PULMONARY IMPEDANCE AND ALVEOLAR INSTABILITY DURING INJURIOUS VENTILATION IN RATS

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Running Header: Impedance and alveolar instability in rats

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ABSTRACT

The mechanical derangements in the acutely injured lung have long been ascribed, in large part, to altered mechanical function at the alveolar level. This has not been directly demonstrated, however, so we investigated the issue in a rat model of over-inflation injury. Following thoracotomy, rats were mechanically ventilated with either 1) high tidal volume (Vt), or 2) low Vt with periodic deep inflations (DIs). Forced oscillations were used to measure pulmonary impedance every min, from which elastance (H) and hysteresivity (\(\eta\)) were derived. Subpleural alveoli were imaged every 15 min using \textit{in vivo} video microscopy. Cross-sectional areas of individual alveoli were measured at peak inspiration (I) and end-exhalation (E), and the percent change was used as an index of alveolar instability (%I-E\(\Delta\)). Low Vt never lead to an increase in %I-E\(\Delta\) but did result in progressive atelectasis that coincided with an increase in H but not \(\eta\). DI reversed atelectasis due to low Vt, returning H to baseline. %I-E\(\Delta\), H and \(\eta\) all began to rise by 30 min of high Vt and were not reduced by DI. We conclude that simultaneous increases in both H and \(\eta\) are reflective of lung injury in the form of alveolar instability, while an isolated and reversible increase in H during low Vt reflects merely derecruitment of alveoli.

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INTRODUCTION

Mechanical ventilation is an often critical intervention in the management of respiratory failure and acute lung injury (ALI). However, over the past two decades, physicians have become increasingly aware that ongoing mechanical ventilation has the potential to worsen existing damage to the lung in a process known as ventilator-induced lung injury (VILI) (7, 43). Research suggests that mechanical ventilation is more likely to be injurious to the lung when baseline mechanical function is already impaired (8), as is usually the case in ALI. Nevertheless, high tidal volume ($V_t$) ventilation (HVV) alone can also create de novo injury in the naïve lung (23, 48). It is therefore of great importance to be able to detect the onset of VILI in critically ill patients, so that immediate steps can be taken to attenuate it.

We have demonstrated that HVV in naïve mice eventually causes a sudden and rapid rise in lung stiffness that is not reversed by deep inflation (DI). In contrast, when mice are ventilated with a protective strategy using low $V_t$ ventilation (LVV), lung stiffness also rises progressively but can be reversed by DI (1). We speculated that these differences are due to the development of irreversible injury in the HVV group as opposed to mere lung closure (atelectasis) in the LVV group. If true, then ongoing measurements of lung mechanics should provide a means of monitoring for the onset of VILI in patients. However, there are other potential explanations for a steady rise in lung stiffness during mechanical ventilation, including stress adaptation of tissues and changes in surfactant function and dispersal at the air-liquid interface (3). Clearly, until the relative roles of these various mechanisms are understood, the usefulness of lung mechanics measurements for tracking VILI will remain unclear.
The aim of the present study was therefore to determine how early changes in global lung mechanics during protective and injurious modes of mechanical ventilation reflect changes in alveolar stability (32). We pursued this aim in rats by exploring the temporal relationship between changes in pulmonary impedance and subpleural alveolar instability and closure during both protective and injurious modes of mechanical ventilation. We also examined how impedance measurements following DI reflect the effect of recruitment maneuvers on alveolar closure.

**METHODS**

*Surgical Preparation:* Male Sprague-Dawley rats (290 to 569 grams) received intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg) with additional dosing as needed to maintain anesthesia. A 12 gauge rigid plastic cannula was inserted into the trachea, sutured, and coupled to a *flexiVent* (SCIREQ, Montreal) small animal ventilator. Paralysis was achieved by intravenous pancuronium (0.8 mg/kg) while ventilating with room air and a *Vt* of 8.0 ml at 35 breaths/min. Positive end-expiratory pressure (PEEP) was achieved by submerging the expiratory limb of the ventilator circuit to 3 cmH₂O. The carotid artery and internal jugular vein were surgically catheterized. A midline sternotomy was performed with removal of the right third through sixth ribs, and lung volume history was standardized by delivering two slow 20 ml DIs with a pressure limit (*P*_limit) of 45 cmH₂O. All protocols were approved by the Committee for the Humane Use of Animals at SUNY Upstate Medical University, Syracuse, NY.

*Experimental Groups:* Rats were assigned to one of two constant flow ventilation settings. The LVV group (*n*=7) received a programmed *Vt* of 8.0 ml (*P*_limit 12 cmH₂O).
The HVV group ($n=6$) received a programmed $V_t$ of 20 or 25 ml ($P_{\text{limit}}$ of 45 cmH\textsubscript{2}O). Delivered $V_t$, when accounting for gas compression, ranged from 6.5 to 7.0 ml in the LVV group (14 to 20 ml/kg), and 15 to 17 ml in the HVV group (40 to 50 ml/kg). Respiratory rate was then reduced to 20 breaths/min to facilitate video microscopy, and PEEP was kept at 3 cmH\textsubscript{2}O. *Time 0* was designated as the time point immediately following initiation of the HVV or LVV strategy. We performed this study in two parts, using separate groups of rats. The first part was designed to study the relationship between pulmonary impedance ($Z_p$) and alveolar stability during the development of VILI. The second part of the study was designed to determine the relationship between pulmonary impedance and alveolar recruitment during DI.

**Pulmonary Impedance:** $Z_p$ was determined using a *flexiVent* small animal ventilator (SCI REQ, Montreal, Quebec). Ventilator piston volume displacement and cylinder pressure were measured during delivery of an 8 sec oscillatory volume perturbation to the airway opening. These perturbations were composed of 13 superimposed sine waves having frequencies, ranging from 0.5 to 19.75 Hz, chosen to be mutually prime in order to reduce the harmonic distortion that can occur in nonlinear systems (16). Before beginning each set of measurements, dynamic calibration signals were obtained to correct for the physical characteristics of the *flexiVent* itself and its connecting tubing (i.e. resistance of the tubing, and elastance and inertance of the gas in the cylinder and tubing) (17, 41). $Z_p$ was determined via Fourier transformation of the measurements of ventilator piston volume and cylinder pressure as described previously (13, 17). $Z_p$ was interpreted by being fit with the model
\[ Z_p = Raw + i2\pi f Iaw + \frac{G - iH}{(2\pi f)^\alpha} \]

where
\[ \alpha = \frac{2}{\pi} \arctan \left( \frac{H}{G} \right) \]

The parameters \( Raw \) and \( Iaw \) characterize the resistive and inertive properties, respectively, of the airways, while \( G \) and \( H \) characterize the dissipative and elastic properties, respectively, of the lung tissues (16). In particular, the parameter \( H \) is equal to respiratory elastance at an oscillation frequency of \( 1/2\pi \) Hz. Hysteresivity (\( \eta \)) is the quotient \( G/H \). Changes in \( \eta \) can be caused by changes in intrinsic tissue properties. However, \( G \) and \( \eta \) has also been shown to increase with increases in regional heterogeneity of lung function (21, 25). Following initiation of the protocol, \( Z_p \) was measured immediately after the first 2 DIs, then subsequently every min. DIs were repeated every 15 min during LVV to reverse atelectasis.

**In Vivo Microscopy:** Every 15 min, an epi-objective, epi-illumination microscope (Olympus), fitted with a specially designed coverslip stage apparatus, was gently brought to rest upon the anterior aspect of the right lower lobe visceral pleura. Suction (\(<5\) cmH\(_2\)O) was applied at end-inspiration in order to keep the lung in place. Immediately prior to each set of measurements, the apparatus was reattached to the lung. The lung tissue within the stage apparatus was filmed field-by-field from one edge of the coverslip to the other at a magnification of 130X using a color video camera (model CCD SSC-S20, SONY, Japan) and recorded on a videocassette recorder (model SVO-9500 MD, SONY, Taiwan).
The area of each imaged field was $1.22 \times 10^6 \, \mu m^2$. In the first part of the study, continuous filming of individual alveoli was performed throughout 5 complete $V_t$ deliveries and the results averaged. In the second part of the study, both LVV and HVV groups were ventilated with similar $V_t$ just prior to image collection ($8.0 ml \, V_t, 20 \, \text{breaths/min, PEEP 3cmH}_2O$), and video footage was obtained before, during, and immediately following three consecutive DIs ($V_t 20 \, ml, P_{\text{limit}} 45 \, \text{cmH}_2O$). The injurious ventilation group was returned to HVV immediately following acquisition of the video images, but before measuring $Z_p$ over each subsequent 15 min interval.

**Image Analysis of Alveoli:** We assessed alveolar mechanics by replaying the video frame-by-frame and capturing still images of alveoli at peak inspiration (I) and end-exhalation (E). For each visual field, the subset of alveoli analyzed consisted of those that contacted a vertical line bisecting the field, amounting to approximately 10 alveoli per field. Five microscopic fields were analyzed for each animal at each time point. Measurements of alveolar area were made by manually tracing the outer wall of individual alveoli at both I and E. Image analysis software (Empire Imaging Systems, Image Pro, Syracuse, NY) was then used to calculate the cross-sectional area of each traced alveolus. The degree of alveolar stability was quantified as the percent change in alveolar area from I to E relative to alveolar area at I (%I-E $\Delta$). The formula used for this derivation was: $(\text{Area(I)} - \text{Area(E)}) / \text{Area(I)} \times 100$.

**Impedance and alveolar recruitment:** In the second part of the study, LVV ($n = 7$) and HVV ($n = 5$) protocols were applied to separate groups of rats as described above. $Z_p$ was measured every min for 15 min, and then 2 DIs were administered. This sequence was...
repeated 4 times for a total study duration of 1 hour. *In vivo* microscopy images were obtained immediately before, during, and after each set of DIs. We later analyzed each image using a color thresholding application bundled within IMAQ Vision Builder software (Version 6.1, National Instruments, Austin, TX) to convert the color images to black&white such that white regions represented aerated portions of lung. The threshold parameters were set to capture pixels that fell within set intensity windows for the colors blue, green and red. The number of black pixels in the thresholded images were then counted and expressed as a % of the total number of pixels (%NBP) in the image (see Fig. 6). The red, blue and green intensity windows were kept constant, when possible, while analyzing images from different time points. However, following the onset of alveolar instability in the HVV group, the red, blue and green spectra changed. Thus, the intensity windows were occasionally adjusted in order to account for obvious color differences and to adequately convert the aerated portions of lung to white pixels. Despite this adjustment, the settings were always kept constant when analyzing pre, post, and peak-DI images at any one time point, thus eliminating any potential bias when calculating the change in %NBP during and after DI.

*Hemodynamic Measurements:* Systemic arterial pressures were continuously transduced (TruWave™, Baxter Healthcare Corp., Irvine, CA) via carotid arterial line. Fluid resuscitation with a 1cc bolus of warmed lactated Ringer’s solution was delivered when mean arterial pressure fell below 60 mmHg.

*Arterial blood gas measurements:* Seventy-five to 100 µL of arterial blood was obtained from the carotid arterial line every 15 min prior to DI and analyzed by a blood-gas
Impedance and alveolar instability in VILI: Results from in vivo microscopy image analysis are shown in Figure 1A and demonstrate that %I-EΔ in the HVV group began to increase by 15 min and became significantly elevated from baseline by 45 min, but never significantly changed in the LVV group. Furthermore, %I-EΔ in the HVV group began to diverge from that of the LVV group by 15 min, becoming significantly different by 30 min (p<0.05).
$H$ and $\eta$ versus time are shown in Figures 1B and C, respectively. $H$ increased steadily over time in the LVV group, but returned to near-baseline levels immediately following each DI such that the post-DI values were not significantly changed from baseline by the end of the protocol ($p=0.149$). Conversely in the HVV group, $H$ remained low for the first 30 min but then began to increase, becoming significantly elevated by 40 min and reaching nearly 3 times baseline by 60 min (Fig. 1B). Values for $H$ in the HVV group began to diverge from post-DI values in the LVV group by 30 min, becoming significantly different by 40 min (Fig. 1B). Values for $\eta$ began to rise by 30 min in the HVV group but remained unchanged throughout the protocol in the LVV group (Fig. 1C). $\eta$ became statistically different between the two groups by 60 min (Fig. 1C).

PaO$_2$ for the HVV and LVV groups is shown in Figure 2A. These data demonstrate that PaO$_2$ was actually higher during the first 30 min of HVV when compared to the LVV group, but then gradually declined throughout the HVV protocol up until 90 min when there was an unexpected rise (likely an artifact reflecting the reduced number of arterial blood samples obtained at this time point). PaO$_2$ was similar in both groups at 45, 60, and 90 min, at which times $\%I-E\Delta$, $H$, and $\eta$ were all on the rise (Fig. 1). Panel B in Figure 2, however, demonstrates that while PaO$_2$ was not significantly worsening, the alveolar-arterial oxygen gradient (A-aDO$_2$) progressively increased throughout the HVV protocol while it remained essentially unchanged throughout the LVV protocol. The slightly negative early values of A-aDO$_2$ are physically impossible and thus reflect an artifact in measurement.
Impedance and alveolar recruitment: The changes in pulmonary mechanics observed during the second part of the study mirrored those of the first part, with $H$ and $\eta$ in the HVV group both becoming significantly elevated compared to the post-DI values in the LVV group by 35 min. Examples of threshold images obtained by in vivo microscopy pre-DI, at peak DI, and post-DI are shown underneath their respective color images in Figure 3 from both LVV (Panel A) and HVV (Panel B) groups.

The mean percent number of black pixels (%NBP) for thresholded binary images at each time point is shown in Figure 4. Compared to pre-DI, %NBP was significantly decreased both at peak-DI and post-DI in the LVV group, as would be expected with recruitment of closed lung units. The same was true in the HVV group at 0 and 15 min. However, beyond 15 min of HVV, the decrease in %NBP seen at peak-DI was not maintained post-DI. Furthermore, %NBP both pre-DI and post-DI in the HVV group became significantly increased compared to the LVV group by 30 min. This relationship is explored further in Figure 5, which shows the change in %NBP from pre-DI to post-DI, for each rat at each time point, versus the corresponding % change in $H$. The changes in %NBP and $H$ following DI are positively correlated ($R=0.71$, $p<0.001$), though not evenly matched quantitatively, indicating that the amount of decrease in $H$ at least partly reflects the amount of lung recruited by DI.

**DISCUSSION**

To our knowledge, this study is the first to link changes in lung mechanics to specific events at the alveolar level during potentially injurious modes of mechanical ventilation. Previously, conclusions regarding the relationship between changes in global lung function and mechanics at the alveolar level have been inferred either from pressure
volume curves (24, 36), computed-tomography images (5, 30, 34, 38), or end-expiratory lung volume measurements (20, 26, 33). By using the technique of in vivo microscopy, we have been able in the present study to examine this relationship directly.

The principle finding from the first part of our study is that derangements in lung mechanics, manifest in the HVV group as a rise in both $H$ and $\eta$ (Figs. 1B and CB), temporally coincided with the development of alveolar instability (Fig. 1A), which has previously been linked to lung injury at the alveolar level (14, 40, 44). Our results also indicate that HVV caused lung injury rather than simple atelectasis because the progressive rise in A-aDO$_2$ (Fig. 2B) was not reversed by DI (by definition, every breath in the HVV group was a DI). Also, $\eta$ rose along with $H$ (Fig. 1C). Increases in $\eta$ have been shown to occur both with changes in the intrinsic mechanical properties of the lung tissue (9, 39) and with increased regional heterogeneity throughout the lung (21, 25), both of which would be expected to accompany damage to the lung tissue. It should be noted that since $\eta$ is the quotient of $G/H$, it is primarily a disproportionate rise in $G$ relative to $H$ that drives the rise in $\eta$. This could be due to increased heterogeneity(25). By contrast, the rise in $H$ in the LVV group was reversible with DI and was not accompanied by a rise in $\eta$, suggesting that both $G$ and $H$ rose proportionately and merely reflected the development of progressive lung closure or atelectasis (15, 25).

The above conclusions are further supported by the second part of our study in which we showed DI to be effective at reversing the increases in $H$ and %NBP associated with LVV, but not those associated with HVV (Figs. 4 and 5). Insofar as %NBP is an index of the amount of derecruited lung, these results confirm the hypothesis that a rise in $H$
without a concomitant rise in $\eta$ reflects gradual derecruitment of the lung, while an accompanying rise in $\eta$ is indicative of the changes in tissue rheology and heterogeneity likely to accompany lung injury.

Another interesting result of our study was that PaO$_2$ during injurious HVV was only marginally diminished compared to the LVV group at 45, 60, and 75 min (Fig. 2A), despite the fact that substantial changes in both alveolar stability and lung mechanics had already occurred by these times (Fig. 1). In fact, PaO$_2$ was actually higher in the HVV group for the first 45 min of the protocol, likely attributable to the higher peak and mean airway pressures involved. Nevertheless, PaO$_2$ did fall gradually during the HVV protocol, while A-aDO$_2$ rose (Fig. 2B). These changes are more in keeping with a pattern of gradual injury onset and mirrored the changes seen in $H$, $\eta$, and %I-E$\Delta$ (Fig. 1). Thus, while the acutely injured lung is usually characterized by increases in shunt, A-aDO$_2$, physiologic dead space, and PaCO$_2$ (31), it seems likely that the unusually high $V_t$ we used in the HVV group served to continually hyperventilate the lung and minimize dead space throughout the protocol. In any case, our results clearly show that the changes in $H$ and $\eta$ were more pronounced than those in PaO$_2$ and also were evident at an earlier time point. This suggests that forced oscillation measurements of lung impedance may be more sensitive than monitoring oxygenation as an early indicator of alveolar instability and incipient VILI.

It is interesting to note that following the onset of VILI in the HVV group, alveolar instability progressed to the point of repeated alveolar collapse and re-expansion with each breath, even at a moderate level of PEEP (Fig. 1A). Hence, although repeated
alveolar closure and re-expansion did not appear to occur to any significant degree in the uninjured lung, either during LVV or HVV, it did occur with the onset of VILI, in agreement with previous studies using in vivo microscopy (6, 14, 40). Indeed, it is widely recognized that repeated alveolar closure and re-expansion provides a potential mechanism for exacerbating lung injury (35, 46), even though its occurrence in the acutely injured lung is still debated (18). Supported by CT studies (11, 12) but contradicted by data obtained using a parenchymal marker technique (29), this debate is perpetuated by the practical difficulties of characterizing lung architecture in vivo during mechanical ventilation (4, 28). The technique of in vivo microscopy used in the present study has helped to overcome these limitations, and to sway the argument in favor of repeated alveolar closure and re-expansion occurring in the injured lung (6, 14, 40), and potentially contributing to the development of subsequent injury.

Our study thus appears to have answered some important questions concerning the changes in lung function wrought by various modes of mechanical ventilation. Nevertheless, the conclusions from this study must be viewed in light of its potential limitations, which are both technical and theoretical in nature. Perhaps the foremost issue to address is the novel analysis we developed to quantify the degree of atelectasis in terms of %NBP obtained from thresholded in vivo microscopy images. We chose this method of analysis because it involves a straightforward algorithm devoid of any pattern recognition issues with their related complications. Consequently, it can be performed in an automated manner using a computer, thereby helping minimize potential biases involved in image analysis. Nevertheless, the question remains as to how effectively %NBP really does quantify non-aerated lung. Obviously, any dense structure, be it
consolidated parenchyma or airway or vessel wall will contribute to %NBP. Therefore, at best, this quantity can be expected to merely follow changes in the degree of atelectasis rather than to be an actual quantitative measure of it. The calculation of %NBP also involves the initial setting of color intensity windows so that the white areas in the resulting binary images correspond to aerated portions of the lung. However, since these settings were always kept constant when analyzing pre-, post-, and peak-DI images from any single time point, subjective bias was eliminated from the change in %NBP calculation.

A related caveat to our conclusions involves certain technical considerations related to the *in vivo* microscopy technique itself. Although we found an absence of repeated alveolar closure and re-expansion in the uninjured lung, this may have been partly due to the negative pleural pressure that was applied by the optical stage of our video microscope, which could have lead to an underestimation of %I-EΔ during tidal ventilation. This stage stabilizes the lung field during ventilation so that the same alveoli are viewed at I and E, but this could have limited local expansion of the lung parenchyma. This may have contributed to our finding of minimal differences in %I-EΔ between the HVV and LVV groups early in the protocol despite large differences in VT. In an attempt to minimize these effects, the stage was lowered manually onto the pleural surface in such a way as to allow the lung free lateral movement as it expanded. Furthermore, we found the diameters of individual alveoli to measure between 50 and 100µm (see superimposed micrometer in Fig. 3), a number in keeping with previous published work from morphometric studies based on histology (19, 47). Thus, the stage did not appear to significantly influence alveolar size. Nevertheless, we cannot discount
the influence of the stage altogether. Additionally, since our technique visualizes only subpleural alveoli we must be wary of drawing conclusions about the stability of alveoli deeper below the pleural surface. This limitation may explain the observation in Figure 5 that the change in %NBP and % change in $H$ for the LVV group, although correlated, are not equal. This may reflect the fact that $H$ is a global measure of lung stiffness while %NBP is a local measure limited to the pleural surface.

It should also be noted that we used very large $V_t$ in our HVV rats in order to create overdistension injury within the 1 hour time scale of our experiments. Indeed, we expanded the lungs well beyond normal total lung capacity with each breath (22) in these animals in order to accelerate the onset of lung injury (42). Although such $V_t$ have been used to create VILI in other animals models (10, 48), they are much greater than the levels of lung overdistension thought to contribute to VILI in patients. Even the $V_t$ used in the LVV group was substantially larger than the low $V_t$ regimen of 6 ml/kg ideal body weight clinically recommended for patients with ALI. This $V_t$ was necessary to maintain adequate minute ventilation while using the much reduced respiratory frequency required for implementation of in vivo microscopy. Thus, our definitions of HVV and LVV in the present study differ somewhat from their clinical counterparts.

With respect to our arterial blood gas measurements, although the samples were all collected and analyzed in the same manner, the calculated A-aDO$_2$ was negative for some samples, particularly early in the HVV group. Since a negative A-aDO$_2$ is a physiological impossibility, it must represent some artifact. Potential explanations for this include incomplete humidification of air by the time it reaches the distal airways, underestimation of alveolar oxygen tension (from added mean airway pressures), or an
underestimation of the respiratory quotient. Although previous research in rats supports a respiratory quotient of 0.8 (45), a higher quotient in our rats, perhaps due to stress, would underestimate the A-aDO₂ in both groups. Nevertheless, initial values of A-aDO₂ were only slightly less than zero, and it is the actual change in A-aDO₂ over time that is most crucial to the conclusions of this study.

The measurement of lung mechanics also deserves some discussion. The quantities $H$ and $\eta$ are derived from a model fit to $Z_p$. $H$ is the equivalent of conventional lung elastance (2, 3), but has the advantage of being independent of the frequency of breathing. $\eta$ is the ratio of energy dissipation to storage in the lung tissue, something which has been shown to remain remarkably constant with frequency of oscillation (9) and which represents the viscoelastic properties of the tissues. We have shown previously that the theoretical model underlying $H$ and $\eta$ provides an excellent fit to measurements of respiratory impedance in mouse models of acute lung injury (2, 3), and others have had similar success in rats (37). Nevertheless, the calculation of $Z_p$ itself is predicated on the assumption that the lung behaves as a linear dynamic system. This assumption tends to be more valid when the amplitude of the signal (i.e. volume) used to perturb the system is small, and was met reasonably well in the present study in the LVV group. The volume perturbation amplitudes did not exceed normal tidal volume, and measurements of impedance were made at end-exhalation at a PEEP of 3 cmH₂O. However, when the lungs became significantly damaged in the HVV group, it is possible that the resulting regional collapse lead to overdistension of the remaining open lung during impedance measurement, with the coincident appearance of nonlinear mechanical effects. This might partly explain why $H$ plateaued toward the end of the experiment.
(Fig. 1B) instead of continuing to increase. That is, although the existence of the plateau might indicate stabilization of the level of injury, it could also reflect the vagaries of a linear analysis applied to a frankly nonlinear system. To examine this possibility, we examined the percent power in pressure that fell outside of the delivered frequencies in volume, and found that although harmonic distortion increased slightly toward the end of the protocol in 3 of 6 rats in the HVV group, this effect was very small (from 3% to 5%). This would seem to discount nonlinear conditions as the principle cause of the plateau in $H$. However, when the lung became significantly injured in the HVV animals, it is likely that considerable recruitment and derecruitment of lung units was taking place during the application of the volume perturbations used to determine $Z_p$. If this opening and closing of units took place progressively with changes in lung volume, the system could still appear reasonably linear, which might explain why we found only little harmonic distortion.

Recruitment and derecruitment may also explain the features of $H$ and $\eta$ we observed during development of acute lung injury (Fig. 1). Worsening lung injury would be expected to increase the amount of lung recruited during inspiration because more lung would have collapsed during the preceding expiration. This could cause the stiffness of the lung (i.e. $H$) to remain unaltered or even decrease, because now the imposed volume would be going into the opening of new units rather than expanding already open units. On the other hand, each time a unit opens it dissipates energy due to the breaking of surface tension bonds, etc., so an increase in recruitment would also be expected manifest as an increase in tissue resistance (i.e. $G$). This could explain why we observed a progressive increase in $\eta$ (Fig. 1C) with little change in $H$ (Fig. 1B). Another point is that
we noted slow declines in pressure during the delivery of forced oscillations at the plateau in $H$, suggesting that small leaks due to micro-tears may have developed at the pleural surface, which could have prevented $H$ from rising further. Regardless of the explanation, however, these effects do not alter the basic conclusions of our study, which center on the point at which $H$ and $\eta$ begin to increase. What is important is that we have shown this point to temporally coincide with the appearance of alveolar instability and the onset of VILI.

In summary, we have demonstrated that progressive rises in both $H$ and $\eta$ during injurious mechanical ventilation occur in concert with increases in alveolar instability, and that all three quantities start to change before measurable derangements in gas exchange. This provides new evidence at the microscopic level that a gradual rise in $H$ without a corresponding rise in $\eta$ represents the development of progressive derecruitment, while an irreversible rise in both $H$ and $\eta$ together may signify the onset of actual lung injury. Furthermore, this study demonstrates that a decrease in $H$ following DI represents, at least in part, recruitment at the alveolar level. Our results further suggest that frequent measurements of respiratory impedance during mechanical ventilation could be useful in the early detection of impending VILI in a clinical setting, which could be invaluable for ensuring the expedient transition to a more protective ventilation strategy.
REFERENCES


**Figure 1:** A) %I-EΔ (mean +/- SEM) at all 15 min intervals for HVV (grey bars) and LVV (white bars) groups. * represents significant difference between groups. # represents significant difference for HVV group compared to baseline at 0 min. B) H versus time for HVV (grey circles) and LVV (white circles) groups (mean +/- SEM). The regularly spaced sudden drops in H for the LVV group correspond to DI. C) η versus time for HVV (grey circles) and LVV (white circles) groups (mean +/- SEM).

**Figure 2:** PaO2 (mean +/- SEM) for HVV (grey bars) and LVV (white bars) groups in top panel A. Alveolar-arterial O2 gradient (A-aDO2)(mean +/- SEM) for HVV (grey bars) and LVV (white bars) groups in bottom panel B. Baseline values were obtained both during closed and open chest. Subsequent values were obtained at 15 min intervals.

**Figure 3:** Representative *in vivo* microscopy images (magnification 130x) from LVV (panel A) and HVV (panel B) groups, together with their corresponding black&white thresholded images (beneath each color image). The black&white images were used to quantify %NBP at pre-DI, peak-DI, and post DI time points. Scale in first figure represents 0 to 0.5 mm (500µm).

**Figure 4:** DI-induced changes in percent number of black pixels (%NBP) in HVV (grey circles) and LVV (white circles) groups (mean +/- SEM). # represents a significant difference in %NBP between LVV and HVV groups. * represents a significant reduction in %NBP in post-DI images compared to pre-DI images.
**Figure 5:** Individual values for change in percent number of black pixels (%NBP) following DI versus corresponding % change in $H$ for HVV (grey circles) and LVV (white circles) groups. Graph demonstrates a direct correlation between recruitment (negative change in %NBP) and decrease in $H$ (negative % change in $H$) following DI ($R=0.74$, $p<0.0001$).
Figure 2

A

PaO₂ (mmHg)

0 40 80 120 160

Closed Open 15 30 45 60 75 90

Chest Chest Time (minutes)

B

A-aDO₂ (mmHg)

-10 0 10 30 50 70

Closed Open 15 30 45 60 75 90

Chest Chest Time (minutes)
Figure 3
Figure 4
Figure 5

![Graph showing %NBP change after DI vs. % change in H after DI for HVV and LVV with correlation coefficient R=0.72, slope 0.74, and p<0.0001.]