Pulmonary impedance and alveolar instability during injurious ventilation in rats

Gilman B. Allen,1,2 Lucio A. Pavone,3 Joseph D. DiRocco,3 Jason H. T. Bates,1,2 and Gary F. Nieman3

1Department of Medicine, Vermont Lung Center, University of Vermont, Burlington, Vermont; 2Fletcher Allen Health Care, Burlington, Vermont; and 3Department of Surgery, State University of New York Upstate Medical University, Syracuse, New York

Submitted 1 December 2004; accepted in final form 11 April 2005

Allen, Gilman B., Lucio A. Pavone, Joseph D. DiRocco, Jason H. T. Bates, and Gary F. Nieman. Pulmonary impedance and alveolar instability during injurious ventilation in rats. J Appl Physiol 99: 723–730, 2005. First published April 14, 2005; doi:10.1152/japplphysiol.01339.2004.—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 overinflation injury. After thoracotomy, rats were mechanically ventilated with either high tidal volume (VT) or low VT with periodic deep inflations (DIs). Forced oscillations were used to measure pulmonary impedance every minute, from which elastance (H) and hysteresivity (η) were derived. Subpleural alveoli were imaged every 15 min in vivo video microscopy. Cross-sectional areas of individual alveoli were measured at peak inspiration and end exhalation, and the percent change was used as an index of alveolar instability (%I-EΔ). Low VT never led to an increase in %I-EΔ but did result in progressive atelectasis that coincided with an increase in H but not η. DI reversed atelectasis due to low VT, returning H to baseline. %I-EΔ, H, and η 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 η are reflective of lung injury in the form of alveolar instability, whereas an isolated and reversible increase in H during low VT reflects merely derecruitment of alveoli.

METHODS

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 (VT) ventilation (HVV) alone can also create de novo injury in the naive 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 naive 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 VT 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 (Zp) 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.

Surgical preparation. Male Sprague-Dawley rats (290–569 g) 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, Quebec, Canada) 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 (Plim) 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 (Plim of 12 cmH2O). The HVV group (n = 6) received a programmed VT of 20 or 25 ml (Plim of 45 cmH2O). Delivered VT, when accounting for gas compression, ranged from 6.5 to 7.0 ml in the LVV group (14–20 ml/kg) and from 15 to 17 ml in the HVV group (40–50 ml/kg). Respiratory rate was then reduced to 20 breaths/min to facilitate video microscopy, and PEEP was kept at 3 cmH2O. 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 Zp and alveolar stability during the development of VILI. The second part of the study was designed to determine the relationship between Zp and alveolar recruitment during DI. Zp. Zp was determined using a flexiVent small animal ventilator (SCIREQ). Ventilator piston volume displacement and cylinder pressure were measured during delivery of an 8-s 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 to reduce the harmonic distortion that can occur in nonlinear systems (16). Before each set of measurements was begun, 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 inerterance of the gas in the cylinder and tubing) (17, 41). Zp was determined via Fourier transformation of the measurements of ventilator piston volume and cylinder pressure as described previously (13, 17). Zp was interpreted by fitting with the model

$$Zp = \frac{Raw + i2\pi fIaw}{G - iH} \frac{G - iH}{(2\pi f)^2}$$

(1)

where

$$\alpha = \frac{2}{\pi} \arctan \left( \frac{H}{G} \right)$$

(2)

where the parameters Raw and Iaw characterize the resistive and inertive properties, respectively, of the airways, whereas 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. The symbol f represents frequency, after 1/(2\pi)Hz, and i is the square root of -1. Hysteresivity (\( \eta \)) is the quotient G/H. Changes in \( \eta \) can be caused by changes in intrinsic tissue properties. However, G and \( \eta \) have also been shown to increase with increases in regional heterogeneity of lung function (21, 25). After initiation of the protocol, Zp was measured immediately after the first two DIs, then subsequently every minute. DIs were repeated every 15 min during LVV to reverse atelectasis.

In vivo microscopy. Every 15 min, an epibiojective, epi-illumination microscope (Olympus), fitted with a specially designed coverslip stage apparatus, was gently brought to rest on the anterior aspect of the right lower lobe visceral pleura. Suction (\( \leq 5 \) cmH\(_2\)O) was applied at end inspiration to keep the lung in place. Immediately before 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 \( \times 130 \) using a color video camera (model CCD SSC-S20, Sony) and recorded on a videocassette recorder (model SVO-9500 MD, Sony).

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 five complete VT deliveries, and the results were averaged. In the second part of the study, both LVV and HVV groups were ventilated with similar VT just before image collection (8.0-ml VT; 20 breaths/min, PEEP of 3 cmH\(_2\)O), and video footage was obtained before, during, and immediately after three consecutive DIs (VT of 20 ml, Pa\(_{\text{a}}\) of 45 cmH\(_2\)O). The injurious ventilation group was returned to HVV immediately after acquisition of the video images but before Zp was measured 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 exhale (E). For each visual field, the subset of alveoli analyzed consisted of those that contacted a vertical line bisecting the field, amounting to \( \sim 10 \) 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-EA). The formula used for this derivation was [area(I) - area(E)]/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. Zp was measured every min for 15 min, and then two DIs were administered. This sequence was repeated four times for a total study duration of 1 h. 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 IM AQ Vision Builder software (version 6.1, National Instruments, Austin, TX) to convert the color images to black and 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 percentage 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, after the onset of alveolar instability in the HVV group, the red, blue, and green spectra changed. Thus the intensity windows were occasionally adjusted 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, Irvine, CA) via carotid arterial line. Fluid resuscitation with a 1-ml bolus of warmed lactated Ringer solution was delivered when mean arterial pressure fell below 60 mmHg.

Arterial blood gas measurements. Seventy-five to 100 \( \mu \)l of arterial blood was obtained from the carotid arterial line every 15 min before DI and analyzed by a blood-gas analyzer and cooximeter (ABL5, Radiometer, Copenhagen, Denmark) for determination of arterial \( pH \), oxygen tension [arterial Po\(_2\) (Pa\(_{\text{a}}\)], and carbon dioxide (arterial PCO\(_2\)) tension. The alveolar-arterial oxygen gradient (A-Do\(_2\)) was calculated by subtracting Pa\(_{\text{a}}\) from the alveolar oxygen tension (Pao\(_2\), calculated using the alveolar gas equation: \( \text{Pao}_2 = (\text{Pb} - \text{Pb}_{\text{H}2\text{O}}) \times \text{FAO}_2 - (\text{PAO}_2/\text{RQ}) \)), where Pb is the barometric pressure (760 mmHg), Pb\(_{\text{H}2\text{O}}\) is the water vapor pressure of fully saturated air (47 mmHg), Pb\(_{\text{O}_2}\) is the inspired oxygen fraction (20.9%), Pao\(_2\) is the alveolar CO\(_2\) tension (assumed equal to arterial PCO\(_2\)), and RQ is the respiratory quotient (assumed to be 0.8) (27, 45).

Statistics. All graphing and statistical analyses were performed using StatView (version 5.0, Cary, NC), Origin (version 7.03, Northampton, MA), or Microsoft 2002 Excel software. When overall differences between the LVV and HVV groups were compared at post-DI time points, repeated-measures ANOVA was used, followed by post hoc Bonferroni tests for means comparison. When within-group differences were compared at separate time points, paired t-tests were used. Differences were considered significant when \( P \) values were <0.05.

RESULTS

Impedance and alveolar instability. Results from in vivo microscopy image analysis are shown in Fig. 1A and demonstrate that %I-E\( \Delta \) 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-E\( \Delta \) in the HVV group began to diverge from that of
the LVV group by 15 min and became significantly different by 30 min (P < 0.05). H and η vs. time for HVV (shaded ovals) and LVV (open ovals) groups. The regularly spaced sudden drops in H for the LVV group correspond to deep inhalation (DI). C: η vs. time for HVV (shaded ovals) and LVV (open ovals) groups. Values are means ± SE.

Fig. 1. A: Percent change was used as an index of alveolar instability (%I-EΔ) at all 15-min intervals for high (HVV; shaded bars) and low tidal volume ventilation (LVV; open bars) groups. *Significant difference between groups (P < 0.05). #Significant difference for HVV group compared with baseline at 0 min (P < 0.05). B: elastance (H) vs. time for HVV (shaded ovals) and LVV (open ovals) groups. The regularly spaced sudden drops in H for the LVV group correspond to deep inhalation (DI). C: η vs. time for HVV (shaded ovals) and LVV (open ovals) groups. Values are means ± SE.

The changes in pulmonary mechanics observed during the second part of the study mirrored those of the first part, with H and η in the HVV group both becoming significantly elevated compared with 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 Fig. 3 from both LVV (Fig. 3A) and HVV (Fig. 3B) groups.

The mean %NBP for thresholded binary images at each time point is shown in Fig. 4. Compared with 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 with the LVV group by 30 min. This relationship is explored further in Fig. 5, which shows the change in %NBP from pre-DI to post-DI, for each rat at each time point, vs. the corresponding % change in H. The changes in %NBP and H following DI are positively correlated (R = 0.71, P < 0.001), although 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 from either pressure volume curves (24, 36), computed-tomography images (5, 30, 34, 38), or end-expiratory lung volume measurements (20, 26, 33). Using the technique of in vivo microscopy, we have been able in the present study to examine this relationship directly.

The principal 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 \( \eta \) (Fig. 1, B and C), 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

---

**Fig. 3.** Representative in vivo microscopy images (magnification \( \times 130 \)) from LVV (A) and HVV (B) groups, together with their corresponding black-and-white threshold images (under each color image). The black-and-white images were used to quantify percent number of black pixels (%NBP) at pre-DI, peak-DI, and post-DI time points. Scale in first figure represents 0–0.5 mm (500 \( \mu \)m).
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, whereas 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 $P_{aO_2}$ during injurious HVV was only marginally diminished compared with 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, $P_{aO_2}$ was actually higher in the HVV group for the first 45 min of the protocol, which was likely attributable to the higher peak and mean airway pressures involved. Nevertheless, $P_{aO_2}$ did fall gradually during the HVV protocol, whereas 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, although the acutely injured lung is usually characterized by increases in shunt, A-aDO$_2$, physiological dead space, and arterial PCO$_2$ (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 $P_{aO_2}$ 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, after the onset of VILI in the HVV group, alveolar instability progressed to the point of repeated alveolar collapse and reexpansion with each breath, even at a moderate level of PEEP (Fig. 1A). Hence, although repeated alveolar closure and reexpansion 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 reexpansion 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 computer tomography 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 reexpansion 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 threshold 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 nonaerated 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

![Graph](image-url)
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 reexpansion 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 led 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 Vr. 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 100 μm (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 Fig. 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 whereas %NBP is a local measure limited to the pleural surface.

It should also be noted that we used very large Vr in our HVV rats to create overdistension injury within the 1-h time scale of our experiments. Indeed, we expanded the lungs well beyond normal total lung capacity with each breath (22) in these animals to accelerate the onset of lung injury (42). Although such Vr have been used to create VILI in other animal models (10, 48), they are much greater than the levels of lung overdistension thought to contribute to VILI in patients. Even the Vr used in the LVV group was substantially larger than the low Vr regimen of 6 ml/kg ideal body weight clinically recommended for patients with ALI. This Vr 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-aDO2 was negative for some samples, particularly early in the HVV group. Because a negative A-aDO2 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 η are derived from a model fit to Zp. H is the equivalent of conventional lung elastance (2, 3) but has the advantage of being independent of the frequency of breathing. η is the ratio of energy dissipation to storage in the lung tissue, something that has been shown to remain remarkably constant with frequency of oscillation (9) and represents the viscoelastic properties of the tissues. Our laboratory has shown previously that the theoretical model underlying H and η provides an excellent fit to measurements of respiratory impedance in mouse models of ALI (2, 3), and others have had similar success in rats (37). Nevertheless, the calculation of Zp 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 Vr, and measurements of impedance were made at E 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 led 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 three of six rats in the HVV group, this effect was very small (from 3 to 5%). This would seem to discount nonlinear conditions as the principal 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 Zp. 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 η we observed during development of ALI (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 to manifest as an increase in tissue resistance (i.e., G). This could explain why we observed a progressive increase in η (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 occur. 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, whereas 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.

**GRANTS**

This study was funded by grant COBRE P20RR15557 and National Heart, Lung, and Blood Institute Grant HL-67273.

**REFERENCES**


