# Biologic Impact of Mechanical Power at High and Low Tidal Volumes in Experimental Mild Acute Respiratory Distress Syndrome

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## ABSTRACT

**Background:** The authors hypothesized that low tidal volume ($V_T$) would minimize ventilator-induced lung injury regardless of the degree of mechanical power. The authors investigated the impact of power, obtained by different combinations of $V_T$ and respiratory rate (RR), on ventilator-induced lung injury in experimental mild acute respiratory distress syndrome (ARDS).

**Methods:** Forty Wistar rats received *Escherichia coli* lipopolysaccharide intratracheally. After 24 h, 32 rats were randomly assigned to be mechanically ventilated (2 h) with a combination of different $V_T$ (6 ml/kg and 11 ml/kg) and RR that resulted in low and high power. Power was calculated as energy ($\Delta P_L^2/E_{,L}$) × RR ($\Delta P_L$ = transpulmonary driving pressure; $E_{,L}$ = lung elastance), and was threefold higher in high than in low power groups. Eight rats were not mechanically ventilated and used for molecular biology analysis.

**Results:** Diffuse alveolar damage score, which represents the severity of edema, atelectasis, and overdistension, was increased in high $V_T$ compared to low $V_T$, in both low (low $V_T$: 11 [9 to 14], high $V_T$: 18 [15 to 20]) and high (low $V_T$: 19 [16 to 25], high $V_T$: 29 [27 to 30]) power groups. At high $V_T$, interleukin-6 and amphiregulin expressions were higher in high-power than in low-power groups. At high power, amphiregulin and club cell protein 16 expressions were higher in high $V_T$ than in low $V_T$. Mechanical energy and power correlated well with diffuse alveolar damage score and interleukin-6, amphiregulin, and club cell protein 16 expression.

**Conclusions:** In experimental mild ARDS, even at low $V_T$, high mechanical power promoted ventilator-induced lung injury. To minimize ventilator-induced lung injury, low $V_T$ should be combined with low power. **(Anesthesiology 2018; 128:1193-206)**



MECHANICAL ventilation potentially worsens or even initiates lung injury.[1,2] High distending pressures (barotrauma), as well as repetitive collapse and reopening of lung units (atelectrauma), may trigger an inflammatory response (biotrauma) that can result in ventilator-induced lung injury,[3] organ dysfunction,[4,5] and death.[6]

The role of low tidal volume ($V_T$) in lung protection has been well established in both experimental[7] and clinical studies.[8] In addition, the importance of choosing adequate levels of positive end-expiratory pressure (PEEP) has been well documented, especially in patients with severe acute respiratory distress syndrome (ARDS).[2,9] More recently, the importance of limiting the so-called driving pressure (ΔP = inspiratory plateau pressure—PEEP) has been emphasized even when combinations of low $V_T$ and adequate PEEP are used.[10] Furthermore, experimental studies have shown that the respiratory rate (RR),[11] as well as the inspiratory[12,13] and expiratory[14] airflow, modulate the severity of lung injury. When combined, all of these variables determine the mechanical energy, or power, that is transferred from the mechanical ventilator to the lungs.[15,16]

Mechanical power (energy over time) is associated with deterioration of lung function and formation of lung edema

### What We Already Know about This Topic

- It has generally been believed that excess tidal volume (or distending pressure) are the main causes of ventilator-associated lung injury in acute respiratory distress syndrome

### What This Article Tells Us That Is New

- In an *in vivo* study of experimental acute respiratory distress syndrome, different combinations of tidal volume and respiratory rate were used to demonstrate that mechanical power and tidal volume can independently contribute to ventilator-induced lung injury

This article is featured in "This Month in Anesthesiology," page 1A. Corresponding article on page 1062. 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). This article has a video abstract. R.S.S. and L.d.A.M. contributed equally as first authors.

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in pigs without previous lung injury and ventilated with increased $V_T$.[16] While the contribution of RR to mechanical power and its impact on lung injury has been described,[17] it is still unclear whether low and high $V_T$ result in comparable lung injury when similar levels of mechanical power are achieved. Based on this aforementioned, we hypothesized that low $V_T$ would minimize ventilator-induced lung injury regardless of the degree of mechanical power.

In the present study, we investigated the impact of mechanical power, as obtained by combinations of different $V_T$ and respiratory rate (RR), on lung function and surrogates of ventilator-induced lung injury (histologic lung damage, inflammation, and mechanical alveolar stretch) in a rat model of mild ARDS.

## Materials and Methods

This study was approved by the Ethics Committee of the Carlos Chagas Filho Institute of Biophysics, Health Sciences Center, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences, USA.

### Animal Preparation and Experimental Protocol

Forty Wistar rats (weight 408 ± 63g) were anesthetized by inhalation of sevoflurane 2% (Sevorane; Cristália, Brazil) and underwent intratracheal instillation of *Escherichia coli* lipopolysaccharide (O55:B5, LPS Ultrapure; Invivogen, France), 200 μg suspended in saline solution to a total volume of 200 μl,[18] to induce mild-to-moderate ARDS. After 24 h, animals were premedicated intraperitoneally with 10 mg/kg diazepam (Compaz; Cristália, Brazil), followed by 100 mg/kg ketamine (Ketamin-S+; Cristália, Brazil) and 2 mg/kg midazolam (Dormicum; União Química, Brazil). After local anesthesia with 2% lidocaine (0.4 ml), a midline neck incision and tracheostomy were performed.

An intravenous (iv) catheter (Jelco 24G, Becton, Dickinson and Company, USA) was inserted into the tail vein, and anesthesia induced and maintained with midazolam ($2\ mg \cdot kg^{-1} \cdot h^{-1}$) and ketamine ($50\ mg \cdot kg^{-1} \cdot h^{-1}$). Additionally, $10\ ml \cdot kg^{-1} \cdot h^{-1}$ Ringer's lactate (B. Braun, Crissier, Switzerland) was administered iv; Gelafundin (B. Braun, São Gonçalo, Brazil) was administered (iv, in 0.5 ml boluses) to maintain mean arterial pressure (MAP) greater than 60 mmHg. A second catheter (18G; Arrow International, USA) was then placed in the right internal carotid artery for blood sampling and arterial blood gas analysis (ABL80 FLEX; Radiometer Medical, Denmark), as well as monitoring of MAP (Networked Multiparameter Veterinary Monitor LifeWindow 6,000 V; Digicare Animal Health, USA). A 30–cm-long water-filled catheter (PE-205, Becton, Dickinson and Company) with side holes at the tip, connected to a differential pressure transducer (UT-PL-400, SCIREQ, Canada), was used to measure the esophageal pressure. The catheter was passed into the stomach and then slowly returned into the esophagus; its proper positioning was assessed with the "occlusion test," as described elsewhere.[19] Heart rate (HR), MAP, and rectal temperature were continuously monitored (Networked Multiparameter Veterinary Monitor LifeWindow 6000V, Digicare Animal Health, USA). Body temperature was maintained at 37.5 ± 1°C using a heating bed.

Animals were paralyzed with pancuronium bromide (2 mg/kg, iv), and their lungs mechanically ventilated (Servo-i, MAQUET, Sweden) in volume-controlled mode (VCV) with constant inspiratory flow, $V_T$ = 6 ml/kg, RR to maintain normocapnia ($Pa_{CO_2}$ = 35 to 45 mmHg), PEEP = 3 cm $H_2O$, $F_{IO_2}$ = 1.0, and inspiratory-expiratory ratio of 1:2 (fig. 1). After 5 min (BASELINE), arterial blood gases (300 μl) were determined (Radiometer ABL80 FLEX, Denmark). $F_{IO_2}$ was reduced to 0.4 to prevent possible iatrogenic effects, and lung mechanics were assessed. Thirty-two rats were then randomly assigned to be mechanically ventilated with a combination of different $V_T$ (6 ml/kg and 11 ml/kg) and RR settings that resulted in low and high mechanical power (N = 8/group): (1) low power/low $V_T$: $V_T$ = 6 ml/kg and RR adjusted to maintain normocapnia; (2) low power/high $V_T$: $V_T$ = 11 ml/kg and RR to maintain normocapnia; (3) high power/low $V_T$: $V_T$ = 6 ml/kg and RR to increase mechanical power by threefold compared to the first two groups; and (4) high power/high $V_T$: $V_T$ = 11 ml/kg and RR also adjusted to increase mechanical power by threefold compared to the first two groups. The "low" mechanical power levels were derived from a previous publication,[7] whereas the "high" mechanical power levels were defined according to pilot studies (data not shown). The different levels of $V_T$ corresponded approximately to the lower limits of protective and nonprotective ventilation (6 and 11 ml/kg, respectively). Settings were kept for 2 h. Arterial blood gases were determined after randomization (INITIAL) and at the end of the experiment (FINAL). Lung mechanics were assessed after randomization (INITIAL), at 60 min, and at the end of the experiment (FINAL). Eight rats were used for molecular biology analysis and were not mechanically ventilated (nonventilated). At BASELINE and FINAL, the endotracheal tube was clamped, and animals were connected to a flexiVent mechanical ventilator (SCIREQ); standardized pressure-volume curves at the same $F_{IO_2}$ and PEEP as implemented by the manufacturer were then obtained. Heparin (1000 IU) was injected into the tail vein and animals were euthanized by overdose of sodium thiopental (60 mg/kg). The lungs were extracted at PEEP = 3 cm $H_2O$ for histology and molecular biology analysis.

To rule out the potential effects of a mismatch in $Pa_{CO_2}$ between the high and low mechanical power groups on diffuse alveolar damage score and the expression of biomarkers of inflammation, 10 additional animals were ventilated with high mechanical power and low as well as high $V_T$, but the dead space adapted to yield normocapnia (Supplemental Digital Content 1, http://links.lww.com/ALN/B640).

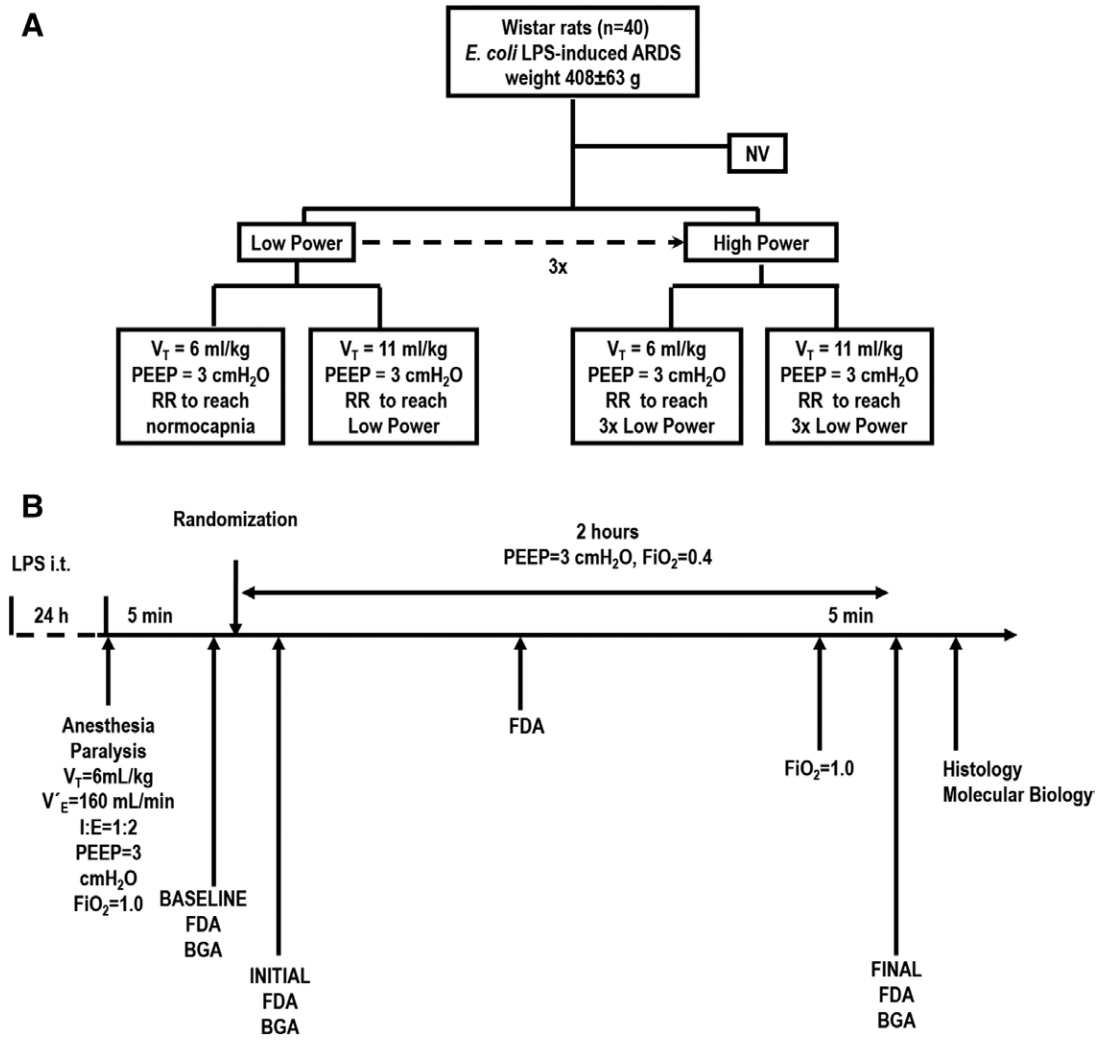


**Fig. 1.** Schematic flowchart of study design (top) and timeline representation of the experimental protocol (bottom). Lung mechanics were assessed every 20 min. Pressure-volume (PV) curve and arterial blood gases were evaluated at INITIAL and FINAL. ARDS = acute respiratory distress syndrome; BGA = blood gas analysis; FDA = functional data acquisition; $F_{IO_2}$ = fraction of inspired oxygen; I:E = inspiratory-to-expiratory ratio; i.t. = intratracheally; LPS = *Escherichia coli* lipopolysaccharide; MV = minute ventilation; NV = nonventilated; PEEP = positive end-expiratory pressure; RR = respiratory rate; $V'_E$ = minute ventilation; $V_T$ = tidal volume.

### *Data Acquisition and Processing*

Airflow, airway pressure (Paw), and esophageal pressure were continuously recorded throughout the experiments with a computer running custom-made software written in LabVIEW (National Instruments, USA).[20,21] Inspiratory flow was measured from the recorded curve. Briefly, $V_T$ was calculated by digital integration of the airflow signal obtained from a custom-made pneumotachograph[22] that was connected to the Y-piece of the ventilator tubing, while RR was calculated from the esophageal pressure swings as the frequency per minute of each type of breathing cycle. All signals were amplified in a four-channel signal conditioner (SC-24, SCIREQ), and sampled at 200 Hz with a 12-bit analog-to-digital converter (National Instruments, USA). Mechanical data were computed offline by a routine written in MATLAB (Version R2007a; The Mathworks Inc., USA).

Respiratory system plateau pressure was measured in occluding airways at end-inspiration during 5 s.[20] Respiratory system driving pressure was calculated as the difference between respiratory system plateau pressure and PEEP. Transpulmonary pressure was calculated as the difference between the pressure in the alveoli and the pressure in the pleural cavity (transpulmonary pressure at end-inspiration), and transpulmonary driving pressure as the difference between transpulmonary pressure at end-inspiration and at end-expiration. Intrinsic PEEP was measured by occluding the airway at end-expiration.[23]

The mechanical energy was calculated based on: (1) the equation described by Guerin *et al.*[24] and Marini and Jaber[25] (simplified formula); (2) a quasi-static pressure-volume curve (flexiVent); and (3) the equation proposed by Gattinoni *et al.*,[26] based on the equation of motion.

The first approach to compute energy is presented in equation 1:

$$\text{Energy}_{,L} = \Delta P_{,L}^{2} / E_{,L} \quad (1)$$

where $\Delta P_{,L}$ is the transpulmonary driving pressure and $E_{,L}$ is the lung elastance.

In order to perform the quasi-static pressure-volume curve, a stepwise increase in airway pressure up to 30 cm $H_2O$ was done. The area of the pressure-volume curve was computed (Supplemental Digital Content 2, http://links.lww.com/ALN/B641). The third method to compute mechanical energy was based on the formula presented in equation 2:

$$\text{Energy}_{,L} = \Delta V^{2} \times [(0.5 \times E_{,RS} + RR \times (1 + I{:}E) / 60 \times I{:}E \times Raw) + \Delta V \times PEEP] \quad (2)$$

where $\Delta V$ is the variation of tidal volume, $E_{,RS}$ is the respiratory system elastance, I:E is the inspiratory to expiratory ratio, and Raw is the airway resistance. All values were converted to mJ. Mechanical power was calculated as the product of mechanical energy multiplied by RR.

### *Histology*

**Light Microscopy.** The lungs and heart were removed *en bloc*. The left lung was frozen in liquid nitrogen and immersed in formaldehyde solution (4%), embedded in paraffin, cut longitudinally in the central zone by means of a microtome into slices 4 μm thick, and stained with hematoxylin-eosin for histologic analysis.[26] Photomicrographs at magnifications of ×100, ×200, and ×400 were obtained from eight non-overlapping fields of view per section using a light microscope (Olympus BX51; Olympus Latin America Inc., Brazil). Diffuse alveolar damage was quantified by two investigators (V.M. and V.L.C.) blinded to group assignment and independently, using a weighted scoring system, as described elsewhere.[27] Briefly, scores of 0 to 4 were used to represent edema, atelectasis and overdistension, with 0 standing for no effect and 4 for maximum severity. Additionally, the extent of each scored characteristic per field of view was determined on a scale of 0 to 4, with 0 standing for no visible evidence and 4 for complete involvement. Scores were calculated as the product of severity and extent of each feature, on a range of 0 to 16. The cumulative diffuse alveolar damage score was calculated as the sum of each score, and thus ranged from 0 to 48.

**Immunohistochemistry.** To analyze the adherens junction protein E-cadherin, immunohistochemical procedures were performed on 4-μm-thick, paraffin-embedded lung sections using a mouse polyclonal antibody against E-cadherin (1:250; cat. number, 610181; BD Transduction Laboratories, Becton, Dickinson and Company).[18] After dewaxing and rehydrating, endogenous peroxidase was blocked with 3% $H_2O_2$ in methanol for 15 min. Heat-mediated antigen retrieval and enzymatic techniques were performed according to the specific antibody. After blocking the non-specific binding of immunoglobulins to the tissue, primary antibodies were then incubated overnight at 4°C in a humidified chamber for approximately 16 h. The sections were then washed in 0.25% Tween/phosphate-buffered saline solution for 5 min, and the secondary antibodies were incubated (Nichirei-Histofine Simple Stain Rat MAX-PO-Mouse; Nichirei Bioscience Inc., Germany). The chromogen substrate was diaminobenzidine (cat. number, K3468; Liquid DAB, Dako Denmark A/S, Denmark). Negative control slides were incubated with mouse isotype immunoglobulins or with antibody diluent solution. Visualization and image capture were performed using a light microscope (Eclipse E800; Nikon, Japan) coupled to a digital camera (Evolution; Media Cybernetics, USA) with the Q-Capture 2.95.0 graphic interface software (version 2.0.5; Quantitative Imaging, Canada). High-quality images (2,048 × 1,536 pixel buffer) were captured away from airways. After calibration of program settings, images were analyzed using the Image Pro Plus software (version 4.5.1; Media Cybernetics, USA).[18]

**Transmission Electron Microscopy.** Three slices (2 × 2×2 mm) were cut from three different segments of the right lung and fixed for electron microscopy. On each electron micrograph (20 fields per animal), type II epithelial cell damage, basement membrane thickness, and endothelial cell damage 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.[20,28] Electron microscopy analyses were performed by two investigators (V.M. and V.L.C.) blinded to group assignment.

**Biologic Markers Associated with Mechanical Cell Stress, Inflammation, and Epithelial and Endothelial Cell Damage.** Quantitative real-time reverse transcription polymerase chain reaction was performed to measure biomarkers associated with alveolar pulmonary stretch (amphiregulin), inflammation [interleukin (IL)-6], epithelial cell damage [club cell protein 16 (CC16)], and endothelial cell damage [intercellular adhesion molecular (ICAM)-1]. The primers used are shown in the Supplemental Digital Content 3 (http://links.lww.com/ALN/B642). Central slices of the right lung were cut, collected in cryotubes, flash-frozen by immersion in liquid nitrogen, and stored at −80°C. Total RNA was extracted from frozen tissues using the RNeasy Plus Mini Kit (Qiagen, Germany), following the manufacturer's recommendations. RNA concentrations were measured by spectrophotometry in a Nanodrop ND-1000 system (ThermoScientific, USA). First-strand cDNA was synthesized from total RNA using a Quantitec reverse transcription kit (Qiagen). Messenger RNA (mRNA) levels were measured with a SYBR green detection system in an ABI 7500 real-time polymerase chain reaction system (Applied Biosystems, USA). Samples were run in triplicate. For each sample, the expression of each gene was normalized to the

acidic ribosomal phosphoprotein P0 (*36B4*) housekeeping gene[29] and expressed as fold change relative to respective nonventilated animals, using the $2^{-\Delta\Delta Ct}$ method, where $\Delta Ct = Ct_{(target\ gene)} - Ct_{(reference\ gene)}$.[30]

### Statistical Analysis

Sample size was based on pilot studies and on our past experience with ventilator strategies in small animals.[20] We tested the hypothesis that low $V_T$ would decrease IL-6 gene expression regardless of the degree of mechanical power. Accordingly, a sample size of eight animals per group (providing for one animal as dropout) would provide the appropriate power ($1\text{-}\beta = 0.8$) to identify significant ($\alpha = 0.05$) differences in the symmetry score, taking into account an effect size d = 1.9, a two-sided test, and a sample size ratio = 1 (G*Power 3.1.9.2, University of Düsseldorf, Germany). Data were tested for normality using the Kolmogorov-Smirnov test with Lilliefors' correction, while the Levene median test was used to evaluate homogeneity of variances. If both conditions were satisfied, Mauchly's test of sphericity with repeated-measures ANOVA ($P < 0.05$) was used.[31] If epsilon was higher than 0.75, the Huynh-Feldt *P* value was shown; otherwise, the Greenhouse-Geisser *P* value was provided. Additionally, to compare all parameters among groups at each time point, a mixed linear model based on a random intercept for each animal followed by Bonferroni's test was used.

Molecular biology, diffuse alveolar damage, and electron microscopy variables were assessed with the Mann–Whitney *U* test and Bonferroni correction for 4 comparisons ($P = 0.0125$) (low power/low $V_T$ *vs.* low power/high $V_T$ and high power/low $V_T$, as well as high power/low $V_T$ *vs.* high power/high $V_T$). Association and agreement between energy calculated from the pressure-volume curve (flexiVent) and mechanical power equation were determined by Pearson correlation and Bland-Altman analysis, respectively. Associations of diffuse alveolar damage score, IL-6, amphiregulin, and club cell protein 16 with energy and mechanical power were assessed with Spearman coefficients. Multiple linear regression analysis was performed to predict associations with the dependent variables (diffuse alveolar damage, IL-6, amphiregulin, and club cell protein 16) and independent variables ($V_T$, RR, and transpulmonary driving pressure). Parametric data were expressed as mean ± SD, while nonparametric data were expressed as median (interquartile range). The impact

**Table 1.** Mean Arterial Pressure and Arterial Blood Gas Analysis during Mechanical Ventilation

| Parameter | Power | $V_T$ | INITIAL | FINAL | Time Effect | Group Effect | Time *vs.* Group Effect |
|---|---|---|---|---|---|---|---|
| MAP (mmHg) | | | | | $P = 0.38$ | $P = 0.12$ | $P = 0.59$ |
| | Low | Low | 98 ± 17 | 70 ± 27 | | | |
| | | High | 101 ± 19 | 105 ± 22 | | | |
| | High | Low | 76 ± 4 | 74 ± 27 | | | |
| | | High | 100 ± 43 | 94 ± 41 | | | |
| Blood gas analysis | | | | | | | |
| $PaO_2/FIO_2$ (mmHg) | | | | | $P = 0.47$ | $P = 0.16$ | $P = 0.61$ |
| | Low | Low | 410 ± 69 | 375 ± 105 | | | |
| | | High | 332 ± 95 | 300 ± 129 | | | |
| | High | Low | 337 ± 80 | 358 ± 100 | | | |
| | | High | 395 ± 115 | 394 ± 62 | | | |
| pHa | | | | | $P = 0.38$ | $P < 0.001$ | $P = 0.99$ |
| | Low | Low | 7.33 ± 0.10 | 7.35 ± 0.13 | | | |
| | | High | 7.32 ± 0.08 | 7.32 ± 0.07 | | | |
| | High | Low | 7.55 ± 0.10* | 7.60 ± 0.11* | | | |
| | | High | 7.53 ± 0.06† | 7.54 ± 0.05† | | | |
| $PaCO_2$ (mmHg) | | | | | $P = 0.17$ | $P < 0.001$ | $P = 0.74$ |
| | Low | Low | 42 ± 10 | 32 ± 11 | | | |
| | | High | 45 ± 11 | 44 ± 7 | | | |
| | High | Low | 19 ± 6* | 17 ± 6* | | | |
| | | High | 18 ± 6† | 17 ± 5† | | | |
| Bicarbonate (mmol/l) | | | | | $P = 0.49$ | $P < 0.001$ | $P = 0.48$ |
| | Low | Low | 21.2 ± 3.8 | 18.5 ± 5.4 | | | |
| | | High | 22.2 ± 2.0 | 23.4 ± 2.0 | | | |
| | High | Low | 17.8 ± 2.0 | 17.8 ± 2.1 | | | |
| | | High | 15.7 ± 4.7† | 14.7 ± 2.8† | | | |

Mean arterial pressure (MAP) and arterial blood gas analysis during mechanical ventilation in the following groups: (1) low power/low tidal volume ($V_T$): $V_T$ = 6 ml/kg and respiratory rate (RR) adjusted to maintain normocapnia; (2) low power/high $V_T$: $V_T$ = 11 ml/kg and RR to maintain the same power as in the low power/low $V_T$ group; (3) high power/low $V_T$: $V_T$ = 6 ml/kg and RR set to obtain a power three times that of the low-power groups; and (4) high power/high $V_T$: $V_T$ = 11 ml/kg, with RR set to obtain a power three times that of the low-power groups. Values are mean ± SD of 8 animals/group. Comparisons were done using a mixed linear model followed by Bonferroni's multiple comparisons ($P < 0.05$).

**versus* low power/low $V_T$; †*versus* low power/high $V_T$.

$PaO_2/FIO_2$ = the ratio of partial pressure arterial oxygen and fraction of inspired oxygen; $PaCO_2$ = partial pressure of carbon dioxide; pHa = arterial pH.

**Table 2.** Respiratory Parameters during Mechanical Ventilation

| Parameter | Power | | INITIAL | 60 min | FINAL | Huynh-Feldt*; Greenhouse-Geisser† |
|---|---|---|---|---|---|---|
| $V_T$ (ml/kg) | | | | | | $P < 0.001$* |
| | Low | Low | 6.0 ± 0.6 | 6.0 ± 0.7 | 6.0 ± 0.6 | |
| | | High | 10.3 ± 0.4‡ | 10.1 ± 1.0‡ | 10.8 ± 0.9‡ | |
| | High | Low | 7.4 ± 0.2 | 7.6 ± 1.3 | 7.3 ± 1.3 | |
| | | High | 11.7 ± 1.3§ | 11.6 ± 1.3§ | 10.8 ± 0.8§ | |
| RR (bpm) | | | | | | $P = 0.192$* |
| | Low | Low | 69 ± 6 | 69 ± 6 | 73 ± 16 | |
| | | High | 29 ± 6‡ | 29 ± 5‡ | 29 ± 2‡ | |
| | High | Low | 126 ± 24‡ | 123 ± 25‡ | 117 ± 22‡ | |
| | | High | 84 ± 27‖§ | 75 ± 20‖§ | 71 ± 14‖§ | |
| $V'_E$ (ml/min) | | | | | | $P = 0.467$† |
| | Low | Low | 160 ± 18 | 161 ± 19 | 169 ± 28 | |
| | | High | 129 ± 26 | 127 ± 25 | 136 ± 18 | |
| | High | Low | 321 ± 138‡ | 356 ± 69‡ | 325 ± 58‡ | |
| | | High | 401 ± 135‖ | 353 ± 78‖ | 314 ± 60‖ | |
| Flow (ml/s) | | | | | | $P = 0.136$* |
| | Low | Low | 13.2 ± .9 | 12.4 ± 1.0 | 12.6 ± 1.3 | |
| | | High | 14.2 ± 3.1 | 14.5 ± 1.9 | 16.2 ± 1.6 | |
| | High | Low | 32.8 ± 4.4‡ | 28.6 ± 4.5‡ | 29.0 ± 5.6‡ | |
| | | High | 31.3 ± 8.2‖ | 33.2 ± 10.6‖ | 27.8 ± 6.6‖ | |
| $Pplat,_L$ (cm $H_2O$) | | | | | | $P = 0.374$† |
| | Low | Low | 7.8 ± 1.7 | 7.8 ± 1.3 | 9.5 ± 3.3 | |
| | | High | 12.4 ± 1.5‡ | 12.0 ± 1.5‡ | 11.5 ± 1.9 | |
| | High | Low | 12.5 ± 2.0‡ | 12.0 ± 1.6‡ | 13.2 ± 2.5‡ | |
| | | High | 13.5 ± 3.6 | 13.9 ± 2.8 | 14.8 ± 3.6‖ | |
| $Pplat,_{RS}$ (cm $H_2O$) | | | | | | $P = 0.168$† |
| | Low | Low | 9.7 ± 1.6 | 9.6 ± 1.2 | 11.1 ± 3.2 | |
| | | High | 15.0 ± 2.6‡ | 14.7 ± 1.1‡ | 13.8 ± 1.6 | |
| | High | Low | 15.2 ± 2.2‡ | 14.5 ± 2.0‡ | 14.8 ± 2.4‡ | |
| | | High | 16.0 ± 2.9 | 15.9 ± 2.6 | 18.0 ± 4.8‖ | |
| $\Delta P,_L$ (cm $H_2O$) | | | | | | $P = 0.476$† |
| | Low | Low | 4.8 ± 1.7 | 4.8 ± 1.3 | 6.5 ± 3.3 | |
| | | High | 10.1 ± 2.4‡ | 9.9 ± 1.4‡ | 8.9 ± 1.7 | |
| | High | Low | 10.1 ± 2.4‡ | 9.7 ± 2.3‡ | 10.2 ± 2.4‡ | |
| | | High | 10.5 ± 3.4 | 10.9 ± 2.8 | 11.8 ± 3.6‖ | |
| $\Delta P,_{RS}$ (cm $H_2O$) | | | | | | $P = 0.365$† |
| | Low | Low | 6.7 ± 1.6 | 6.6 ± 1.6 | 8.0 ± 3.2 | |
| | | High | 11.9 ± 2.4‡ | 11.7 ± 1.0‡ | 10.8 ± 1.5 | |
| | High | Low | 12.0 ± 2.4‡ | 10.4 ± 3.9‡ | 10.3 ± 4.4‡ | |
| | | High | 13.0 ± 2.9 | 12.9 ± 2.6 | 15.0 ± 4.8‖§ | |
| Energy (mJ) | | | | | | $P = 0.829$† |
| | Low | Low | 0.55 ± 0.2 | 0.57 ± 0.20 | 0.78 ± 0.46 | |
| | | High | 2.22 ± 0.37‡ | 2.16 ± 0.35‡ | 2.06 ± 0.36‡ | |
| | High | Low | 1.54 ± 0.43‡ | 1.42 ± 0.36‡ | 1.46 ± 0.51‡ | |
| | | High | 2.45 ± 0.73§ | 2.56 ± 0.65§ | 2.59 ± 0.84§ | |
| Power (mJ/min) | | | | | | $P = 0.569$* |
| | Low | Low | 38 ± 13 | 39 ± 13 | 54 ± 27 | |
| | | High | 63 ± 13 | 62 ± 15 | 59 ± 11 | |
| | High | Low | 167 ± 19‡ | 163 ± 27‡ | 144 ± 23‡ | |
| | | High | 190 ± 48‖ | 183 ± 43‖ | 177 ± 48‖ | |
| Intrinsic PEEP (cm $H_2O$) | | | | | | $P = 0.009$* |

(*Continued*)

**Table 2.** Continued

| Parameter | Power | | INITIAL | 60 min | FINAL | Huynh-Feldt*; Greenhouse-Geisser† |
|---|---|---|---|---|---|---|
| | Low | Low | 0.16 ± 0.23 | 0.21 ± 0.41 | 0.09 ± 0.15 | |
| | | High | 0.03 ± 0.06 | 0.06 ± 0.07 | 0 ± 0 | |
| | High | Low | 1.49 ± 0.59‡ | 1.36 ± 0.37‡ | 1.25 ± 0.48‡ | |
| | | High | 1.03 ± 0.41‖§ | 0.75 ± 0.43‖§ | 0.63 ± 0.32‖§ | |

Respiratory parameters during mechanical ventilation in the following groups: (1) low power/low tidal volume ($V_T$): $V_T$ = 6 ml/kg and respiratory rate (RR) adjusted to maintain normocapnia; (2) low power/high $V_T$: $V_T$ = 11 ml/kg and RR to maintain the same power as in the low power/low $V_T$ group; (3) high power/low $V_T$: $V_T$ = 6 ml/kg and RR set to obtain a power three times that of the low-power groups; and (4) high power/high $V_T$: $V_T$ = 11 ml/kg, with RR set to obtain a power three times that of the low-power groups. Values are mean ± SD of 8 animals/group. Comparisons were done using Mauchly's test of sphericity with repeated-measures ANOVA ($P < 0.05$).

If epsilon was higher than 0.75, the Huynh-Feldt *P*-value was shown (*); otherwise, the Greenhouse-Geisser *P*-value was shown (†). Additionally, to compare all parameters among groups at each time point, a mixed linear model followed by Bonferroni's test was used. ‡*versus* low power/low $V_T$ ($P < 0.05$); §*versus* high power/low $V_T$ ($P < 0.05$); ‖*versus* low power/high $V_T$ ($P < 0.05$).

PEEP = positive end-expiratory pressure; $Pplat_{,L}$ = transpulmonary plateau pressure; $Pplat_{,RS}$ = respiratory system plateau pressure; $V'_E$ = minute ventilation; $\Delta P_{,L}$ = transpulmonary driving pressure; $\Delta P_{,RS}$ = respiratory system driving pressure.

of hypocapnia on histologic damage and biologic markers was evaluated with the Student's *t* test and Mann–Whitney *U* tests, as appropriate. The mixed linear models, Mauchly's sphericity test, and multiple linear regression analyses were performed using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., USA). All the other tests were performed in GraphPad Prism version 6.00 (GraphPad Software, USA). Significance was established at $P < 0.05$.

## Results

The survival rate was 100% in all groups. A comparison of mechanical energy computed by equation 1 and according to the quasi-static volume-pressure curve (flexiVent) is presented as Supplemental Digital Content 4 (http://links.lww.com/ALN/B643). Even though the energy level calculated by different methods differed due to the final pressure achieved in each method, the variation of energy from FINAL to INITIAL was not different between the two methods (69% with flexiVent, 72% with the formula).

At BASELINE, no significant differences were observed in MAP and gas exchange variables among groups (Supplemental Digital Content 5, http://links.lww.com/ALN/B644). MAP did not differ over time or among groups (table 1). Cumulative fluid volumes administered were comparable among groups: low power/low $V_T$ = 10.0 ± 1.7 ml, low power/high $V_T$ = 13.1 ± 3.1 ml, high power/low $V_T$ = 15.3 ± 5.2 ml, and high power/high $V_T$ = 14.5 ± 6.8 ml. $P_{AO_2}/F_{IO_2}$ did not differ significantly either among groups or between INITIAL and FINAL. In the high mechanical power groups, $P_{ACO_2}$ was lower and arterial pH was higher compared to low mechanical power (table 1).

At BASELINE, no significant differences were observed in respiratory parameters among groups (Supplemental Digital Content 6, http://links.lww.com/ALN/B645). As depicted in table 2, high $V_T$ was accompanied by lower RR in high mechanical power and low mechanical power groups alike. $V'_E$ and flow increased in high mechanical power compared to low mechanical power groups, regardless of $V_T$. Transpulmonary plateau pressure, respiratory system plateau pressure, transpulmonary driving pressure, and respiratory system driving pressure were increased in high power/low $V_T$ compared to low power/low $V_T$, as well as high power/high $V_T$ compared to low power/high $V_T$. Additionally, in the high mechanical power groups, mechanical energy increased with high compared to low $V_T$. As desired, mechanical power was approximately three times higher in high mechanical power compared to low mechanical power groups. Intrinsic PEEP was higher in high power/low $V_T$ compared to low power/low $V_T$, as well as in high power/high $V_T$ compared to low power/high $V_T$ and high power/low $V_T$ (table 2).

Figure 2 depicts light microscopy images of representative animals. As shown in table 3, high $V_T$ resulted in increased diffuse alveolar damage scores in both low and high mechanical power groups. Furthermore, despite low $V_T$, diffuse alveolar damage scores were increased in high *versus* low power groups. E-cadherin expression in lung tissue was lower in low power/high $V_T$ compared to low power/low $V_T$ group (table 3, Supplemental Digital Content 7, http://links.lww.com/ALN/B646).

Electron microscopy images of one representative animal per group are shown in figure 3. In both low and high mechanical power groups, high $V_T$ increased type II epithelial cell damage and basement membrane thickness compared to low $V_T$ (table 4). In the high mechanical power group, high $V_T$ also led to greater endothelial cell damage.

Levels of IL-6, amphiregulin, and club cell protein 16 mRNA expressions, but not ICAM-1 expression, increased in all groups compared to nonventilated, as shown in figure 4. However, IL-6 and amphiregulin mRNA expressions were higher in high power/high $V_T$ than in low power/high $V_T$. Moreover, high power/high $V_T$ animals exhibited increased amphiregulin and club cell protein 16 expression compared to high power/low $V_T$ animals.

Correlation analyses revealed significant associations of diffuse alveolar damage score, IL-6, amphiregulin, and club cell protein 16 mRNA expressions with mechanical energy (calculated by a simplified formula) and power (fig. 5). Similar associations of diffuse alveolar damage score, IL-6, amphiregulin, and club cell protein 16 mRNA expressions with mechanical energy computed by flexiVent and the

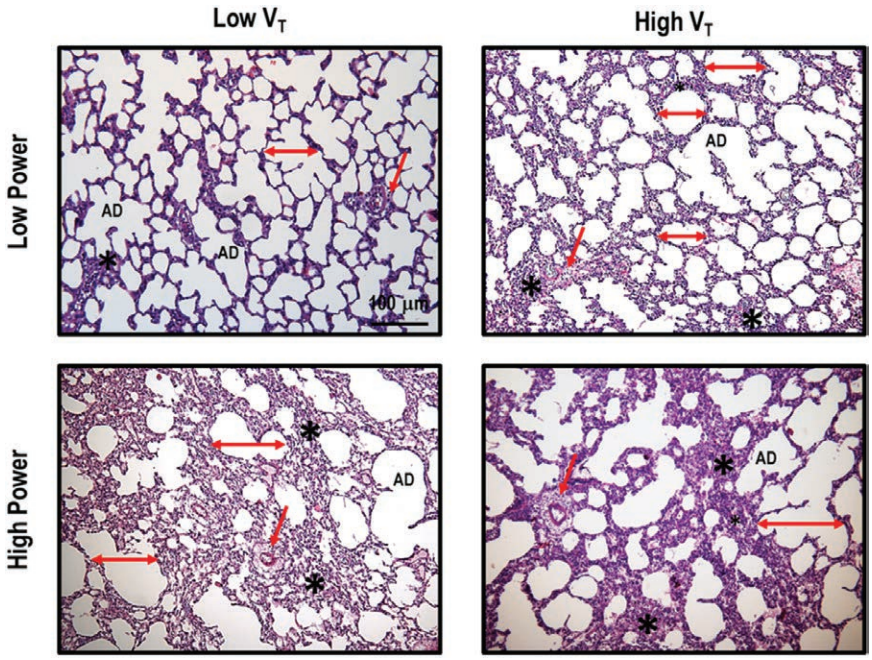


**Fig. 2.** Representative photomicrographs (light microscopy) of lung parenchyma stained with hematoxylin and eosin. *Asterisks* show alveolar collapse. *Arrows* indicate alveolar overdistension. Photomicrographs are representative of data obtained from lung sections of seven animals (original magnification, ×200). *Bars* = 100 µm. AD = alveolar duct; $V_T$ = tidal volume.

**Table 3.** Diffuse Alveolar Damage Score Variables and Fraction Area of E-cadherin

| | Low Power | | High Power | |
|---|---|---|---|---|
| | Low $V_T$ | High $V_T$ | Low $V_T$ | High $V_T$ |
| Edema | 6 (4–6) | 6 (4–8) | 8 (6–9) | 9 (9–12)* |
| Atelectasis | 3 (2–4) | 6 (4–6) | 6 (5–12)† | 9 (6–12) |
| Overdistension | 3 (2–4) | 6 (4–8) | 4 (2–9) | 8 (8–12) |
| Cumulative DAD score | 11 (9–14) | 18 (15–20)† | 19 (16–25)† | 29 (27–30)*‡ |
| E-cadherin (%) | 17.2 (12.8–28.9) | 3.1 (2.2–10.5)† | 8.1 (3.4–19.8) | 3.4 (1.6–6.0) |

Diffuse alveolar damage score (scores arithmetically averaged from two independent investigators) representing injury from edema, atelectasis, and overdistension, as well as the fraction area of E-cadherin in lung tissue in the following groups: (1) low power/low tidal volume ($V_T$): $V_T$ = 6 ml/kg and respiratory rate (RR) adjusted to maintain normocapnia; (2) low power/high $V_T$: $V_T$ = 11 ml/kg and RR to maintain the same power as in the low power/low $V_T$ group; (3) high power/low $V_T$: $V_T$ = 6 ml/kg and RR set to obtain a power three times that of the low-power groups; and (4) high power/high $V_T$ (11 ml/kg), with RR set to obtain a power three times that of the low-power groups. Values are given as medians, interquartile ranges, and minimum/maximum of 8 animals in each group. Comparisons among all groups were done using the Mann–Whitney *U* test and Bonferroni correction for 4 comparisons ($P < 0.0125$).

**versus* low power/high $V_T$ ($P < 0.0125$); †*versus* low power/low $V_T$ ($P < 0.0125$); ‡*versus* high power/low $V_T$ ($P < 0.0125$).

DAD = diffuse alveolar damage.

formula based on the equation of motion (Supplemental Digital Content 8, http://links.lww.com/ALN/B647) were observed.

On multiple linear regression analyses, variances in diffuse alveolar damage score and E-cadherin, Il-6 and amphiregulin gene expressions were better predicted by $V_T$ (Supplemental Digital Content 9, http://links.lww.com/ALN/B648).

Mean arterial pressure (Supplemental Digital Content 10, http://links.lww.com/ALN/B649), gas exchange (Supplemental Digital Content 11, http://links.lww.com/ALN/B650), and diffuse alveolar damage scores (Supplemental Digital Content 12, http://links.lww.com/ALN/B651; and Supplemental Digital Content 13, http://links.lww.com/ALN/B652) in experiments performed with matched $Paco_2$ at high mechanical power are reported as Supplemental Digital Content. Diffuse alveolar damage score variables (edema, atelectasis, and overdistension) and cumulative diffuse alveolar damage score did not differ between high-power groups with low levels of $Paco_2$ and high-power groups with normal $Paco_2$. On comparison of molecular biology analysis of high-power groups at low $Paco_2$ with those which presented similar levels of $Paco_2$ at low mechanical power, expression of IL-6, amphiregulin, club cell protein 16, and ICAM-1 behaved as in high- power groups at low $Paco_2$ levels (Supplemental Digital Content 14, http://links.lww.com/ALN/B653).

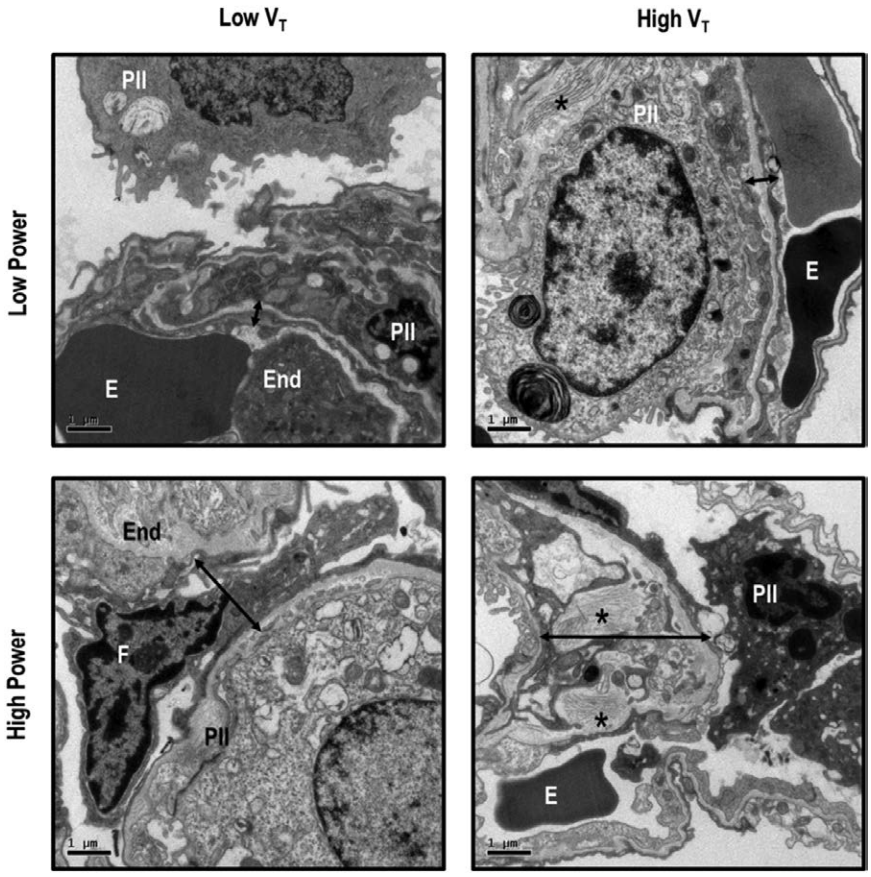


**Fig. 3.** Electron microscopy of lung parenchyma. Photomicrographs are representative of data obtained from lung sections of eight animals per group. Ultrastructural features of the alveolar–capillary barrier in each of the following groups: (1) low power/low tidal volume ($V_T$; 6 ml/kg) and respiratory rate (RR) to maintain a minute ventilation ($V'_E$) of 160 ml; (2) low power/high $V_T$ (11 ml/kg) and RR to maintain the same power as in the low power/low $V_T$ group; (3) high power/low $V_T$ (6 ml/kg) and RR set to obtain a power three times that of the low-power groups; and (4) high power-high $V_T$ (11 ml/kg), with RR set as in high power/low $V_T$. All groups exhibited type II epithelial cell (PII) damage and apoptosis of epithelial and endothelial cells (End). At low power, high $V_T$ was associated with further epithelial cell damage and interstitial edema, with an increase in basement-membrane thickness. At high power, low $V_T$ protected the lung from additional epithelial and endothelial cell damage; however, at high $V_T$, there were prominent changes in the alveolar–capillary barrier, characterized by interstitial edema, increased collagen fiber (*asterisk*) and basement membrane thickness (*double arrows*), and epithelial and endothelial cell damage. E = erythrocyte.

## Discussion

In the model of mild ARDS used herein, we found that, at low mechanical power, low $V_T$ reduced diffuse alveolar damage score, without changing expression of biomarkers associated with inflammation, alveolar pulmonary stretch, or epithelial cell damage. At high mechanical power, high $V_T$ increased diffuse alveolar damage, promoted ultrastructural impairment in alveolar epithelial and endothelial cells and alveolar–capillary membrane, as well as loss of cell-cell adhesion. Diffuse alveolar damage score and expression of IL-6, amphiregulin, and club cell protein 16 were associated with energy and power, and these findings were independent of $Paco_2$ levels.

To the best of our knowledge, this was the first experimental study to investigate the impact of low and high mechanical power on ventilator-induced lung injury. Different methods have been proposed to calculate mechanical power. We chose a simplified equation in order to facilitate its routine use in the clinical setting.[29] This equation computes the most important component (driving mechanical power), without taking into account resistive properties and PEEP, unlike the equation proposed by Gattinoni *et al.*[15] The resistive properties depend on flow magnitude and profile, and may contribute to lung damage. However, it is difficult to directly link the mechanical power dissipated in the proximal airways to alveolar injury. Additionally, the contribution of PEEP to mechanical power was comparable among groups. We calculated mechanical energy using a quasi-static pressure-volume curve and observed that, even though the degree of energy differs between the two methods, due to the maximum pressure achieved in the pressure-volume curve (30 cm $H_2O$), the variation from INITIAL to FINAL was similar. We also calculated mechanical power based on the aforementioned

**Table 4.** Electron Microscopy

| | Low Power | | High Power | |
|---|---|---|---|---|
| | Low $V_T$ | High $V_T$ | Low $V_T$ | High $V_T$ |
| Type II epithelial cell damage | 2 (1–2) | 3 (2–3)* | 2 (2–3) | 4 (3–4)†‡ |
| Basement membrane thickness | 2 (1–2) | 3 (2–4)* | 3 (2–3) | 4 (4–4)‡ |
| Endothelial cell damage | 2 (2–2) | 3 (2–3) | 2 (2–3) | 4 (3–4)‡ |

A five-point semiquantitative severity-based scoring system was used. Findings were graded as follows: 0 = normal lung parenchyma; 1 to 4 = damage to 1 to 25%, 26 to 50%, 51 to 75%, and 76 to 100% of examined tissue, respectively. Values are median (interquartile range) of 8 animals in each of the following groups: Respiratory parameters during mechanical ventilation in the following groups: (1) low power/low tidal volume ($V_T$): $V_T$ = 6 ml/kg and respiratory rate (RR) adjusted to maintain normocapnia; (2) low power/high $V_T$: $V_T$ = 11 ml/kg and RR to maintain the same power as in the low power/low $V_T$ group; (3) high power/low $V_T$: $V_T$ = 6 ml/kg and RR set to obtain a power three times that of the low-power groups; and (4) high power/high $V_T$ (11 ml/kg), with RR set to obtain a power three times that of the low-power groups. Comparisons among all groups were done using the Mann–Whitney *U* test and Bonferroni correction for 4 comparisons ($P < 0.0125$).

**versus* low power/low $V_T$ ($P < 0.0125$); †*versus* low power/high $V_T$ ($P < 0.0125$); ‡*versus* high power/low $V_T$ ($P < 0.0125$).

Gattinoni equation, which includes resistive properties and PEEP. Regardless of the method used to calculate energy, its correlation with diffuse alveolar damage, IL-6, club cell protein 16, and amphiregulin did not differ. Cressoni *et al.* calculated energy as the area between the inspiratory limb of the delta-transpulmonary pressure–volume curve. Direct comparison between our data and those of Cressoni *et al.* was not feasible due to differences in animal size (piglets *vs.* rats), use of healthy *versus* ARDS animals, positioning (supine *vs.* prone-Trendelenburg position), duration of mechanical ventilation (2 *vs.* 54 h), and the combination of $V_T$ and RR. In our study, not only RR but also $V_T$ was modulated to ensure tight control of the two levels of mechanical power and maintained within a safe range to keep animals hemodynamically stable and alive during the experiments. Even the highest $V_T$ values used were within ranges reported in clinical practice.[32] Additional groups were subjected to ventilation with high power and low as well as high $V_T$, but the dead space was adapted to yield normocapnia, in order to exclude the potential effects of a mismatch in $Paco_2$ between high and low power groups on diffuse alveolar damage score and expression of biomarkers.

It is worth noting that, despite an approximately threefold increase in mechanical power, transpulmonary plateau pressure, transpulmonary driving pressure, and respiratory system driving pressure differed between low $V_T$, but not high $V_T$ groups. This finding might be explained by the fact that, in high power groups, RR was increased substantially under low $V_T$, resulting in a small level of intrinsic PEEP.

Both RR[17] and inspiratory flow[13] have been recognized as potential determinants of ventilator-induced lung injury. We observed that, at the same power (low or high), $V_T$ increased and RR reduced but inspiratory flow did not differ significantly between low and high $V_T$. Inspiratory flow was higher at high compared to low power, suggesting the participation of airflow as a factor promoting ventilator-induced lung injury, since lung damage was observed even at low $V_T$. However, the damage induced by high $V_T$

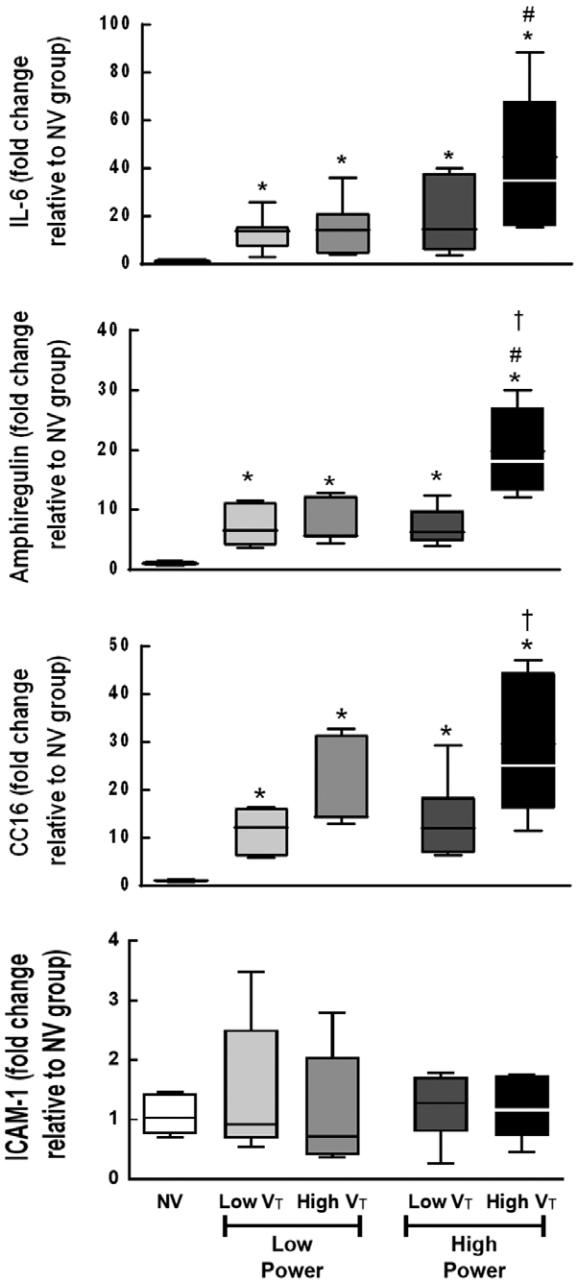


**Fig. 4.** Expression of biologic markers associated with inflammation (interleukin [IL]-6), alveolar pulmonary stretch (amphiregulin), epithelial cell damage (club cell protein 16 [CC16]), and endothelial cell damage (intercellular adhesion molecule [ICAM]-1). Relative gene expression was calculated as a ratio of the average gene expression levels compared with the reference gene (*36B4*) and expressed as fold change relative to nonventilated animals (NV). Values are medians and interquartile ranges of eight rats in each group. The comparisons among all groups were done by Bonferroni's procedure adjusted for four comparisons ($P < 0.0125$). **versus* NV ($P < 0.0125$); #*versus* low power/high $V_T$ ($P < 0.0125$); †*versus* high power/low $V_T$ ($P < 0.0125$).

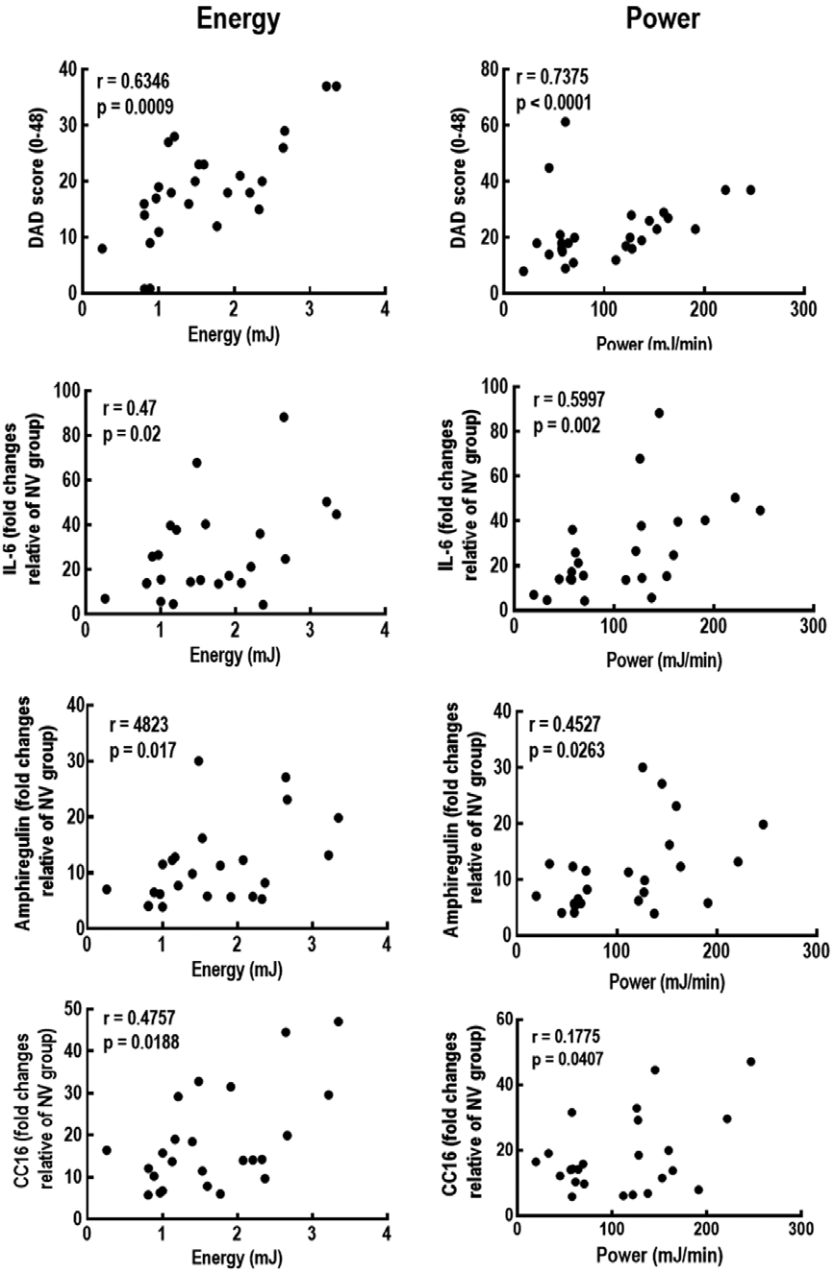


**Fig. 5.** Spearman's correlation analyses of diffuse alveolar damage (DAD) score and interleukin (IL)-6, amphiregulin, and club cell protein (CC)16 messenger RNA expressions with mechanical energy (calculated based on simplified formula) and power. NV = nonventilated; *r* = correlation coefficient with respective *P* value.

at high power was greater than that induced by low $V_T$. Thus, at high power, the role of airflow as a determinant of ventilator-induced lung injury was minor compared to that of $V_T$. Even though the high RR observed at low $V_T$ and high power resulted in intrinsic PEEP, it was not high enough to affect transpulmonary plateau pressure, transpulmonary driving pressure.

Diffuse alveolar damage score was lower in low than high $V_T$, regardless of the power level. In fact, at low $V_T$, energy did not differ, while RR increased in the high, compared to low, power group. In the first proof-of-concept study[16] investigating the association between mechanical power and ventilator-induced lung injury, lung edema increased while oxygenation decreased proportionally to RR. In contrast, in our study, the histologic damage resulting from high $V_T$ was greater than that associated with low $V_T$ at comparable levels of low and high power, suggesting that the proportional role of $V_T$ in promoting lung damage is greater than predicted by the mechanical power model. In line with these results, E-cadherin expression was further reduced with high $V_T$ compared to low $V_T$ at low power, suggesting increased mechanical stress/strain at the alveolar epithelial layer, leading to loss of cell–cell adhesion.[18,33] High $V_T$ might also have exceeded the plasticity limits of the lungs, resulting in

disproportionally high parenchymal strain.[34,35] In rats, respiratory system plateau pressure exceeding 14 cm $H_2O$ can trigger expression of biomarkers of inflammation,[20] which supports this hypothesis. We were also unable to rule out the possibility that inhomogeneity of injury might have amplified the effects of $V_T$ on lung damage. Intratracheal instillation of LPS results in heterogeneous distribution of diffuse alveolar damage features, as also reflected by altered viscoelastic properties.[36] In a physical model of ARDS, inhomogeneities in lung structure can increase the local forces generated during lung expansion, amplifying regional stress.[37]

Oxygenation did not differ among groups at FINAL. One might expect worse oxygenation in the presence of high $V_T$ and high power due to greater lung damage. However, oxygenation depends not only on the pulmonary structure, but also on regional ventilation and perfusion, including effects on pulmonary vascular resistance and venous return yielding differences in $\dot{V}/\dot{Q}$ and dead space.[38] We hypothesize that, in our study, oxygenation probably did not decline in the presence of high $V_T$ and high power due to increased dead space.

Although all ventilated groups showed increased gene expression of biomarkers of inflammation, alveolar stretch, and alveolar epithelial and endothelial cell damage as compared to nonventilated, the combination of high power with high $V_T$ led to more pronounced responses. As observed for lung damage, the contribution of high $V_T$ to triggering the pro-inflammatory response was higher than predicted by mechanical power. Moreover, the mechanical energy and power showed association with variables of ventilator-induced lung injury. As mechanical energy, unlike mechanical power, is not determined by RR, the intratidal phenomenon of extracellular matrix deformation and anchored endothelial and epithelial cells seem to play a central role in ventilator-induced lung injury. Multiple linear regression analyses revealed that $V_T$ predicted most of the impact of mechanical ventilation on diffuse alveolar damage score, E-cadherin, and gene expressions of amphiregulin and IL-6.

### *Possible Implications*

Our findings reinforce the concept that mechanical power should be measured in ARDS patients under protective mechanical ventilation. However, the hypothesis that mechanical power is the main determinant of ventilator-induced lung injury could inadvertently lead to acceptance of higher $V_T$, even when RR and/or transpulmonary driving pressure are within a "safe" range. Although this range has yet to be defined clearly, our data suggest that mechanical ventilation at low power may promote lung injury in the presence of inappropriately high $V_T$. Therefore, despite use of low RR and transpulmonary driving pressure, $V_T$ should always be kept in the low protective range. This claim certainly remains to be confirmed in clinical studies.

### *Limitations*

Several limitations of the present study must be acknowledged. First, experimental ARDS was induced by endotoxin, and our results cannot be extrapolated to other models or to human ARDS. Second, the observation time was relatively short, precluding extrapolation of our findings to longer periods of ventilation. To keep small animals with ARDS alive for 6 h would require larger fluid volumes, perhaps vasopressors to maintain MAP greater than 70 mmHg, and bicarbonate to counteract intense metabolic acidosis. Therefore, as a primary study design, even though a 2-h duration represents a short period of observation, it allowed us to evaluate the gene activation induced by different $V_T$ and power without any interference from therapies that would be needed to keep the animals alive in a longer experiment. Third, PEEP was constant during the experiments and we cannot exclude different results with different PEEP levels. Fourth, $Paco_2$ and arterial pH were not matched between low and high-power groups. There is a controversy on the potential harmful[39] or beneficial[40,41] effects of these variables in terms of lung protection. Nevertheless, in additional experiments performed with high power, $Paco_2$ and arterial pH were kept in normal ranges, but no differences were observed in lung damage nor in gene expression of biomarkers of inflammation, alveolar mechanical stretch, epithelial and endothelial cell damage.

### *Conclusions*

In this model of mild ARDS, high mechanical power was associated with ventilator-induced lung injury, even at low $V_T$. The impact of high $V_T$ on ventilator-induced lung injury was greater than predicted by mechanical power. To minimize ventilator-induced lung injury, low $V_T$ should be combined with low mechanical power.

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## Competing Interests

The authors declare no competing interests.

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