Effects of reduced tidal volume ventilation on pulmonary function in mice before and after acute lung injury

Apiradee Thammanomai,1 Arnab Majumdar,1 Erzsébet Bartolák-Suki,1,2 and Béla Suki1
1Department of Biomedical Engineering, Boston University, Boston; and 2CelluTraf Sci., Boston, Massachusetts

Submitted 2 January 2007; accepted in final form 2 August 2007

Mechanical ventilation (MV) is used to assist breathing when spontaneous breathing is either insufficient or absent. Patients suffering from respiratory failure, such as acute respiratory distress syndrome (ARDS), often require MV (33, 42). However, prolonged MV can lead to ventilator-induced lung injury (VILI), which, in turn, can worsen the lung conditions, possibly leading to systemic failure (35, 43). Numerous studies have been conducted using animal models to investigate the causes of VILI and to develop protective MV protocols that minimize injuries (1–3, 8, 11, 12, 16, 36). The currently accepted ventilation strategy is to use low tidal volumes (Vt) with moderate positive end-expiratory pressure (PEEP), which has been previously shown to lower mortality rates in ARDS patients (4, 41). However, some studies have also shown that the success of the low-Vt moderate PEEP approach depends on the optimal parameter setting for a particular individual (13). This optimum Vt/PEEP setting or whether there exists a state is still a subject of debate (32, 37). In any case, when the Vt and PEEP levels are not appropriately set, patient condition can quickly deteriorate, which is particularly important during MV of newborns. Since the ventilator settings have such enormous effects on the evolution of the ARDS condition itself, it is important to conduct studies in animal models of ARDS to better understand the interaction between the Vt/PEEP combination delivered by the ventilator and lung function.

The mouse has been the preferred choice in medical research, mostly because of the widely available reagents and the possibility of genetic manipulations to generate various disease models. To study the effect of ventilation in mouse models of ARDS, it is important to be able to accurately control the ventilation parameters. However, in small animals such as the mouse, complications can arise from the fact that the respiratory impedance, which is the load impedance (Zload) to the ventilator, is high. As a consequence, depending on the ventilator type and the tubing setup, the Vt actually delivered to a mouse may be significantly smaller than the prescribed value. Such a situation may occur when mice undergo a given treatment, such as lavage to mimic conditions of ARDS in which respiratory impedance is considerably higher than in normal mice. To our knowledge, the issue of how the delivered Vt depends on the conditions of the mice and whether that might further influence subsequent changes in lung function have not been systematically investigated.

The purpose of this study was to map the load dependence of the delivered Vt in a commercially available mouse ventilator and to examine the effects of ventilation without compensating for any reduction in Vt on lung function in mice. To this end, we first tested the load dependence of the delivered Vt with known impedances and examined how the load dependence influenced lung function in normal mice and in a mouse lavage model of ARDS. The results showed that the delivered Vt can be substantially smaller than the prescribed Vt, which, in turn, has a significant impact, not only on lung function, but also on surfactant secretion, resistance to injury, and survival rate.

**METHODS**

**Ventilator.** In this study, all experiments were performed using a computer-controlled, small-animal ventilator (flexiVent, SCIREQ, Montreal, Quebec, Canada). The outlet of the ventilator was connected to the load with a tube (polyvinyl chloride, inner diameter 0.432 cm). The lengths of inspiratory and expiratory limbs were 4.5 and 7.5 cm, respectively, with the dead space of 3.81 cm³.

**Test loads.** To assess how the Zload influences the delivered Vt, a 60-ml syringe was connected to the flexiVent ventilator. The syringe volume was set to one of the following values: 2.5, 5, 7.5, 10, 15, 30, 45, and 60 ml. The corresponding input impedances of the syringe

Address for reprint requests and other correspondence: B. Suki, Dept. of Biomedical Engineering, Boston Univ., Boston, MA 02215 (e-mail: bsuki@bu.edu).

http://www.jap.org 8750-7587/07 $8.00 Copyright © 2007 the American Physiological Society


The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
were measured using two periods of a 1-Hz sine wave. The impedance (Z) is the complex ratio of the Fourier transformed pressure and flow measured at the inlet of the syringe, which is estimated by the flexiVent system after taking into account the effects of gas compression in the valves and the impedance of the connecting tubing. The elastance (E) is the out-of-phase component of the pressure with flow normalized with flow and was calculated in the frequency domain from the imaginary part of the impedance [Im(Z)] as $E = -\omega \text{Im}(Z)$, where $\omega = 2\pi f$, where $f$ is the frequency. Theoretical upper and lower limits of the elastance were calculated, assuming adiabatic and isothermal compressions, respectively (31). The $V_t$ was set to be in between 0.2 and 0.4 ml for each load. The actual delivered volume to the load was reported by the flexiVent. The delivered volume was independently obtained by integrating the flow measured using a calibrated small-animal flowmeter (TSD137, Harvard Apparatus, Holliston, MA).

**Animal preparation.** Male C57BL/6 mice ($N = 20$, weight 24–27 g, Charles River Laboratories, Wilmington, MA) were anesthetized with an intraperitoneal injection of 70 mg/kg of pentobarbital sodium, tracheostomized with an 18-gauge metal cannula, and placed on a heated pad to maintain a constant body temperature (37°C) throughout the experiment. The tracheal cannula was connected to the outlet of the flexiVent ventilator. Extra doses of pentobarbital sodium (20 mg/kg) were administered every 20 min to keep the animal in a deeply anesthetized state. The protocol was approved by the Animal Care and Use Committee of Boston University.

**Measurement protocol.** Mice were assigned to one of two groups ventilated using either an uncontrolled ($N = 10$) or a controlled ($N = 10$) ventilation mode on room air. In the uncontrolled group, animals were ventilated by prescribing the piston displacement volume to be 8 ml/kg throughout the experiment. In the controlled group, the displacement volume of the piston was manually adjusted using the delivered $V_t$ reported by the flexiVent on a regular basis to make sure that the delivered $V_t$ was 8 ml/kg. In both groups, the breathing rate was 240 breaths/min, and the desired PEEP level was maintained by placing the expiratory port of the ventilator in a water trap. In each group, six animals were ventilated at a PEEP of 3 cmH$_2$O, and the remaining four animals were ventilated at a PEEP of 6 cmH$_2$O. To standardize volume history, two consecutive deep inspirations (DI) were administered to the animals at the beginning of the experiment. Each DI was a ramp increase in volume to 1 ml in 2 s. During the baseline ventilation, the mice were ventilated for 30 min. Lung injury was then induced by instilling 0.75 ml of warm saline via the tracheal cannula. During the baseline ventilation, the mice were ventilated for 30 min. Lung injury was then induced by instilling 0.75 ml of warm saline via the tracheal cannula, and placed on a heated pad to maintain a constant body temperature (37°C) throughout the experiment. The tracheal cannula was connected to the outlet of the flexiVent ventilator. Extra doses of pentobarbital sodium (20 mg/kg) were administered every 20 min to keep the animal in a deeply anesthetized state. The protocol was approved by the Animal Care and Use Committee of Boston University.

**Animal preparation.** Male C57BL/6 mice ($N = 20$, weight 24–27 g, Charles River Laboratories, Wilmington, MA) were anesthetized with an intraperitoneal injection of 70 mg/kg of pentobarbital sodium, tracheostomized with an 18-gauge metal cannula, and placed on a heated pad to maintain a constant body temperature (37°C) throughout the experiment. The tracheal cannula was connected to the outlet of the flexiVent ventilator. Extra doses of pentobarbital sodium (20 mg/kg) were administered every 20 min to keep the animal in a deeply anesthetized state. The protocol was approved by the Animal Care and Use Committee of Boston University.

**Measurement protocol.** Mice were assigned to one of two groups ventilated using either an uncontrolled ($N = 10$) or a controlled ($N = 10$) ventilation mode on room air. In the uncontrolled group, animals were ventilated by prescribing the piston displacement volume to be 8 ml/kg throughout the experiment. In the controlled group, the displacement volume of the piston was manually adjusted using the delivered $V_t$ reported by the flexiVent on a regular basis to make sure that the delivered $V_t$ was 8 ml/kg. In both groups, the breathing rate was 240 breaths/min, and the desired PEEP level was maintained by placing the expiratory port of the ventilator in a water trap. In each group, six animals were ventilated at a PEEP of 3 cmH$_2$O, and the remaining four animals were ventilated at a PEEP of 6 cmH$_2$O. To standardize volume history, two consecutive deep inspirations (DI) were administered to the animals at the beginning of the experiment. Each DI was a ramp increase in volume to 1 ml in 2 s. During the baseline ventilation, the mice were ventilated for 30 min. Lung injury was then induced by instilling 0.75 ml of warm saline via the tracheal cannula and slowly retrieving 0.6 ml. The lavage sample from each mouse was first centrifuged, and the supernatant was stored for later analysis. Immediately after the lavage, the mice were given two DIs to recruit the lung. The animals were then ventilated for another 10 min using the same ventilation setup. If the animal survived, they were ventilated for about another 20 min and killed using pentobarbital injection to the heart.

Another group of C57BL/6 ($N = 3$, weight: 23–25 g) was anesthetized with an intraperitoneal injection of 70 mg/kg of pentobarbital sodium, tracheostomized, and immediately lavaged as described above. The lavage samples were centrifuged, and the supernatants were used for total protein and Western blot analysis.

**Impedance measurement.** Respiratory mechanics were determined using the optimum ventilator waveform (OVW), which is a broadband waveform designed to estimate lung impedance using forced oscillation while simultaneously delivering tidal-like volume excursions (31a). The energy in the OVW was distributed among five discrete frequencies ranging from 2 to 31 Hz, which were selected in a manner to eliminate harmonic distortion and minimize cross talk, according to a nonsum nondifference principle (39). The frequencies, relative amplitudes, and phases of the OVW are given in Table 1. The phase angles were chosen to minimize the peak-to-peak pressure of the oscillatory waveform. For each mouse, the amplitude of the oscillatory waveform was adjusted to match the $V_t$ delivered during MV.

### Table 1. Frequencies, amplitudes, and phases of the optimum ventilator waveform used to measure mouse respiratory impedance

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Amplitude</th>
<th>Phase, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.623</td>
<td>11.94</td>
</tr>
<tr>
<td>5</td>
<td>0.187</td>
<td>-55.62</td>
</tr>
<tr>
<td>11</td>
<td>0.156</td>
<td>69.19</td>
</tr>
<tr>
<td>19</td>
<td>0.156</td>
<td>195.75</td>
</tr>
<tr>
<td>31</td>
<td>0.109</td>
<td>-45.93</td>
</tr>
</tbody>
</table>

Every 5 min, two complete cycles of OVWs were delivered, with each cycle being 2 s long. The piston displacement and pressure in the ventilator cylinder were reported by the flexiVent software, and the data were corrected for the effects of gas compression and the resistance of the cannula. The cross- and autopower spectra of pressure and flow were calculated off-line from overlapping data segments using fast Fourier transform. The respiratory input impedance ($Z_{rs}$) was then obtained at each input frequency as the ratio of the cross-power spectrum of pressure and flow and autopower spectrum of flow.

**Modeling.** The $Z_{rs}$ spectra were fitted by a model consisting of an airway compartment, characterized by the airway impedance ($Z_{aw}$), and a tissue compartment, described by a tissue impedance ($Z_{ti}$), connected in series. The $Z_{rs}$ is calculated as $Z_{aw} + Z_{ti}$. The $Z_{aw}$ is partitioned to air resistance ($Raw$) and inertance ($Iaw$).

$$Z_{aw} = Raw + j\omega Iaw$$

where $j$ is the square root of $-1$. The $Z_{ti}$ was modeled as a constant-phase impedance, which was introduced by Hantos et al. (23) and has been widely used in both animal models (22–24) and humans (29, 30). The constant-phase model characterizes $Z_{ti}$ by a tissue damping coefficient ($G$) and a tissue elastance coefficient ($H$) as follows:

$$Z_{ti}(\omega) = \frac{G - jH}{(\omega)^n}, \text{ where } \alpha = \frac{2}{\pi} \arctan \left( \frac{H}{G} \right)$$

where $\omega_0 = \omega/\omega_0$ is the normalized circular frequency with $\omega_0 = 1 \text{ rad/s}$ (7). The exponent $\alpha$ characterizes the frequency dependence of tissue resistance, $Rti = G/\omega_0^n$, and tissue elastance, $Eti = H/\omega_0^n$. All parameters were estimated using a global optimization algorithm, which minimized the root mean squared error between data and model (14).

Additionally, a model with distributed elastance introduced by Ito et al. (26) was also used to characterize heterogeneity of tissue elasticity of the lung, which generally occurs after lung injury (28). In this model, the airway tree is represented by parallel pathways, each composed of Raw and Iaw and a tissue compartment. The values of Raw and Iaw are assumed to be the same for every pathway, whereas each tissue compartment is described by the constant-phase model. The tissue hysteresivity ($\eta$), which is a material property of tissue (18), is set to be constant in every compartment. The $Z_{ti}$ is now described by

$$Z_{ti}(\omega) = \frac{(\eta - jH)}{(\omega_0)^n}$$

where $\eta = G/H$. The $H$ is assumed to be distributed according to a probability density function $n(H)$, which is proportional to $1/H$ between a maximum ($H_{max}$) and a minimum ($H_{min}$) value of $H$. An expression for $Z_{rs}$ thus obtained (26) can be used to fit the measured impedance using the parameters Raw, Iaw, $\eta$, $H_{min}$, and $H_{max}$ which characterize the mechanical properties of the respiratory system.

**Total protein and Western blot analysis.** The amount of protein in the supernatant of the lavage sample was measured using the protein
assay (BCA reagent kit, Pierce, Rockford, IL), according to the manufacturer’s instructions. The protein content was determined by measuring absorbance at 562 nm using a microplate reader (ELx 800; BioTek, Winooski, VT).

To test whether the ventilation mode had an effect on the development of injury, Western blot analysis was carried out for epithelial cadherin (E-cadherin) that was shown to correlate with epithelial cell damage (19, 20) and surfactant protein B (SP-B), as follows. The lavage can probe the lung to different depths, especially when regions of the lung are collapsed as a result of ventilation and/or treatment. Hence the amount of lavage return can vary from animal to animal. To eliminate the uncertainty due to this variability, equal amounts of protein were prepared from each lavage sample (1.06 and 0.83 μg for samples at PEEP levels of 3 and 6 cmH2O, respectively) and processed with reducing SDS-PAGE using precasted 4–20% gradient gels (BioRad Laboratories, Hercules, CA). After electrophoresis, proteins were transferred to polyvinylidene difluoride membrane (BioRad Laboratories) and blocked in 5% milk in phosphate-buffered saline containing 0.05% Tween 20 (pH 7.4). Immunoblot analysis was performed with either monoclonal antibody to mouse E-cadherin (1:500, Abcam, Cambridge, MA) or a polyclonal anti-rabbit SP-B antibody (1:3,000, Chemicon, Temecula, CA) for 1 h. Blots were washed four times for 15 min with PBS-Tween 20. For E-cadherin and SP-B, the membrane was incubated with a secondary horseradish peroxidase-conjugated anti-mouse antibody (Pierce, Rockford, IL) and peroxidase-labeled anti-rabbit IgG (Pierce), respectively, for 1 h. Both membranes were washed four times for 15 min with PBS and developed using a chemiluminescent reagent (SuperSignal West Pico, Pierce), followed by autoradiography. The relative intensity was quantified by densitometry following the removal of the background intensity using software developed in our laboratory.

Statistical analysis. Statistical differences among parameters under different conditions were tested using t-test and ANOVA (one-way ANOVA, two-way repeated-measure ANOVA, and Tukey test for pairwise comparison). When data were not normally distributed, a log transformation was carried out before applying the statistical tests. A value of P < 0.05 was used to establish statistical significance. SigmaStat (SPSS, Chicago, IL) was used for all statistical analysis.

RESULTS

Load dependence of delivered Vr. The real part of the Zload, the resistance of the syringe, was negligible compared with the magnitude of the imaginary part, given by the product of the elastance and ω. The measured values of elastance were 287, 203, 150, 79, 41, 28, and 21 cmH2O/ml when the syringe volumes were set to 2.5, 5, 7.5, 10, 15, 30, 45, and 60 ml, respectively. The measured elastances were within the limits of the theoretical elastance values computed, assuming either adiabatic or isothermal gas compressions (31), as shown in Fig. 1A.

Additionally, the delivered Vr was also measured when the Zload was practically zero, consisting of only the resistance and inertance of the independent flowmeter. The delivered volume was then calculated by integrating the flow for each prescribed piston displacement volume in the range 0.2–0.4 ml. The average ratio of delivered to prescribed volume as a function of the elastance of the load is shown in Fig. 1B, indicating that the efficiency of the ventilator decreases significantly from 100% with near zero load to slightly below 50% when the elastance of the syringe was 200 cmH2O/ml. These results were independent of the value of the prescribed Vr in the range of 0.2–0.4 ml.

Effects of uncontrolled ventilation. Examples of the real part of Zrs and respiratory elastance as a function of frequency are shown in Fig. 2, A and B, in two representative animals corresponding to the controlled and uncontrolled ventilation protocols, respectively, both before and after lavage. The lines show the corresponding constant-phase model fits. Although the fitting error increased approximately two to five times after lavage, the model was still able to fit the measured Zrs reasonably in both cases. Before lavage, the delivered Vr values in the uncontrolled group ranged from 0.13 to 0.18 ml compared with 0.2–0.22 ml in the controlled group for both PEEP levels. Immediately after lavage, the delivered Vr values for the controlled group were maintained, while the Vr in the uncontrolled group dropped to 0.08–0.10 ml. After 10 min of uncontrolled ventilation following the lavage, the delivered Vr further decreased to 0.06–0.09 ml at 3-cmH2O PEEP, which is less than one-half of the prescribed Vr, and 0.11–0.16 ml at 6-cmH2O PEEP.

The time course of the H during 30 min of ventilation is shown in Fig. 3. There is a significant increase in H as well as in the percent increase of H (%H, not shown) independent of PEEP and ventilation mode. There is no significant difference in H between the controlled and uncontrolled groups at either PEEP levels. However, two-way repeated-measures ANOVA
spectra from 10 min after baseline ventilation; Raw, G, and 10 min after lavage (L10) are summarized in Fig. 4. After baseline ventilation using controlled (C0) and uncontrolled groups (A). The symbols are the measured values, and the lines represent model fits. Impedance spectra from 10 min after baseline ventilation: ◦, impedance spectra from 10 min after lavage. The model parameters are as follows: before lavage, airway resistance (Raw) = 0.18 and 0.23 cmH2O·s·ml−1, damping coefficient (G) = 4.2 and 4.3 cmH2O·s·ml−1, elastance coefficient (H) = 18.3 and 19.8 cmH2O·s·ml−1 for controlled and uncontrolled, respectively; after lavage, Raw = 0.25 and 1.27 cmH2O·s·ml−1, G = 4.3 and 6.8 cmH2O·s·ml−1, H = 19.75 and 50.15 cmH2O·s·ml−1 for controlled and uncontrolled, respectively.

Fig. 2. Examples of the real part of the respiratory system impedance [Re(Z)] and respiratory elastance and the corresponding model fits as a function of frequency in an animal from the controlled (A) and uncontrolled groups (B). The symbols are the measured values, and the lines represent model fits. Impedance spectra from 10 min after baseline ventilation: ◦, impedance spectra from 10 min after lavage. The model parameters are as follows: before lavage, airway resistance (Raw) = 0.18 and 0.23 cmH2O·s·ml−1, damping coefficient (G) = 4.2 and 4.3 cmH2O·s·ml−1, elastance coefficient (H) = 18.3 and 19.8 cmH2O·s·ml−1 for controlled and uncontrolled, respectively; after lavage, Raw = 0.25 and 1.27 cmH2O·s·ml−1, G = 4.3 and 6.8 cmH2O·s·ml−1, H = 19.75 and 50.15 cmH2O·s·ml−1 for controlled and uncontrolled, respectively.

Fig. 3. Time course of the H during baseline ventilation using controlled (solid symbols) and uncontrolled (open symbols) ventilation modes at 3-cmH2O (circles) and 6-cmH2O positive end-expiratory pressure (PEEP) (inverted triangles). Values are means ± SD.

showed that the %H increased significantly more in the uncontrolled group (P = 0.046) at 6-cmH2O PEEP. The Raw and G significantly increased over the 30-min ventilation at 3-cmH2O PEEP, but, at 6-cmH2O PEEP, these parameters stayed constant in time (not shown). Additionally, G was also affected by ventilation mode: it was significantly higher (P < 0.02) during uncontrolled ventilation.

The respiratory mechanical parameters from the constant-phase model at baseline (C0), immediately after lavage (L0), and 10 min after lavage (L10) are summarized in Fig. 4. After lavage, both G and H of the uncontrolled group were significantly higher (P < 0.05 and P < 0.03, respectively) than those of the controlled group at both L0 and L10 and at both PEEP levels. The Raw was significantly higher in the uncontrolled group for both L0 and L10 at 3-cmH2O PEEP (P < 0.001). The values of Raw were small in both groups and are not reported. When the data were combined, including C0, L0, and L10, the results of the two-way repeated-measures ANOVA showed that all three parameters (Raw, G, and H) were significantly increased during ventilation between C0 and L10 (due to lavage), independent of ventilation mode and PEEP (P < 0.001). Moreover, H was significantly higher during uncontrolled ventilation, independent of time and PEEP (P < 0.001 for PEEP = 3 cmH2O and P = 0.03 for PEEP = 6 cmH2O).

To test whether heterogeneity could be assessed as a result of the mode of ventilation and/or lavage, the heterogeneous tissue model was also fitted to the Zrs spectra at both PEEP levels at 30 min after baseline ventilation (C30) and after 10-min ventilation following lavage (L10). The comparison of the Hmin and Hmax values between the controlled and uncontrolled groups is shown in Fig. 5. Two-way repeated-measures ANOVA showed that Hmin was significantly higher in the uncontrolled group at both 3- and 6-cmH2O PEEP (P = 0.008 and P = 0.009, respectively), independent of the condition (C30 or L10), whereas Hmax was significantly higher in the uncontrolled group only at 3-cmH2O PEEP (P < 0.001). Lavage significantly increased both Hmin and Hmax independent of the mode of ventilation (P < 0.02). Additionally, the width of the distribution of H, ΔH = Hmax − Hmin, that characterizes functional heterogeneity showed that the ventilation mode had a significant effect on ΔH (P < 0.001) at PEEP = 3 cmH2O. However, at 6-cmH2O PEEP, there was no significant difference in ΔH between controlled and uncontrolled ventilation. When analyzed separately using one-way ANOVA, both Hmax and ΔH were significantly higher in the uncontrolled group (P < 0.01) before lavage (C30) at 3-cmH2O PEEP. In summary, these results demonstrate that mean lung elastance was significantly higher during uncontrolled ventilation at both PEEP levels, whereas, at the lower PEEP, uncontrolled ventilation also increased the functional heterogeneity of the lung.
The total protein levels were not different between the unventilated, controlled, and uncontrolled groups at either PEEP levels. Examples of Western blot analysis for E-cadherin and SP-B are shown in Fig. 6. In the supernatant of the lavage fluid, Western blot analysis detected only the soluble form of E-cadherin (85 kDa) in all groups. At 3-cmH2O PEEP, one-way ANOVA showed a significant difference among the groups for both E-cadherin and SP-B (P ≤ 0.021 and P ≤ 0.001, respectively). When examined individually, there was significantly more soluble E-cadherin in the lavage fluid of the lungs of the uncontrolled group compared with the controlled group (P ≤ 0.017), but the unventilated group was not different from either group (Fig. 7A). Lavage fluid samples from the controlled group also had significantly more SP-B compared with both the unventilated and uncontrolled groups (P ≤ 0.001 and P ≤ 0.002, respectively), as shown in Fig. 7A. At 6-cmH2O PEEP, there was no difference in the levels of E-cadherin or SP-B among the unventilated, controlled, and uncontrolled groups, as shown in Fig. 7B.

Both the mode of ventilation and the applied PEEP level had a significant impact on the survival rate following lavage. At 3-cmH2O PEEP, three out of six animals in the controlled group survived the 10 min of ventilation subsequent to the lavage. In the uncontrolled group, none of the animals survived more than 10 min of ventilation following lavage. At 6-cmH2O PEEP, all animals that underwent controlled ventilation survived the protocol, while only one out of four animals survived 10 min of uncontrolled ventilation.

**DISCUSSION**

Currently, there is no clinically accepted pharmacological therapy for ARDS patients. Hence, these patients must rely mostly on the specific ventilator strategy, despite the known adverse effects of prolonged MV. The ARDS network (41) and Amato et al. (4) have conducted randomized, controlled trials and shown that lung-protective ventilation, low VT accompanied with PEEP, can significantly reduce mortality rate. However, there are several other randomized trials that showed no significant difference between low VT and the conventional
high-Vt ventilation (9, 10, 38). Nevertheless, there are several discrepancies in how “low Vt” and “conventional Vt” ventilation settings are administered in various studies. In clinical practice, the currently accepted Vt range is between 6 and 8 ml/kg, but the actual ventilation strategy depends on the specific patient condition. The effects of further reducing Vt or the optimum PEEP/Vt combination are still under investigation.

In this study, we investigated the effects of Zload on ventilator performance, as well as the effects of low-Vt ventilation on lung physiology, surfactant secretion, as well as epithelial adhesion integrity during a 30-min ventilation of normal mice and a 10-min additional ventilation following lavage-induced injury. The main results are that 1) the delivered Vt from the small-animal ventilator is highly dependent on the Zload; 2) ventilating normal mouse lungs with a Vt lower than prescribed resulted in increased parenchymal heterogeneity, lower SP-B, and higher E-cadherin levels at the lower PEEP level; 3) at the higher PEEP level, these parameters were less affected by the ventilation mode; and 4) in a lavage model of ARDS, ventilating mice with lower than prescribed Vt results in severely compromised lung function and poor survival rate, even at the higher PEEP level. We first discuss the technical aspects of the load dependency followed by an analysis of the physiological implications of using small-Vt ventilation.

**Load dependence.** An important finding of this study was that the delivered Vt significantly depended on the load at the outlet of the ventilator (Fig. 1B). The flow of air leaving the piston enters the load through the internal circuitry of the ventilator, including valves and tubing. A schematic of the ventilator’s internal impedance (Zinternal), the impedances of the tubing (Ztube), and the Zload are shown in Fig. 8. The actual air volume delivered to the load mostly depends on the ratio of the Zload and Zinternal. The former can be the impedance of a test object, such as a syringe, or the impedance of the ventilated mouse. The latter is primarily determined by the gas compression in the system. Usually, the Ztube, including resistance and inertance, is much smaller than Zload or Zinternal, and, for simplicity, Ztube will be neglected from the following analysis. Thus the flow leaving the piston is partitioned between these two impedances, such that the flow entering the load relative to the total airflow is equal to Zinternal/(Zload + Zinternal). Therefore, when Zload is equal to Zinternal, the load receives only one-half of the total airflow. In the healthy mouse, the elastance of the respiratory system (H) ranges between 18 and 40 cmH2O/ml. This load results only in a 10–20% reduction of the delivered Vt compared with the prescribed value. However, following injury, H can reach values higher than 250 cmH2O/ml (Fig. 4), which can result in a >50% reduction in the delivered Vt with significant physiological consequences (see below). It is important to note that, since Zinternal is specific for a given ventilator, the load dependence of the delivered Vt shown in Fig. 1 is only applicable for the ventilator used in the present study. Nevertheless, these are important issues, since tubing and changes in Zload as a consequence of, for example, alterations in the conditions of a subject during the development of VILI can influence the delivered Vt by neonatal ventilators (17, 27).

**Effects of Vt on lung physiology before lavage.** The constant-phase model was able to fit the impedance data well in all experimental groups, with examples shown in Fig. 2. While the fitting errors significantly increased after lavage, the constant-phase model still accounted for the impedance spectra reasonably. During 30 min of ventilation before lavage, all respiratory mechanical parameters (Raw, G, and H) increased steadily from their baseline values at the start of ventilation (C0). Except for the percent increase in H at 6-cmH2O PEEP, no significant difference was found between the mechanical pa-

---

**Image Descriptions:**

- **Fig. 6.** Example Western blots for E-cadherin and surfactant protein B (SP-B) in lavage samples from animals in the unventilated (UV), controlled (C), and uncontrolled groups (UC) at PEEP of 3 and 6 cmH2O.

- **Fig. 7.** Amounts of E-cadherin and SP-B in lavage samples normalized with the corresponding mean values of the unventilated group for PEEP of 3 cmH2O (A) and 6 cmH2O (B). *Significant difference between groups (P < 0.05).

- **Fig. 8.** Schematic of the ventilator circuit and the attached load. Pcyl, piston cylinder pressure; V0, piston displacement volume; Zinternal, ventilator’s internal impedance; Zload, load impedance; Ztube, impedance of tubing.
rameters corresponding to the controlled and uncontrolled ventilation mode, despite the fact that, in the uncontrolled group, the average delivered VT decreased by 25% (6 vs. 8 ml/kg) and 15% (7 vs. 8 ml/kg) at PEEP levels of 3 and 6 cmH$_2$O, respectively.

Although the values of Raw, G, and H from the single-compartment, constant-phase model were not different between the controlled and uncontrolled groups before lavage, using a model that accounts for tissue heterogeneity, we found that, following 30 min of ventilation, the uncontrolled group had a significantly higher $H_{\text{max}}$ as well as a wider distribution of elastance, at 3-cmH$_2$O PEEP. Thus the tissue heterogeneous model that partitions $H$ into $H_{\text{min}}$ and $H_{\text{max}}$ is able to pick up subtle signs of abnormal lung physiology similarly to the results reported in a mouse model of early emphysema (25). The higher $H_{\text{max}}$ in the uncontrolled group compared with the controlled group (Fig. 5A, left) suggests that the uncontrolled ventilation also increases the maximum stiffness of the lung. This increase in $H_{\text{max}}$ was most likely due to alveolar collapse, which occurred more at the lower PEEP level. Indeed, after 30 min of ventilation, $H$ on average increased by 36.5 and 42% in the controlled and uncontrolled groups, respectively, at 3-cmH$_2$O PEEP, whereas, at 6-cmH$_2$O PEEP, $H$ increased only 21.3% in the controlled group and 23.6% in the uncontrolled group. It is interesting to note that the smaller percent increase in $H$ at 6-cmH$_2$O PEEP was statistically significant due probably to the stabilizing effect of the higher PEEP that resulted in a smaller interanimal variability than at 3-cmH$_2$O PEEP. Nevertheless, this steady increase in $H$ could be reversed by a single DI in both cases (not shown). Thus these results suggest that the gradual development of larger heterogeneity at the lower PEEP and higher elastance at the higher PEEP during uncontrolled ventilation would likely lead to respiratory complications and perhaps failure, if such ventilation were continued over longer time periods.

Analysis of the lavage fluid. The slightly faster rate of increase of $H$ at both PEEP levels and the increases in $H_{\text{max}}$ and $\Delta H$ at the lower PEEP in the uncontrolled group did not result in an overall organ level injury that would lead to plasma leakage or edema, since, at both PEEP, the total protein levels from the lavage fluids were not significantly different between the unventilated and the ventilated groups. However, Western blot analysis for SP-B, which is the main surfactant protein that contributes to lowering the surface tension at the alveolar air-liquid interface (21, 34), showed that, at the PEEP of 3 cmH$_2$O, there was more SP-B in the lavage fluids of the controlled group than in those of the unventilated mice or the uncontrolled group (Fig. 7A). Additionally, the SP-B levels were similar in the unventilated and the uncontrolled groups.

As argued above, the uncontrolled ventilation at the lower PEEP resulted in a gradual collapse and increased $H_{\text{max}}$ and $\Delta H$ and hence functional heterogeneity. Therefore, the smaller 6 ml/kg VT and lung collapse in the uncontrolled group did not significantly alter the SP-B levels in the lavage compared with the unventilated mice, despite the applied PEEP. On the other hand, the presence of a moderate PEEP level of 3 cmH$_2$O and a steady 8 ml/kg VT in the controlled group led to an increased secretion of SP-B. Nevertheless, at 6-cmH$_2$O PEEP, there was no difference in SP-B levels of the two ventilation groups. This is probably due to the reduced difference in delivered VT between controlled and uncontrolled (8 vs. 7 ml/kg), as well as the protection against derecruitment at the higher PEEP. Compared with the unventilated group, both the controlled and the uncontrolled groups at 6-cmH$_2$O PEEP had similar SP-B levels. The percent increases in $H$ implied that, at 3-cmH$_2$O PEEP, the animals’ lung collapsed more than at 6-cmH$_2$O PEEP. Therefore, it is possible that the combination of partially collapsed lung and higher VT delivered to the animals in the controlled group at 3-cmH$_2$O PEEP resulted in larger stretches of the alveoli and consequently higher SP-B secretion compared with the situation at 6-cmH$_2$O PEEP, where the lung was more homogeneous and hence overstretching may have been less important.

The E-cadherin is an important regulatory receptor involved in epithelial cell-to-cell adhesion (40). In this study, we detected only the smaller soluble form of the molecule, which was shown to be associated with epithelial cell injury (19, 20). Since the level of E-cadherin in the unventilated group was similar to that of the uncontrolled and controlled groups, we believe that the lavage procedure itself caused some epithelial cell damage. Nevertheless, at the lower PEEP of 3 cmH$_2$O, the E-cadherin level of the uncontrolled group was significantly higher than that of the controlled group (Fig. 7A). Therefore, the uncontrolled ventilation must have resulted in a reduction in the integrity of epithelial cell-cell adhesion. It is conceivable that a ventilation mode without adequate recruitment in which repeated airway closure and opening occurs will lead to localized epithelial cell injury. Indeed, this has been demonstrated in model systems (6). The fact that, at 6-cmH$_2$O PEEP, there was no significant difference between the E-cadherin levels of any groups suggests that the increased PEEP helped keep the lung open and avoid epithelial cell adhesion damage independent of ventilation mode.

Effects of VT on lung physiology after lavage. The lavage process itself induces injury mostly by depletion of surfactant, which, in turn, increases alveolar surface tension and hence makes the lung prone to collapse. As a consequence of the lavage, all respiratory mechanical parameters increased significantly from their baseline values ($L_0$ in Fig. 4), suggesting that the lungs were indeed severely injured and collapsed. Due to the increased magnitude of $Z_{rs}$, the delivered VT in the uncontrolled group decreased to ~4 ml/kg at both PEEP levels, which was one-half of the prescribed value. This suggests that $Z_{rs}$ was almost equal to $Z_{external}$. More importantly, the smaller than prescribed VT ventilation induced a much stronger increase in the parameters corresponding to the uncontrolled group than in the controlled group, with the largest differences in Raw and $H$ (Fig. 4). The difference between the mechanical parameters of the two groups immediately after the lavage and the recruitment maneuver ($L_0$ in Fig. 4) demonstrates that the effect of recruitment was less effective and more transient during the uncontrolled ventilation. Indeed, even a very short period of uncontrolled ventilation resulted in a severe deterioration of lung function. After an additional 10 min of ventilation, the parameters in the controlled group stabilized, while Raw and $H$ in the uncontrolled group further increased significantly ($L_{10}$ in Fig. 4).

The heterogeneous tissue model was also fitted to the impedance spectra obtained at 10 min of ventilation after lavage ($L_{10}$), in addition to impedance measured immediately before lavage ($C_{30}$), as we discussed earlier. The fitting errors using the heterogeneous tissue model were comparable to those
obtained by the single-compartment, constant-phase model for both data sets. However, the heterogeneous model provides additional insight into lung physiology. After lavage and 10 min of ventilation \((L_{10})\), \(H_{\text{min}}, H_{\text{max}}\), as well as the width \(\Delta H\) of the distribution were significantly higher in the uncontrolled group at PEEP = 3 cmH\(_2\)O, while only \(H_{\text{min}}\) was significantly higher at PEEP = 6 cmH\(_2\)O. Moreover, the \(H_{\text{max}}\) of the controlled group at both PEEP levels was similar in magnitude as the \(H_{\text{min}}\) of uncontrolled group at PEEP 3 cmH\(_2\)O (Fig. 5B).

Thus regional lung function in the stiffest areas of the lung in the controlled group was similar to that of the most compliant regions in the uncontrolled group at the lower PEEP level. These results then indicate that, especially at low-PEEP levels, the application of a smaller than prescribed VT can significantly increase heterogeneity in the lung, which is an important contributor to the increase in the magnitude of Zrs and the development of ventilation-perfusion mismatch.

The progressive worsening of lung function in the uncontrolled group likely resulted from the gradual collapse of the lung, because the delivered VT was too small to keep the lung open after the lavage injury. Historically, collapse of lung units has been thought to occur at end-expiration, and the addition of PEEP has been the primary method to minimize such collapse (4). In our study, the effect of controlled and uncontrolled ventilation was investigated at PEEP levels of 3 and 6 cmH\(_2\)O. Following lavage, there was a significant reduction in VT at both PEEP levels, and hence the animals undergoing uncontrolled ventilation experienced a lower lung volume at end-inspiration. It is reasonable to assume that a significant amount of collapse can occur, if the lung does not experience a certain minimum volume distension. A possible explanation of this phenomenon is related to the long-term transients during recruitment/derecruitment that have been reported in several studies (1, 15) and theoretically modeled by Bates and Irvin (5). According to this model, periodic excursions to high lung volumes result in overcoming certain critical threshold pressures, with a tendency to open airways and alveoli. Once open, these lung units require a finite time, often the course of several breaths, before they collapse again. During uncontrolled ventilation following lavage, the VT and hence the maximum lung volume are lower than during controlled ventilation. Thus fewer lung units see volume excursions beyond their critical threshold and gradually collapse, leading to the slow increase in Zrs. Since the process of collapse is necessarily heterogeneous, this mechanism also explains the increased heterogeneity observed during uncontrolled ventilation.

During the short-ventilation period following lavage, the increase in the magnitude of Zrs leads to a decrease in the delivered VT, which, in turn, can generate more collapse and further increase Zrs. Therefore, the load dependency of the ventilator, coupled with the gradually worsening lung function in lavaged mice, form a positive feedback loop, and the ventilator/mouse system can get into a vicious cycle. Indeed, as a result of the smaller than prescribed VT ventilation following lung injury, in the uncontrolled groups, all six animals at 3-cmH\(_2\)O PEEP and three of four animals at 6-cmH\(_2\)O PEEP died within several minutes of the conclusion of the experiment. This is in sharp contrast to the 50 and 100% survival rate of the animals in the controlled group at 3- and 6-cmH\(_2\)O PEEP, respectively, for at least another 30 min. It is likely that the mice in the uncontrolled group did not survive the ventilation due to the severe collapse of the lung as well as the insufficient VT delivered after injury that caused circulatory complications and failure in these animals.

Summary. The goal of this study was to investigate how the VT actually delivered by the ventilator to the lung depends on the lung impedance and to examine the extent to which ventilation with smaller than prescribed VT leads to deterioration in lung function in mice. We found that, in normal mice, the delivered VT can be up to 25% less than the prescribed value, depending on the PEEP applied. In injured mice, the delivered VT can be up to 50% less than the prescribed value. Additionally, we also found that, if the VT is left uncompensated, continued ventilation can significantly alter physiological and biological responses in mice. These results highlight the importance of delivering appropriate VT to both the normal and injured lungs. The possible implications of these results to ventilating patients, especially preterm and full-term neonates, with high lung impedance warrant further investigation.

GRANTS
This study was funded by National Heart, Lung, and Blood Institute Grant HL-076372.

REFERENCES


