6 ml/kg, PEEP = 11 cm H_2O). In other groups, at low V_T, PEEP was adjusted to obtain a Pplat,rs similar to that achieved with ΔP_LMEAN and ΔP_LHIGH at high V_T.

Results: At ΔP_LLOW expressions of interleukin (IL)-6, receptor for advanced glycation end products (RAGE), and amphiregulin were reduced, despite morphometric evidence of alveolar collapse. At ΔP_LHIGH (V_T = 6 ml/kg and PEEP = 11 cm H_2O), lungs were fully open and IL-6 and RAGE were reduced compared with ΔP_LMEAN (27.4 ± 12.9 vs. 41.6 ± 14.1 and 0.6 ± 0.2 vs. 1.4 ± 0.3, respectively), despite increased hyperinflation and amphiregulin expression. At ΔP_LMEAN (V_T = 6 ml/kg and PEEP = 9.5 cm H_2O), when PEEP was not high enough to keep lungs open, IL-6, RAGE, and amphiregulin expression increased compared with ΔP_LLOW (41.6 ± 14.1 vs. 9.0 ± 9.8, 1.4 ± 0.3 vs. 0.3 ± 0.5, and 6.7 ± 0.8 vs. 2.2 ± 1.0, respectively). At Pplat,rs similar to that achieved with ΔP_LMEAN and ΔP_LHIGH, higher V_T and lower PEEP reduced IL-6 and RAGE expression.

Conclusion: In the acute respiratory distress syndrome model used in this experiment, two strategies minimized ventilator-induced lung injury: (1) low V_T and PEEP yielding low ΔP_L and Pplat,rs; and (2) low V_T associated with a PEEP level sufficient to keep the lungs open. (Anesthesiology 2015; 123:423-33)

A CUTE respiratory distress syndrome (ARDS) is a severe inflammatory condition characterized by heterogeneous pulmonary injury with both normal and diseased areas throughout the lung. Patients with ARDS require mechanical ventilation (MV), which improves gas exchange while minimizing harm to already injured tissue. The selection of adequate tidal volume (V_T), positive end-expiratory pressure (PEEP), respiratory system plateau pressure (Pplat,rs), and transpulmonary driving pressure (ΔP_L; difference between transpulmonary pressure at end-inspiration and end-expiration) settings is crucial to reducing ventilator-induced lung injury (VILI).2-5

A lung-protective strategy (V_T ≤6 ml/kg predicted body weight and plateau pressures ≤30 cm H_2O) and adequate levels of PEEP have been proposed to reduce VILI and mortality.

What We Already Know about This Topic

- Recent retrospective analysis of clinical acute respiratory distress syndrome trials suggested that driving pressure was an important factor associated with mortality.

What This Article Tells Us That Is New

- Different combinations of tidal volume and positive end-expiratory pressure (PEEP) were used to create a range of driving pressures in a rat model of acute respiratory distress syndrome due to tracheal instillation of endotoxin for 24 h. Low transpulmonary driving pressure was associated with alveolar collapse and high driving pressure was associated with hyperinflation. The combination of a tidal volume of 6 ml/kg predicted body weight and the lowest PEEP and driving pressure to maintain oxygenation in a normal range minimized ventilator-induced lung injury even in the presence of alveolar collapse.

This article is featured in “This Month in Anesthesiology,” page 1A. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal’s Web site (www.anesthesiology.org).

Submitted for publication September 14, 2014. Accepted for publication March 20, 2015. From the Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.); Laboratory of Experimental Surgery, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (C.S.S., R.S.S., C.L.S., N.S.F., M.B., C.S.N.B.G., P.L.S., P.R.M.R.);

Copyright © 2015, the American Society of Anesthesiologists, Inc. Wolters Kluwer Health, Inc. All Rights Reserved. Anesthesiology 2015; 123:423-33

Anesthesiology, V 125 • No 2

August 2015

Downloaded From: http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/934246/ on 11/07/2018
in patients with ARDS.\textsuperscript{3} Controversy remains regarding optimal PEEP, Pplat,rs, and driving pressures across the respiratory system and lungs. The use of lower VT and lower PEEP may be associated with more homogeneous ventilation of aerated lung and maintenance of alveolar collapse, reducing inflation shear stress.\textsuperscript{3,4} However, MV with lower VT and higher PEEP may: (1) not be enough to keep the alveoli open at end-expiration, resulting in less atelectasis compared with lower PEEP but creating lung regions with alveolar instability and repeated opening and closing of lung units; (2) be able to open and keep open most of the collapsed alveoli, thus resulting in less recruitment and derecruitment, but potentially leading to hyperinflation of aerated lungs; and (3) increase Pplat,rs and ΔP, predisposing to VILI. However, thus far, no study has compared the effects of combinations of different VT and PEEP settings resulting in different fixed levels of ΔP and Pplat,rs on lung morphology and biological markers of VILI in experimental ARDS.

We hypothesized in intratracheal endotoxin-induced ARDS that (1) low VT associated with lower PEEP (ΔP\textsubscript{LOW}) would minimize VILI because areas of alveolar collapse would remain unaltered, thus avoiding cyclic recruitment/ derecruitment of distal lung units, whereas in normal lung regions, no hyperinflation would occur, thus reducing end-inspiratory stress and lung inflammation; (2) at the same level of ΔP, low VT combined with a level of PEEP not enough to open the lungs might result in VILI; and (3) the combination of low VT with higher PEEP, when associated with higher Pplat,rs and ΔP, would increase lung damage. For this purpose, we investigated the effects of different fixed levels of ΔP or Pplat,rs induced by different combinations of VT and PEEP on lung morphology and biological markers in experimental ARDS.

Materials and Methods

This study was approved by the Federal University of Rio de Janeiro Health Science Center Research Ethics Committee (Rio de Janeiro, Brazil). All animals received humane care in compliance with the National Society for Medical Research “Principles of Laboratory Animal Care” and the U.S. National Academy of Sciences “Guide for the Care and Use of Laboratory Animals” (Washington, D.C.).

Animal Preparation and Experimental Protocol

Forty-eight adult male Wistar rats (weight 342 ± 13 g) were assigned to ARDS induction by intratracheal instillation of Escherichia coli lipopolysaccharide [O55:B5] (200 μg suspended in 20 μl saline solution).\textsuperscript{6} Twenty-four hours after ARDS induction, rats were anesthetized (ketamine 75 mg/kg and xylazine 2.5 mg/kg intraperitoneally) and tracheotomized. Six rats with ARDS were not mechanically ventilated (nonventilated group) and were used for molecular biology analysis. A polyethylene catheter (PE-50) was introduced into the carotid artery for blood sampling and monitoring of mean arterial pressure (MAP). Changes in esophageal pressure (Pes) were measured with a water-filled catheter (PE205) with side holes at the tip connected to a differential pressure transducer (UT-PL-400; SCIREQ, Canada). The catheter was passed into the stomach and slowly returned into the esophagus; proper positioning was assessed using the “occlusion test.”\textsuperscript{7} MAP was continuously recorded (LifeWindow 6000V Networked Multi-Parameter Veterinary Monitor; DigiCare Animal Health, USA). The tail vein was punctured for continuous infusion of Ringer’s lactate solution (10 ml kg\textsuperscript{-1} h\textsuperscript{-1}). Gelafundin® (B. Braun, Germany) was administered (titrated in 0.5-ml increments) to keep MAP greater than 60 mmHg. Muscle paralysis was achieved by administration of pancuronium (0.4 mg intramuscularly). Animals were then mechanically ventilated (Servo-1; MAQUET, Sweden) in volume-controlled mode with V\textsubscript{T} = 6 ml/kg, minute ventilation = 160 ml/min, inspiratory-to-expiratory ratio = 1:2, fraction of inspired oxygen (Fi\textsubscript{O2}) = 1.0, and PEEP = 3 cm H\textsubscript{2}O for 5 min. Arterial blood (300 μl) was drawn into a heparinized syringe to determine arterial oxygen partial pressure (Pa\textsubscript{O2}), arterial carbon dioxide partial pressure (PaCO\textsubscript{2}), and arterial pH (pHa) (ABL80 FLEX; Radiometer, Denmark) (BASELINE). After blood gas analysis, Fi\textsubscript{O2} was reduced to 0.4 to prevent possible iatrogenic effects, and lung mechanics were assessed. Rats were then assigned to the following groups: (1) according to ΔP: the first group was ventilated with V\textsubscript{T} = 6 ml/kg and PEEP = 3 cm H\textsubscript{2}O (ΔP\textsubscript{LOW} = 7.5 cm H\textsubscript{2}O); the second, with a V\textsubscript{T} that generated sufficient ΔP to keep animals alive during 1 h (ΔP\textsubscript{HIGH} = 12 cm H\textsubscript{2}O, V\textsubscript{T} = 22 ml/kg, PEEP = 3 cm H\textsubscript{2}O); the third, with a V\textsubscript{T} that generated a ΔP\textsubscript{MEAN} = (ΔP\textsubscript{LOW} + ΔP\textsubscript{HIGH})/2 = 10 cm H\textsubscript{2}O (V\textsubscript{T} = 13 ml/kg, PEEP = 3 cm H\textsubscript{2}O); the fourth, with a V\textsubscript{T} = 6 ml/kg and PEEP adjusted to reach the ΔP\textsubscript{HIGH} (V\textsubscript{T} = 6 ml/kg, PEEP = 11 cm H\textsubscript{2}O); and the fifth group was ventilated with a V\textsubscript{T} = 6 ml/kg and a PEEP adjusted to reach ΔP\textsubscript{MEAN} (V\textsubscript{T} = 6 ml/kg, PEEP = 9.5 cm H\textsubscript{2}O); and (2) according to Pplat,rs: V\textsubscript{T} = 6 ml/kg was applied and PEEP was adjusted to achieve Pplat,rs similar to that observed in the mean and high VT groups (14 and 17 cm H\textsubscript{2}O), in which V\textsubscript{T} was 13 and 22 ml/kg, respectively (fig. 1). After this step, animals were ventilated for 1 h, after which Fi\textsubscript{O2} was set at 1.0 for 5 min, and arterial blood gases were analyzed (END). Animals were killed using sodium thiopental injection (60 mg/kg) and lungs were extracted for histological and molecular biology analysis.
Data Acquisition and Processing

Airflow, $V_T$, and tracheal and esophageal pressures were measured. Transpulmonary pressure was calculated as the difference between the pressure in the alveoli and the pressure in the pleural cavity and can be used to estimate lung stress (transpulmonary pressure at end-inspiration), whereas $\Delta P_L$ was the difference between the transpulmonary pressure during end-inspiration and end-expiration. Respiratory system static elastance ($E_{st,rs}$) was calculated as the difference between $P_{plat,rs}$ and $P_{EEP}$ divided by the VT. Signals were filtered (200 Hz), amplified by a 4-channel conditioner (SC-24; SCIREQ), sampled at 200 Hz with a 12-bit analog-to-digital converter (NI-USB-6008; National Instruments, USA), and continuously recorded throughout the experiments.

Fig. 1. Schematic flowchart of study design (top) and timeline of the procedure (bottom). Dashed lines represent additional groups in which tidal volume ($V_t$) = 6 ml/kg was applied and positive end-expiratory pressure (PEEP) adjusted to similar respiratory system plateau pressure ($P_{plat,rs}$) achieved when $\Delta P_L$ was associated with high $V_t$ ($13$ ml/kg [$P_{plat,rs} = 14$ cm H$_2$O] and $22$ ml/kg [$P_{plat,rs} = 17$ cm H$_2$O]). ARDS = acute respiratory distress syndrome; $F_{O2}$ = inspiratory fraction of oxygen; $I:E$ = inspiratory-to-expiratory ratio; i.t. = intratracheally; LPS = *Escherichia coli* lipopolysaccharide; NV = nonventilated group; RR = respiratory rate; $\Delta P_L$ = transpulmonary driving pressure.

**CRITICAL CARE MEDICINE**
Light Microscopy
Laparotomy was performed immediately after blood sampling at END, and heparin (1,000 IU) was injected into the tail vein. The trachea was clamped at end-expiration. Lungs were removed en bloc with end-expiratory volume. The left lung was frozen in liquid nitrogen and submerged in Carnoy solution. Four-micrometer-thick slices were longitudinally cut from the left lung and stained with hematoxylin–eosin. Lung morphometric analysis was performed using an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines of known length coupled to a conventional light microscope (Olympus BX51; Olympus Latin America, Brazil). Both dorsal and ventral areas of the lungs were analyzed. The volume fractions of the lung occupied by collapsed alveoli, normal pulmonary areas, or hyperinflated structures (alveolar ducts, alveolar sacs, or alveoli; maximal chord length in air >120 μm) were determined by the point-counting technique at a magnification of ×200 across 10 random, noncoincident microscopic fields.10

Transmission Electron Microscopy
Three slices (2 × 2 × 2 mm) were cut from three different segments of the left lung and fixed for electron microscopy. On each lung electron microscopy image (20 fields per animal), damage to alveolar capillary membrane, type II epithelial and endothelial cells, and degree of interstitial edema were graded on a five-point, semiquantitative, severity-based scoring system as follows: 0 = normal lung parenchyma, 1 to 4 = changes in 1 to 25%, 26 to 50%, 51 to 75%, and 76 to 100% of examined tissue, respectively.6 Lung morphometry and electron microscopy analysis were performed in a blinded manner (V.L.C. and N.S.F.).

Expressions of Interleukin-6, Amphiregulin, Type 3 Procollagen, and Receptor for Advanced Glycation End Products
Quantitative real-time reverse-transcription polymerase chain reaction was performed to measure the expression of interleukin (IL)-6, type III procollagen (PCIII), receptor for advanced glycation end products (RAGE), and amphiregulin in lung tissue. Central slices of right lung were cut, collected in cryotubes, quick-frozen by immersion in liquid nitrogen, and stored at −80°C. Total RNA was extracted from frozen tissues using SV total RNA Isolation System (Promega Corporation). The survival rate was 100% in all groups during the investigation. Forty-eight animals were used, with 6 animals allocated to each group, including the nonventilated group.

Ventilatory parameters, Est.rs, and arterial blood gases at BASELINE (PEEP = 3 cm H2O) (see table 1, Supplemental Digital Content 2, http://links.lww.com/ALN/B150) showed similar functional impairment among groups before modification of ventilator settings, suggesting a similar degree of lung damage. ΔP,L, Est.rs, V̄T, PEEP, Pplat,rs, and respiratory rate did not differ from INITIAL to END in any group. MAP remained greater than 60 mmHg throughout the experiments (see table 2, Supplemental Digital Content 2, http://links.lww.com/ALN/B150).

Arterial blood gases at END with different ventilator settings are shown in table 1. At similar fixed levels of ΔP,L and Pplat,rs, oxygenation did not differ with changes in V̄T and PEEP. At low V̄T, higher PEEP led to a progressive improvement in oxygenation. PaCO2 markedly increased with VT = 6 ml/kg and PEEP = 11 cm H2O compared with VT = 6 ml/kg and PEEP = 5.5 cm H2O and declined with VT = 22 ml/kg and PEEP = 3 cm H2O compared with VT = 6 ml/kg and PEEP = 11 cm H2O.

The highest degree of alveolar collapse was observed at ΔP,L LOW and the most hyperinflation at ΔP,L HIGH (fig. 2).
At ΔP_L,MEAN, alveolar collapse was lower with VT = 6 ml/kg and PEEP = 9.5 cm H2O compared with VT = 13 ml/kg and PEEP = 3 cm H2O; however, hyperinflation was present with the former strategy. At ΔP_L, HIGH, the extent of alveolar collapse was lower with VT = 6 ml/kg and PEEP = 11 cm H2O than with VT = 22 ml/kg and PEEP = 3 cm H2O, but both strategies produced hyperinflation. At Pplat,rs = 17 cm H2O, ventilation with VT = 6 ml/kg and PEEP = 7.5 cm H2O was associated with less alveolar collapse and hyperinflation compared with VT = 22 ml/kg and PEEP = 3 cm H2O. For VT = 6 ml/kg, the increase in PEEP levels reduced alveolar collapse while increasing hyperinflation.

All animals showed cytoplasmic degeneration of type I and II epithelial and endothelial cells as well as alveolar-capillary damage. At similar levels of ΔP_L and Pplat,rs, regardless of VT and PEEP, no significant differences were observed in damage to the alveolar-capillary membrane, type I and II epithelial cells, or degree of interstitial edema. At VT = 6 ml/kg, ventilation with the highest PEEP to open the lung (PEEP = 11 cm H2O) led to less injury to the alveolar capillary membrane and type I and II epithelial cells compared with PEEP = 7.5 cm H2O and PEEP = 9.5 cm H2O, and less interstitial edema than PEEP = 9.5 cm H2O (see table 3, Supplemental Digital Content 2, http://links.lww.com/ALN/B150, and fig. 1, Supplemental Digital Content 3, http://links.lww.com/ALN/B151).

Gene expression of biological markers associated with inflammation (IL-6), damage inflicted to type I epithelial cells (RAGE), pulmonary stretch (amphiregulin), and fibrogenesis (PCIII) is shown in figure 3.

**Table 1. Arterial Blood Gases at END**

<table>
<thead>
<tr>
<th></th>
<th>NV</th>
<th>ΔP_L,LOW</th>
<th>ΔP_L,MEAN</th>
<th>ΔP_L, HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔP_L (cm H2O)</td>
<td></td>
<td>7.5</td>
<td>8.5</td>
<td>9.2</td>
</tr>
<tr>
<td>VT (ml/kg)</td>
<td></td>
<td>6</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>PEEP (cm H2O)</td>
<td></td>
<td>3</td>
<td>5.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Pplat,rs (cm H2O)</td>
<td></td>
<td>11</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>pHα</td>
<td>7.27±0.06</td>
<td>7.30±0.50</td>
<td>7.30±0.01</td>
<td>7.39±0.10</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>50±9.9</td>
<td>59±8</td>
<td>44±8</td>
<td>47±9</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>142±58.8</td>
<td>302±83</td>
<td>368±110</td>
<td>468±84</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals per group. One-way ANOVA followed by Bonferroni post hoc test. Dashed lines represent Pplat,rs similar to ΔP_L,MEAN and ΔP_L, HIGH at high VT (13 ml/kg [Pplat,rs = 14 cm H2O] and 22 ml/kg [Pplat,rs = 17 cm H2O]). For this purpose, VT was kept low (6ml/kg) and PEEP was adjusted for the level of Pplat,rs. Gas exchange was evaluated at FiO₂ = 1.0.

* vs. VT6-PEEP5.5; † vs. VT13-PEEP3; ‡ vs. VT6-PEEP9.5; § vs. VT22-PEEP3; || vs. VT6-PEEP3.

NV = nonventilated group; PaCO₂ = arterial carbon dioxide partial pressure; PaO₂ = arterial oxygen partial pressure; PEEP = positive end-expiratory pressure; pHα = arterial pH; Pplat,rs = respiratory system plateau pressure; VT = tidal volume; ΔP_L = transpulmonary driving pressure.

Downloaded From: http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/934246/ on 11/07/2018
and amphiregulin expressions were lower in ΔP,L_LOW (V_T = 6 ml/kg and PEEP = 3 cm H_2O) compared with ΔP,L_MEAN (V_T = 6 ml/kg and PEEP = 9.5 cm H_2O). ΔP,LHIGH (V_T = 6 ml/kg and PEEP = 11 cm H_2O) was associated with less RAGE expression than in all other groups ventilated with V_T = 6 ml/kg, except ΔP,L_LOW. At ΔP,L_MEAN, IL-6, amphiregulin, and PCIII expressions were higher with V_T = 6 ml/kg and PEEP = 9.5 cm H_2O than with V_T = 13 ml/kg and PEEP = 3 cm H_2O. At ΔP,LHIGH, different combinations of VT and PEEP did not affect these biological markers. At Pplat,rs = 14 cm H_2O, IL-6 expression was higher with V_T = 6 ml/kg and PEEP = 5.5 cm H_2O than with V_T = 13 ml/kg and PEEP = 3 cm H_2O. At Pplat,rs = 17 cm H_2O, RAGE expression was higher with V_T = 6 ml/kg and PEEP = 7.5 cm H_2O than with V_T = 22 ml/kg and PEEP = 3 cm H_2O. IL-6, RAGE, and amphiregulin expressions were higher with V_T = 6 ml/kg and PEEP = 9.5 than with V_T = 6 ml/kg and PEEP = 3 cm H_2O. Ventilation with V_T = 6 ml/kg and PEEP = 11 cm H_2O reduced the expression of RAGE compared with V_T = 6 ml/kg and PEEP = 9.5 cm H_2O although amphiregulin expression remained high.

Correlation analyses of mechanical, biological, and morphological data among all groups are shown in table 4, Supplemental Digital Content 2, http://links.lww.com/ALN/B150. ΔP,L correlated with alveolar hyperinflation and alveolar collapse. Pplat,rs correlated positively with IL-6 and amphiregulin expressions and alveolar hyperinflation and negatively with alveolar collapse. VT did not present significant correlations with IL-6, RAGE, amphiregulin, or PCIII expressions, alveolar hyperinflation, or alveolar collapse (see table 4, Supplemental Digital Content 2, http://links.lww.com/ALN/B150).

Table 3. Expression of biological markers. Real-time polymerase chain reaction analysis of biological markers associated with inflammation (interleukin [IL]-6), alveolar overdistension (amphiregulin), damage inflicted upon alveolar type I epithelial cells (receptor for advanced glycation end products [RAGE]), and fibrogenesis (type III procollagen [PCIII]). Relative gene expression was calculated as a ratio of the average gene expression levels compared with the reference gene (36B4) and expressed as fold change relative to nonventilated (NV) animals. Values expressed as mean ± SD of six animals per group. Student t test followed by Bonferroni correction was performed between groups with similar fixed levels of ΔP,L and Pplat,rs. One-way ANOVA followed by Bonferroni post hoc test. * Versus V_T6-PEEP3; † versus V_T6-PEEP5.5; § versus V_T6-PEEP9.5 (P < 0.05). PEEP = positive end-expiratory pressure; Pplat,rs = respiratory system plateau pressure; V_T = tidal volume; ΔP,L = transpulmonary driving pressure.

![Fig. 3. Expression of biological markers. Real-time polymerase chain reaction analysis of biological markers associated with inflammation (interleukin (IL)-6), alveolar overdistension (amphiregulin), damage inflicted upon alveolar type I epithelial cells (receptor for advanced glycation end products (RAGE)), and fibrogenesis (type III procollagen (PCIII)). Relative gene expression was calculated as a ratio of the average gene expression levels compared with the reference gene (36B4) and expressed as fold change relative to nonventilated (NV) animals. Values expressed as mean ± SD of six animals per group. Student t test followed by Bonferroni correction was performed between groups with similar fixed levels of ΔP,L and Pplat,rs. One-way ANOVA followed by Bonferroni post hoc test. * Versus V_T6-PEEP3; † versus V_T6-PEEP5.5; § versus V_T6-PEEP9.5 (P < 0.05). PEEP = positive end-expiratory pressure; Pplat,rs = respiratory system plateau pressure; V_T = tidal volume; ΔP,L = transpulmonary driving pressure.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/934246/ on 11/07/2018)
with IL-6 and amphiregulin expressions, as well as with alveolar hyperinflation, and negatively with alveolar collapse (fig. 4).

The following variables were found to be predictors of IL-6, amphiregulin, and PCIII expressions, respectively: (1) $V_T$ (coefficient = 2.646, $P = 0.005$) and PEEP

Fig. 4. Pearson correlations of transpulmonary driving pressure ($\Delta P_L$) and respiratory system plateau pressure ($P_{plat,rs}$) with interleukin (IL)-6 and amphiregulin expressions and alveolar collapse and hyperinflation at fixed tidal volume ($V_T$) = 6 ml/kg. The $r$ value represents the correlation coefficient, and $P$, the respective $P$ value. Statistical significance was accepted at $P < 0.05$. NV = nonventilated group.
(coefficient = 8.977, \( P = 0.002 \)); (2) \( V_T \) (coefficient = 0.393, \( P = 0.005 \)); and (3) \( V_T \) (coefficient = 0.0975, \( P = 0.015 \)) and PEEP (coefficient = 0.496, \( P < 0.001 \)).

**Discussion**

In the model of ARDS used in this study, we found that (1) \( \Delta P_L \)low led to alveolar collapse, preventing cyclic recruitment/derecruitment of distal lung units, and to no hyperinflation, therefore resulting in no end-inspiratory stress and lung inflammation; (2) at fixed \( \Delta P_L \)mean, low \( V_T \) combined with a PEEP level not enough to keep lungs fully open at endexpiration reduced alveolar collapse but increased hyperinflation, consequently increasing lung inflammation and fibrogenesis, probably due to alveolar instability; (3) at fixed \( \Delta P_L \)high, low \( V_T \) with higher PEEP led to alveolar hyperinflation as measured by amphiregulin expression. However, no further lung inflammation and fibrogenesis possibly associated with reduced cyclic recruitment/derecruitment of distal lung units were detected. The latter findings contradict our previous third hypothesis. After multiple linear regression analyses, \( V_T \) was an independent predictor of biological markers of inflammation, lung cell stretch, and fibrogenesis. PEEP was associated with IL-6 and PCIII expression. \( \Delta P_L \) and Pplat,rs seem to have similar effects on the evolution of injury and inflammation in this ARDS model.

Different mechanical factors may promote VILI, such as \( V_T \), PEEP, Pplat,rs, and \( \Delta P_L \). Previous studies found these variables to interact, precluding dissociation of their individual contributions to VILI.8,12–16 In the ARDS model used herein, endotoxin was intratracheally instilled6,17 as a first hit to induce lung inflammation. After 24 h, animals exhibited impaired lung mechanics, atelectasis, damage to epithelium and alveolar-capillary membrane, interstitial edema, and increased IL-6 expression.5 Animals were then randomized to receive different MV parameters as a second hit.18,19 After ventilatory strategies, VILI was defined as histological and mechanical alterations present plus an increase in at least one of the following mediators: IL-6, amphiregulin, RAGE, and PCIII.

To the best of our knowledge, this is the first study describing the individual contributions of \( V_T \) and PEEP at each level of \( \Delta P_L \) and Pplat,rs on lung morphology and molecular biology in experimental ARDS. Furthermore, unlike in previous studies, the effects of ventilator strategies settings were investigated 24 h after the insult (when the morphofunctional and biological changes of ARDS were already present), and an esophageal catheter was used to measure the driving pressure of the lung.

Airway pressure is influenced by chest wall properties and respiratory muscle activity, whereas the transpulmonary pressure at end-inspiration and end-expiration (\( \Delta P_L \)) enables estimation of the actual distending pressure of lungs, unencumbered by chest wall and patient effort on recorded airway pressures.9 The driving pressure is a function not only of \( V_T \) but also of PEEP. In fact, different levels of PEEP are associated with possible changes in lung compliance. For this reason, a given driving pressure can be achieved at higher \( V_T \) with lower PEEP as well as at lower \( V_T \) with higher PEEP. Those conditions have been addressed in the current study.

We measured mRNA expression of IL-6 as a surrogate of inflammation during VILI.20 PCIII expression was evaluated because it is a marker of lung fibrogenesis,21 and RAGE expression reflects alveolar type I cell injury.22 Amphiregulin expression is positively modulated by hyperinflation, activates chemokines, cytokines, and adhesion molecules,23–25 and represents a novel candidate gene in VILI.24,26

Our data suggest that the increase in \( \Delta P_L \) induced by higher PEEP or \( V_T \) promotes VILI. The finding that \( \Delta P_L \)low (\( V_T = 6 \text{ ml/kg} \), PEEP = 3 cm H2O) reduced VILI when compared with \( \Delta P_L \)high (\( V_T = 6 \text{ ml/kg} \), PEEP = 11 cm H2O) may be explained by the maintenance of areas of alveolar collapse (avoiding cyclic recruitment–derecruitment) without hyperinflation, thus minimizing lung inflammation, type I epithelial cell damage, and pulmonary mechanical stress. This is consistent with the concept of “lung rest” or “permissive atelectasis” for lung protection, which has been shown in animals4,27,28 and humans29 to reduce alveolar damage in aerated and atelectatic lung regions. It is worth noting that \( \Delta P_L \)mean, with \( V_T = 6 \text{ ml/kg} \) and PEEP = 9.5 cm H2O was associated with the highest increase in markers of inflammation, epithelial cell damage, lung cell stretch, and fibrogenesis. We hypothesize that if PEEP is not high enough to keep the lungs open at end-expiration, alveolar instability may occur, with cyclic recruitment and derecruitment of lung units and increased shear stress in the unstable zone. At \( V_T = 6 \text{ ml/kg} \) and PEEP = 11 cm H2O, alveolar collapse and markers of epithelial cell damage were reduced, suggesting decreased strain and regional dynamic stress. By using low \( V_T \) but high PEEP, the lungs are kept tonically inflated above their functional residual capacity and thus exposed to an additional static strain.16 \( \text{PaCO}_2 \) also increased, probably due to the associated increase in dead space.

The increased \( \Delta P_L \) resulting from different PEEP levels at fixed \( V_T = 6 \text{ ml/kg} \) yielded less alveolar collapse but higher Est,rs. The lack of correlation between alveolar recruitment and changes in Est,rs has been previously reported29,30 and might be attributed to the prevalence of hyperinflation instead of alveolar reopening with higher PEEP. Our data indicate that during ventilation with \( V_T = 6 \text{ ml/kg} \), the level of PEEP titrated according to the lowest \( \Delta P_L \) and, consequently, the best elastance, may be associated with less activation of biological markers and hyperinflation, as previously shown in experimental and human studies.3,8,31,32

The increase in \( \Delta P_L \) induced by changes in \( V_T \) (from 6 to 22 ml/kg) at fixed low PEEP (3 cm H2O) yielded increased alveolar collapse and hyperinflation. However, the increase in \( \Delta P_L \) obtained by using higher \( V_T \) rather than higher PEEP led to increased IL-6 expression, but no change in amphiregulin, suggesting that the cellular response to inflammation was more pronounced than stretch in the presence of greater strain and dynamic stress.
At fixed ΔP, mean but not at ΔP, high, low VT and high PEEP compared with high VT and low PEEP resulted in increased IL-6, amphiregulin, and PClII expressions. We hypothesize that there could be a threshold of ΔP above which differences in biomarkers of cellular activation are no longer observed by different combinations of VT and PEEP.

Injured lungs have markedly different sizes and variable Pplat,rs. Therefore, the use of high VT may result in greater lung damage compared with low VT. In our study, we observed that, compared with high VT with low PEEP, low VT with high PEEP increased IL-6 expression at fixed Pplat,rs = 14 cm H2O and RAGE expression at Pplat,rs = 17 cm H2O. The mechanotransduction response to Pplat,rs seems to differ from that induced by ΔP, L. This may be due to the different limits of end-inspiratory and end-expiratory stress/strain for each combination of VT and static stress/strain to VILI depend on Pplat,rs.

Multiple linear regressions showed that all ventilator settings investigated herein led to increased biological markers of inflammation, type I alveolar epithelial cell damage, cell stretch, and fibrogenesis. Taken together, these observations suggest that the best approach for protective MV may be to reduce VT, PEEP, ΔP, L, and Pplat,rs. Statistically, the most important ventilator variable associated with VILI was VT, which is in line with experimental and human studies in ARDS.

Possible Clinical Implications
Our data suggest that ventilation with VT = 6 ml/kg combined with the lowest PEEP and ΔP, L to keep oxygenation within a safe range can minimize VILI. Doing so is in accordance with the hypothesis that these ventilator settings may protect the collapsed distal lung units from excessive strain, while avoiding overstretching and inflammation in normal lung regions. This strategy is also known as “permissive atelectasis.” However, if life-threatening hypoxemia with low VT occurs, a defensible compromise would be to set the least PEEP needed to achieve and sustain alveolar recruitment.

In a lung exposed to a first hit (endotoxin), 1 h of MV is enough to modulate different genes associated with VILI depending on the ventilatory strategy (second hit). This is contrary to the behavior observed in healthy lungs subjected to different ventilatory strategies. Therefore, preexisting lung alterations (edema, atelectasis, or pneumonia) make the diseased lung much more susceptible to mechanical injury.

Limitations
First, ARDS was induced by intratracheal administration of endotoxin, and our results can be extended neither to other ARDS models with different degrees of severity nor to human ARDS. Second, the PEEP levels used in the current study, while often used in rats, may not be directly extrapolated to the clinical setting; nevertheless, theoretical analyses have shown that PEEP levels in rats could be equivalent to double those in humans, according to the estimated transpulmonary pressure. Therefore, the range of PEEP levels used in the current study resembles that used in mechanically ventilated critical care patients (6 to 22 cm H2O). Third, we decided to forgo recruitment maneuvers, to avoid possible confounding effects concerning different biological impacts on lung tissue, but cannot rule out that such maneuvers might have further improved lung function and affected different biomarkers. However, the highest PEEP levels used in the current study were able to keep alveolar units open, as demonstrated both by morphometric data and by biological markers. This study was based on a proof of concept, not on the best ventilatory strategy. Fourth, mediators were measured in lung tissue, but not in blood. Fifth, acidosis may modulate the inflammatory process, but its potential effects were not evaluated. Sixth, the high level of variability in physiological data within each group may account for the lack of between-group differences at similar driving or plateau pressures. Finally, the observation time was relatively short as compared with previous studies (1 vs. 2 to 6 h), precluding changes in protein levels of all biological markers analyzed. Furthermore, to keep animals with endotoxin-induced ARDS alive for 4 to 6 h, greater amounts of fluid and/or inotropes would be required, which could interfere with gene expression.

Conclusions
In experimental endotoxin-induced ARDS, a ventilation strategy combining low VT (6 ml/kg) and low PEEP that resulted in low ΔP, L and Pplat,rs mitigated VILI, despite allowing alveolar collapse. Furthermore, VT = 6 ml/kg combined with PEEP at the highest level to open the lungs reduced inflammation and epithelial cell damage, despite allowing hyperinflation. In short, VT = 6 ml/kg with PEEP levels not high enough to keep lungs open can cause alveolar instability with subsequent VILI.

Acknowledgments
The authors thank Andre Silva, B.Sc. (Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil), for animal care; Ana Lucia Silva, B.Sc. (Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro), for her help with microscopy; Moira Schottler (Rio de Janeiro, Brazil) and Filippe Vasconcellos (Porto Alegre, Rio Grande do Sul, Brazil) for their assistance in editing the manuscript; Ronir Luiz, Ph.D. (Institute of Public Health Studies, Federal University of Rio de Janeiro), for his help with statistics, and MAQUET (São Paulo, Brazil) for technical support.

Support was provided by the Brazilian Council for Scientific and Technological Development (CNPq, Brasilia, Distrito Federal, Brazil), Rio de Janeiro a Research Foundation (FAPERJ, Rio de Janeiro, Brazil), São Paulo State Research Foundation (FAPESP, São Paulo, Brazil), National Institute of Science and Technology of Drugs and Medicine
2. Amato MB, Barbas CS, Medeiros DM, Magaldi RB, Schettino


