# Low-volume ventilation causes peripheral airway injury and increased airway resistance in normal open-chest rabbits

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**ABSTRACT**

Lung mechanics and morphometry of 10 normal open-chest rabbits (group A) mechanically ventilated (MV) with physiologic tidal volumes ($V_T$=8-12 ml/kg) at zero end-expiratory pressure (ZEEP) for 3-4 h was compared to that of 5 rabbits (group B) after 3-4 h MV with PEEP of 2.3 $cmH_2O$. Relative to initial MV on PEEP, MV on ZEEP caused a progressive increase in quasi-static elastance (Est; +36%), airway (Rint; +71%) and viscoelastic resistance (Rvisc; +29%) with no change in viscoelastic time constant. After restoration of PEEP, Est and Rvisc returned to control, whilst Rint remained elevated (+22%). On PEEP, MV had no effect on lung mechanics. Gas exchange on PEEP was equally preserved in groups A and B, and the lung wet/dry ratios were normal. Both groups had normal alveolar morphology, whilst only group A had injured respiratory and membranous bronchioles. In conclusion, prolonged MV on ZEEP induces histologic evidence of peripheral airway injury with concurrent increase in Rint, which persists after restoration of normal end-expiratory volumes. This is probably due to cyclic opening-closing of peripheral airways on ZEEP.

keywords: lung elastance, interrupter resistance, viscoelasticity, recruitment-derecruitment of lung units, lung injury scores

In 1984 Robertson (18) suggested that ventilation at low lung volumes may cause lung injury as a result of shear stresses caused by cyclic opening and closing of small airways. Using an *ex vivo* model of lavaged rat lung, Muscedere et al. (17) showed that ventilation with physiologic tidal volumes from zero end-expiratory pressure (ZEEP) resulted in a significant increase of histologic injury scores in the respiratory (RIS) and membranous bronchioles (MIS) relative to ventilation from positive end-expiratory pressure (PEEP) above the lower inflection point on the static inflation volume-pressure curve of the lung. In normal closed-chest rabbits ventilated at low lung volumes for only 1 h, Taskar et al. (22) found no histologic evidence of airways and parenchymal lung injury. In a subsequent study on normal open-chest rabbits ventilated at low lung volumes for 3 h, Taskar et al. (23) again found no histologic evidence of parenchymal lung injury but they did not specifically assess peripheral airway injury with indices such as RIS and MIS. Thus, it is possible that ventilation at low lung volumes for more than 1 h may induce peripheral airway injury in the absence of pre-existent parenchymal lung injury. In fact, to the extent that cyclic opening and closing of peripheral airways is responsible for lung damage, it is likely that the injury should be preferentially located in peripheral airways.

Accordingly, in the present study we have assessed the effects of breathing at low lung volumes for 3-4 h in open-chest rabbits with normal lungs in terms of *a)* histologic indices of peripheral airway and parenchymal injury; and *b)* lung mechanics. The latter was studied not only during the initial period of ventilation on PEEP and next on ZEEP, as in previous studies (17,23), but also after restoration to PEEP from ZEEP in order to assess whether the changes in lung mechanics observed at ZEEP could be reversed.

## METHODS

Fifteen rabbits (weight range 2.2-3.1 kg) were anesthetized with an intravenous injection of a mixture of pentobarbital sodium (20 mg/kg) and urethane (0.5 mg/kg). A brass cannula and a polyethylene catheter were inserted into the trachea and carotid artery, respectively. The animals were paralyzed with pancuronium bromide (0.1 mg/kg) and mechanically ventilated (respirator 660; Harvard Apparatus, Holliston, MA) with a pattern similar to that during spontaneous breathing. Adequate levels of anesthesia and complete muscle relaxation were maintained with additional doses of the anesthetic mixture and pancuronium bromide. The chest was opened via a median

sternotomy and a coronal cut made just above the costal arch. Application of positive end-expiratory pressure (PEEP; 2-2.5 $cmH_2O$) prevented lung collapse. During the measurements, the ribs on the two sides and the diaphragm were pulled widely apart, so that the lungs did not contact the chest wall except in their lowermost parts.

Airflow (V') was measured with a heated Fleisch pneumotachograph no.00 (HS Electronics, March-Hugstetten, Germany) connected to the tracheal cannula and a differential pressure transducer (Validyne MP45, ±2 $cmH_2O$; Northridge, CA). The response of the pneumotachograph was linear over the experimental range of V'. Tracheal pressure (Ptr) was measured with a pressure transducer (model 1290A; Hewlett-Packard, Palo Alto, CA) connected to the side arm of the tracheal cannula; there was no appreciable shift in the signal or alteration in amplitude up to 20 Hz. The signals from the transducers were amplified (model RS3800; Gould Electronics, Valley View, OH), sampled at 200 Hz by a 14-bit A/D converter, and stored on a desk computer. Volume changes ($\Delta V$) were computed by numerical integration of the digitized airflow signal. Arterial blood $P_{O2}$, $P_{CO2}$ and pH were measured by means of a blood gas analyzer (IL 1620; Instrumentation Laboratory, Milan, Italy) on samples drawn at the beginning and at the end of the tests made on PEEP.

After completion of the surgical procedure, the rabbits were ventilated with a specially designed, computer-controlled ventilator, delivering water-saturated air from a high pressure source (4 atm) at constant flows of the selected magnitudes and durations. The inspiratory and expiratory solenoid valves (model S50 and S80; Peter Paul, New Britain, CT) had a closing or opening time of 5 ms: they could be also operated so as to occlude the airways either at end-inspiration or end-expiration for 5 s. The inspiratory and expiratory valves were connected to the pneumotachograph attached to the animal's trachea by means of short rigid tubings. A Fleisch pneumotachograph (no.00) connected to the exhaust valve (model S50) of the inspiratory line and differential pressure transducer (Validyne MP45, ±2 $cmH_2O$) provided the feedback signal to the computer for the fine adjustement of the proportional valve (model PSV1; Aalborg, Orangeburg, NY) setting the inflation flow. A three way stopcock allowed the connection of the expiratory valve either to the ambient or to a drum in which the pressure was set at 2-2.5 $cmH_2O$ by means of a flow-through system. The baseline ventilator setting consisted of fixed tidal volume ($V_T$) of 25 ml (8-12 ml/kg), and inspiratory ($T_I$) and expiratory durations of 1 and 2.2 s, respectively. With this setting no intrinsic

positive end-expiratory pressure was present under any experimental condition, as evidenced by an end-expiratory pause (zero flow) and absence of Ptr changes with airway occlusion at end-expiration.

*Procedure and data analysis*. After opening the thorax, 10 rabbits (group A) were subjected to the following sequence of PEEP and ZEEP while the baseline ventilatory settings remained constant in each rabbit: *a)* 15 min of mechanical ventilation (MV) with PEEP ($PEEP_1$); *b)* 3-4 h of MV at ZEEP; *c)* 15 min of MV with PEEP ($PEEP_2$). Lung mechanics was assessed with the rapid airway occlusion method (2,5) during the $PEEP_1$ and $PEEP_2$ periods, and after 5-10 min ($ZEEP_1$) and at the end of the ZEEP period ($ZEEP_2$). In 5 rabbits (group B) who were subjected only to MV with PEEP for 3-4 h, assessment of lung mechanics was made 5-10 min after the onset of MV with PEEP ($PEEP_1$) and at the end of the PEEP period ($PEEP_2$). Before measurements during MV with PEEP the lungs were inflated 3-4 times up to Ptr of ~25 $cmH_2O$. Two types of experimental procedures were carried out: *a)* while keeping $V_T$ at baseline values, test breaths were intermittently performed with different $V'_I$ and $T_I$ in the range 0.25 to 3 s; and *b)* while keeping $V'_I$ at baseline values, test breaths were intermittently performed with different $V_T$ in the range 8 to 61 ml to obtain quasi-static inflation volume-pressure curves. End-inspiratory occlusions lasting 5 s were made in all test breaths, which were performed in random order and repeated 4-5 times in all experimental conditions. During ventilation at ZEEP, end-inspiratory occlusions were performed only for $V_T$ of 8 and 25 ml. During ventilation with PEEP, the expiratory valve was opened to the ambient for 4-6 expirations in order to measure the difference between the end-expiratory and the resting lung volume ($\Delta EELV$); these breaths were followed by two inflations up to Ptr of 20-25 $cmH_2O$. The animals were from a single cohort and the experiments were done in random order.

The end-inspiratory airway occlusions were followed by a rapid initial drop in Ptr ($\Delta P_1$), and by a slow decay ($\Delta P_2$) to an apparent plateau value (Pst). This pressure, computed as the mean pressure recorded during the interval between 4.5 and 5 s after the occlusion, was taken to represent the quasi-static lung recoil pressure, while $\Delta P_1$ and $\Delta P_2$ divided by $V'_I$ yielded the lung interrupter (Rint) and additional ($\Delta R$) resistances, respectively. Viscoelastic parameters, Rvisc and $\tau visc=Rvisc/Evisc$, were computed by fitting the values of $\Delta R$ and durations of inflation ($T_I$) with the function (5)

$$\Delta R=Rvisc\cdot(1-e^{-T_I/\tau_{visc}}) \qquad (1)$$

while lung quasi-static elastance (Est) was obtained as (Pst-Pee)/VT, Pee being the end-expiratory pressure. After completion of the mechanics measurements, the left or right lung was processed for histologic analysis, while the other one was weighed immediatedly after removal, left overnight in an oven at 120°C, and weighed again to compute the wet/dry ratio.

*Histological analysis.* After excision and isolation, the lungs were fixed by intabronchial infusion of 10% formalin with the pressure maintained at 20 $cmH_2O$ for 24 h. Technically adequate fixation was achieved in seven lungs from rabbits of group A and five from rabbits of group B. Five blocks, 1cm thick, involving both subpleural and para-hilar regions, were obtained in each animal: 2, 1, and 2 blocks from the upper, middle, and lower lobe, respectively, for the right lung, and 2 and 3 blocks from the upper and lower lobe, respectively, for the left lung. Each block was processed through a graded series of alcohols and embedded in paraffin. From each block, sections of 5 μ thickness were cut and stained with hematoxylin-eosin for light miscroscopic analysis. Histologic evaluation was done without knowledge of the mechanical data. The following measurements were performed: *a)* mean linear intercept (Lm), which is a measure of air-space enlargement, as described by Thurlbeck (24); *b)* indices of parenchymal injury, as described by Taskar et al. (23); and *c)* presence of bronchiolar epithelial necrosis and sloughing, which is a measure of bronchiolar injury, as described by Muscedere et al. (17).

For Lm measurements, one section from each block was examined at a magnification of 125, and 40 non-overlapping fields were analyzed on each section, giving a total of 200 fields per animal. The value of Lm was obtained as the ratio between the length in μ of a line passing transversely through each field and the number of alveolar walls intercepting the line, the final result for a given animal being the average Lm of the 200 fields examined. Additional histologic evidence of parenchymal injury was assessed according to the following 5 parameters, namely focal alveolar collapse, intraalveolar edema, hemorrhage, epithelial desquamation in alveoli, and presence of granulocytes in the air spaces (23). Each parameter was evaluated semiquantitatively in a single blind manner, using a four grade scale (absent; mild; moderate; prominent).

Bronchiolar injury was assessed from the presence of epithelial necrosis and sloughing (*i.e.* separation of necrotic tissue) in the respiratory bronchioles*, i.e.* airways with alveolar outpouchings in their walls, and in the membranous bronchioles, *i.e.* airways without cartilage including terminal bronchioles and the parent generation to respiratory bronchioles. At least 50 bronchioles were

examined per animal. Three indices were obtained for each lung: *a)* the respiratory bronchiole injury score (RIS) computed as the percent ratio of injured to total respiratory bronchioles examined; *b)* the membranous bronchiole injury score (MIS) computed as the percent ratio of injured to total membranous bronchioles examined; and *c)* the total injury score (TIS) computed as the percent ratio of injured respiratory and membranous bronchioles to total respiratory and membranous bronchioles (17). *Statistics.* Results from mechanical studies are presented as means ±SE. The least-square regression method was used to assess the parameters in Eq.1 and of the pressure-volume relationship of the lungs. Comparisons among experimental conditions were performed using one-way analysis of variance (ANOVA); when significant differences were found, the Bonferroni test was performed to determine significant differences between different experimental conditions. Results from histologic studies are expressed as median and range, and the statistical analysis was performed using the Mann Whitney-U test. The level for statistical significance was taken at $P \leq 0.05$.

## RESULTS

### *Ventilation on PEEP*

In each animal, the values of arterial $P_{O_2}$, $P_{CO_2}$ and pH obtained at the beginning and at the end of the sessions on PEEP did not differ significantly, and were thus averaged. The mean values of $P_{aO_2}$, $P_{aCO_2}$ and $pH_a$ during $PEEP_1$ and $PEEP_2$ were similar for both group A and B rabbits (Table 1). Also the mean values of the wet/dry ratio assessed at the end of the experiments in the two groups of rabbits did not differ significantly (Table 1) and were virtually the same as that obtained on 29 lungs (4.61±0.07) removed 30 to 40 min after the induction of anesthesia from rabbits in which the only other intervention was the excision of part of the pericardium (3).

The end-expiratory pressure applied to rabbits of both group A and B was almost the same during $PEEP_1$ and $PEEP_2$: its average value was 2.3±0.1cm $H_2O$. Similarly, the mean values of ΔEELV did not differ significantly among the various conditions in both groups of rabbits (Table 2).

*Static V-P relationships.* In each animal, both before and after the prolonged ventilation on ZEEP or PEEP, the inflation volume-pressure curve on PEEP could be closely fitted ($r>0.95$) by a function in the form $V_o \cdot (1-e^{-k \cdot Pst})$, where $V_o$ is maximum volume above the resting volume of the lung and

k=1/P is a shape factor (4,19). The group mean values of these constants during $PEEP_1$ and $PEEP_2$ are reported in Table 2. Since in all animals the values of $V_o$ and k did not change after prolonged ventilation on ZEEP (group A) or PEEP (group B), a unique relationship could be used to describe the quasi-static lung V-P curve above the end-expiratory lung volume with PEEP, as shown in Figure 1.

*Elastance.* On the basis of the $V_o$ and ΔEELV values in Table 2, tidal ventilation with PEEP occurred in the range 30-65 $\%V_o$. The average values of Est obtained under the various conditions in the two groups of animals are given in Table 3. During ventilation with PEEP, Est was almost the same before and after the prolonged period of ventilation on ZEEP (group A), as well as with PEEP (group B).

*Interrupter resistance.* In all animals and conditions, Rint was independent of flow; hence, the values of Rint obtained in each animal and condition were averaged (Table 3). With $PEEP_1$, Rint did not differ significantly between group A and B (P=0.45). In group A rabbits, with $PEEP_2$ Rint increased significantly relative to $PEEP_1$ in seven animals, decreased significantly in one, and was unchanged in two animals: on average, Rint was, however, significantly increased (ΔRint=3.5±1.2 cm $H_2O \cdot s \cdot l^{-1}$; P<0.02) after the prolonged ventilation on ZEEP. On the other hand, in group B rabbits the prolonged ventilation with PEEP did not change Rint significantly (ΔRint=-0.6±0.5 cm $H_2O \cdot s \cdot l^{-1}$; P>0.2).

*Viscoelastic properties.* In all animals and conditions, a unique function in the form of Eq.1 adequately described the experimental ΔR-$T_I$ relations (r>0.975), allowing computation of Rvisc and τvisc. Figure 2 (*upper panel*s) depicts the relationship of ΔR to $T_I$ obtained in one animal during ventilation with PEEP before and after prolonged ventilation on ZEEP (*left*) and the average results obtained from the 10 lungs (*right*). Also shown in that figure (*lower panel*s) are an individual (*left*) and the group mean relationship (*right*) obtained before and after prolonged ventilation on PEEP. No significant changes of Rvisc and τvisc occurred before and after prolonged ventilation on ZEEP or on PEEP (Table 4).

***Ventilation on ZEEP***

*Elastance.* According to the $V_o$ values in Table 2, baseline tidal ventilation ($V_T$=25 ml) on ZEEP occurred in the range 0-35 $\%V_o$. There was both an immediate and a progressive increase of Est with ventilation on ZEEP (Table 3). With $V_T$=8 ml, Est was significantly larger than that for $V_T$=25

ml ($\Delta$Est=6.4±1.8 $cmH_2O \cdot l^{-1}$; P<0.001); this was related to the pronouced "knee" in the lowest part of the dynamic inspiratory V-P curve, that was practically absent during ventilation with PEEP, as shown in Figure 3. Indeed, under the latter condition Est at $V_T$=8 ml was significantly smaller than that with $V_T$=25 ml ($\Delta$Est=-6.6±1.2 $cmH_2O \cdot l^{-1}$; P<0.001).

*Interrupter resistance.* As during ventilation with PEEP, Rint was independent of flow in all animals. The mean values of Rint obtained with $ZEEP_1$ and $ZEEP_2$ are shown in Table 3. Though larger, Rint with $ZEEP_1$ was not significantly different from that with $PEEP_1$ ($\Delta$Rint=4.1±2.5 $cmH_2O \cdot s \cdot l^{-1}$; P>0.05), suggesting that in the volume range 35-65% $V_o$ there is little or no change of Rint. However, Rint increased with $ZEEP_2$, becoming significantly larger than that with both $PEEP_1$ ($\Delta$Rint=11.4±3.6 $cmH_2O \cdot s \cdot l^{-1}$; P<0.01) and $PEEP_2$ ($\Delta$Rint=8.9±3.1 $cmH_2O \cdot s \cdot l^{-1}$; P<0.02).

*Viscoelastic properties.* Figure 2 (*middle panel*s) depicts the relationship of $\Delta$R to $T_I$ pertaining to one animal (*left*) and to the entire group (*right*) obtained with $ZEEP_1$ and $ZEEP_2$. In all animals and conditions, a unique function in the form of Eq.1 adequately described the data points, the mean values of Rvisc and $\tau$visc being reported in Table 4. With $ZEEP_1$, Rvisc increased significantly relative to that with $PEEP_1$ ($\Delta$Rvisc=13.8±4.1 $cmH_2O \cdot s \cdot l^{-1}$; P<0.02), and a further significant increase occurred between $ZEEP_1$ and $ZEEP_2$ ($\Delta$Rvisc=8.7±3.6 $cmH_2O \cdot s \cdot l^{-1}$; P<0.05). In contrast, $\tau$visc remained essentially the same under all conditions.

***Histology***

The results of Lm for the animals that underwent the prolonged period of ventilation on ZEEP (group A) and on PEEP (group B) are shown in Table 5. The Lm did not differ significantly between group A and B, while the membranous, respiratory, and total injury score were significantly greater in group A (P<0.05). There was no histologic evidence of lung edema on specimens from both groups A and B, in line with the normal values of the wet/dry ratio of the lung (Table 1), nor of focal alveolar collapse, hemorrhage, epithelial desquamation in alveoli. Signs of mild inflammation, as judged from the presence of granulocytes in the air spaces, were found only in two out of seven animals of group A, and one animal of group B.

## DISCUSSION

Using an *ex vivo* model of lavaged rat lungs ventilated with physiologic tidal volumes from ZEEP or PEEP above the lower inflection point on the static inflation V-P relationship of the lung,

Muscedere et al. (17) showed that on ZEEP there was a significant increase of RIS and MIS. In line with the latter results, we found that the values of RIS and MIS were significantly higher in group A than B (Table 5). In group A rabbits, however, the bronchiolar injury scores were substantially lower than those obtained in the lavaged rats lungs (17). Since lavaged lungs axiomatically exhibit greater regional structural inhomogeneity, such a discrepancy is predictable based on the concept of parenchymal interdependence postulated by Mead et al. (15). Thus marked regional structural inhomogeneity should enhance the shear stresses and related injury due to cyclic opening-closing of peripheral airways. This has been recently discussed in detail by Marini (14). In the present study we have also measured Lm which did not differ significantly between groups A and B, indicating that mechanical ventilation on ZEEP does not cause enlargement of air spaces when compared with mechanical ventilation on PEEP.

Contrary to Taskar et al. (22), we have found that in normal open-chest rabbits ventilation at low lung volumes elicits significant histologic damage to the peripheral airways. This discrepancy is probably due to the fact that these authors ventilated their rabbits at low-volume for only 1 h as compared to 3-4 h in the present study. In a subsequent study, Taskar et al. (23) found no evidence of lung injury in normal open-chest rabbits ventilated at low lung volume for 3 h. This was based on the following 6 parameters, namely focal alveolar collapse, intraalveolar edema, hyaline membranes, hemorrhage, epithelial desquamation in airways and alveoli, and presence of granulocytes in the air spaces. In both group A and B rabbits there were no histologic signs of alveolar injury, like hemorrhage, focal alveolar collapse, alveolar epithelial desquamation, or intra-alveolar edema, as also evidenced by normal values of lung wet/dry ratio (Table 1), whilst, at variance with Taskar et al. (23) results, there was evidence of epithelial desquamation in the respiratory and membranous bronchioles (Table 5). It should be noted, however, that Taskar et al. (22,23) did not use specific, quantitative indices of peripheral airway injury like those used in the present study. Air-space enlargements, emphysema-like lesions, bronchiectasis and pseudocysts are characteristic feature of baro- and volotrauma in patients with severe respiratory dystress syndrome (7). Such changes, which have also been found in pigs with multifocal pneumonia ventilated at high lung volumes (10), were absent in the present model of low-volume injury.

Lung injury during ventilation at low lung volumes is generally attributed to cyclic opening and closing of relatively small airways with concomitant generation of shear stresses that may be

responsible for some of the lung damage (17,18). With PEEP, there was no evidence of airway closure since, as shown in Figure 1, the static inflation V-P curve of the lung was concave to the pressure axis (8). Accordingly, at PEEP of 2 $cmH_2O$ the static compliance with $V_T$=8 ml was higher than that with $V_T$=25 ml (Fig. 3). In contrast, at ZEEP the initial part of the static inflation V-P curve was convex to the pressure axis, and accordingly the compliance with $V_T$=8 ml was lower than that $V_T$=25 ml (Fig. 3). This change in shape of the static V-P curve at low lung volumes has been attributed to airway closure (8). The site of closure, as determined by serial sections of quick-frozen dog lungs, is in small (<1mm in diameter) airways (11). Thus, based on the above mentioned considerations, it appears that during mechanical ventilation on ZEEP the present rabbits exhibited cyclic airway opening and closing, which should be responsible for the changes in RIS and MIS, as well as the increase in Rint on $PEEP_2$ relative to $PEEP_1$.

On ZEEP, Ptr increased more markedly and rapidly at the onset of inflation than on PEEP, as illustrated in Figure 4, which shows the time course of Ptr, V', and $\Delta V$ in a rabbit at $PEEP_1$ and $ZEEP_2$. During the initial 90 ms of inflation the average rate of rise of Ptr ($\Delta Ptr/\Delta t$) on $PEEP_1$ and $ZEEP_2$ was 6.8 and 32.9 $cmH_2O \cdot s^{-1}$, respectively. The corresponding average values for all rabbits were 6.1±0.8 and 33.7±2.9 $cmH_2O \cdot s^{-1}$, respectively. The high values of $\Delta Ptr/\Delta t$ on ZEEP probably contributed to the histologic damage of the peripheral airways observed after ventilation on PEEP in group A. In contrast, in group B the values of $\Delta Ptr/\Delta t$ were low and almost constant troughout the ventilation period. The increase in the initial $\Delta Ptr/\Delta t$ on ZEEP was due to increased impedance caused by atelectasis and/or airway closure.

On ZEEP there was a significant increase of Est, Rint, and Rvisc relative to $PEEP_1$, which was significantly greater after 3-4 h ($ZEEP_2$) than after 5-10 min of ventilation on ZEEP ($ZEEP_1$). A progressive increase of dynamic lung elastance during mechanical ventilation at low lung volume has been previously reported by Dechman et al. (6) in a normal open-chest dogs and by Taskar et al. (23) in open-chest rabbits. In line with our results, Taskar et al. (23) found a progressive increase in total lung resistance on ZEEP, whereas Dechman et al. (6) found no significant change. It should be noted, however, that in the latter study the lowest PEEP was 1 $cmH_2O$ and the time spent on this PEEP (20 min) was much shorter than in the present investigation.

Two mechanisms can account for the increase of Est that occurs on ZEEP, namely an increase in stiffness of lung tissue due to larger surface forces, and a decrease in the amount of

ventilated tissue caused by airway closure and/or alveolar collapse. Both mechanisms could also be responsible for the progressive increase in Est with time. An increase of surface forces with time at low end-expiratory transpulmonary pressure and lung volume has been advocated to explain the changes of lung compliance in the absence of detectable airway closure (25,26). However, changes in surface forces alone can not account for lung behaviour at very low lung volumes (20). Airway closure and atelectasis represent, therefore, conditions which may contribute to the progressive increase of Est on ZEEP. In fact, a theoretical study of Stamenovic and Wilson (21) indicates that regional mechanical inhomogeneities should lead to diffuse alveolar collapse at low transpulmonary pressures. Presence of focal atelectasis was found, however, only in one out of three additional rabbit lungs fixed after 4 h on ZEEP with a transpulmonary pressure similar to the peak tracheal pressure during mechanical ventilation (~8 $cmH_2O$) to avoid re-expansion of collapsed areas, whilst for essentially the same end-inspiratory pressure the lung volume was about 25 ml larger on PEEP than on ZEEP (Fig.3). Hence, atelectasis alone can not account for such a volume reduction (~30%Vo). Accordingly, it is likely that small airway closure is the main mechanism leading to increased Est during ventilation on ZEEP.

The present study shows for the first time that on ZEEP there is a significant time-dependent increase in Rvisc, while τvisc does not change (Table 4). In principle, the same two mechanisms that have been invoked to explain the increase of Est with ventilation on ZEEP, could account for the concurrent increase of Rvisc (Table 4). In line with previous observations in dog lungs (6,12), most of the resistive properties of the rabbit lung arise from tissue, as Rvisc was markedly larger than Rint under all experimental conditions (Tables 3 and 4), and these mainly reside in the air-liquid interface (1). Changes in the properties of the surface film during ventilation at ZEEP could, therefore, have contributed to the increase in Rvisc. Increased inhomogeneity within the lung due to peripheral airway closure is another mechanism which could have contributed to the increase of Rvisc at ZEEP. A decrease in the amount of ventilated tissue that occurs with airway closure and/or atelectasis, could also cause *per se* an increase in Rvisc without affecting τvisc. The fact that this was the case (Table 4) suggests that airway closure and/or atelectasis may have been the main cause of the changes in Rvisc on ZEEP. Moreover, a reduction in ventilated tissue should have a proportional effect on both Est and Rvisc. In fact, there was a highly significant correlation between changes in Est and Rvisc (Fig. 5).

Airway resistance has been found to increase with acute reductions in lung volume, and this is ascribed to the concomitant decrease in lung recoil (13). Indeed Rint increased with $ZEEP_1$, though not significantly (Table 3). It should be noted, however, that as a result of the reduced lung compliance on $ZEEP_1$, the recoil pressure at end-inflation was only slightly smaller than that on $PEEP_1$. At $ZEEP_2$ Rint became significantly larger than on $PEEP_1$ (Table 3). The increase in Rint between $ZEEP_1$ and $ZEEP_2$ cannot be related to loss of lung recoil as Est became even larger on $ZEEP_2$ than on $ZEEP_1$ (Table 3), and the transpulmonary pressure at end-inflation was essentially the same as that with $PEEP_1$ (Fig. 3). Since the increase of Rint on ZEEP occurred in spite of an increased lung recoil, these changes of Rint should be due to damage of peripheral airways, as evidenced by the injury scores of respiratory and membranous bronchioles (Table 5), and/or to increased brochomotor tone.

After return of group A rabbits to PEEP ($PEEP_2$), Rvisc as well as Est reversed to the initial ($PEEP_1$) values, while Rint remained significantly ($P<0.01$) larger (Table 3). The increase in Rint on $PEEP_2$ could not be related to changes in arterial blood gases or pH, as the latter were not significant (Table 1). Similarly in group B animals the arterial $P_{O_2}$, $P_{CO_2}$ and pH were essentially the same on $PEEP_1$ and $PEEP_2$, indicating that on PEEP gas exchange was stable during the entire experimental period. Since the elastic recoil of the lung was also the same on $PEEP_1$ and $PEEP_2$ (Fig. 1), the increase in Rint was probably due to changes in mechanical properties of the peripheral airways, as reflected by the significant increase of RIS and MIS (Table 5), and/or increased bronchomotor tone caused by release of inflammatory mediators on ZEEP. Although signs of inflammation, as evaluated by the presence of granulocytes in the air spaces, were very modest both in group A and B, this does not exclude the possibility of a different release of inflammatory mediators in the two groups, since the number of inflammatory cells does not necessarily reflect their state of activation. In group A, the increase in Rint between $PEEP_1$ and $PEEP_2$ averaged 3.5 $cmH_2O \cdot s \cdot l^{-1}$ (Table 3). Assuming that under normal conditions peripheral airway resistance (Rp) contributes 20% of Rint (13), Rp with $PEEP_1$ should have amounted to 3.2 $cmH_2O \cdot s \cdot l^{-1}$. Thus, in the absence of changes in bronchomotor tone, Rp should have doubled from $PEEP_1$ to $PEEP_2$ (i.e. from 3.2 to 6.7 $cmH_2O \cdot s \cdot l^{-1}$).

In the present open-chest rabbits, peripheral airway lesions were found throughout the lungs because pleural pressure was essentially uniform. In closed-chest normal lung models, however, the

lesions should be confined to the lower lung zones as a results of the vertical gradient in pleural surface pressure (9). Indeed, with closed chest peripheral airway closure at low volumes occurs preferentially in the dependent lung zones which are subjected to lower transpulmonary pressure (16).

In conclusion, the present study shows for the first time that in normal lungs of open-chest rabbits 3-4 h mechanical ventilation with physiologic tidal volumes at zero end-expiratory pressure induces histologic evidence of peripheral airway injury with a concurrent increase in airway resistance (Rint), which persists after return of mechanical ventilation to a PEEP value that restores normal end-expiratory volumes. In contrast, when shifting from ZEEP to PEEP both Est and Rvisc return to the initial values observed with PEEP. That mechanical ventilation at low lung volume may cause airway damage in normal lungs is of substantial interest. In fact, our open-chest rabbit model may serve to study the effects of acute low volume injury on release of pulmonary inflammatory mediators in the absence of pre-existing alterations or lesions.

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**LEGENDS**

Figure 1. Average relationship between volume above resting lung volume (ΔV) and quasi-static transpulmonary pressure (Pst) obtained (*upper panel*) in 10 open chest rabbits (group A) during ventilation with PEEP of 2.3 cm $H_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$), and in 5 open chest rabbits (group B) during ventilation with PEEP of 2.3 cm $H_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on PEEP (PEEP2). Bars: SE. All data fit a unique monoexponential function.

Figure 2. Relationships of additional lung resistance (ΔR) to duration of inflation obtained at a fixed inflation volume during ventilation with PEEP of 2.3 $cmH_2O$ (*upper panels*) before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$) and during ventilation on ZEEP (*middle panels*) at the beginning ($ZEEP_1$) and end of the 3-4 h period ($ZEEP_2$) in 10 open chest rabbits (group A), and during ventilation with PEEP of 2.3 $cmH_2O$ (*lower panels*) before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$) in 5 open chest rabbits (group B). *Left*: representative animal; *right:* average relationships. Bars: SE. Under all conditions, the data fit a monoexponential function.

Figure 3. Average relationships (continuous lines) between volume above resting lung volume (ΔV) and transpulmonary pressure (Ptp) obtained in 10 open chest rabbits during ventilation with PEEP of 2.3 $cmH_2O$ ($PEEP_1$) and at the end of the 3-4 h of ventilation on ZEEP ($ZEEP_2$). Closed symbols joined by dotted lines represent corresponding static end-expiratory and end-inspiratory conditions for tidal volumes of 25 and 8 ml, respectively.

Figure 4. Ensemble average of records of flow (V'), volume changes (ΔV), and tracheal pressure (Ptr) from ten consecutive breath cycles in an open-chest rabbit during baseline ventilation with PEEP of 2.3 $cmH_2O$ ($PEEP_1$) and after 3 h ventilation on ZEEP ($ZEEP_2$).

Figure 5. Relationship of changes in viscoelastic resistance to those in static elastance occurring after 5-10 min ($ZEEP_1$) and 3-4 h of ventilation on ZEEP ($ZEEP_2$), both expressed relative to the corresponding values during the initial period of ventilation with PEEP of 2.3 $cmH_2O$ ($PEEP_1$), obtained in 10 open chest rabbits.

Table 1. Mean values (±SE) of arterial $P_{O_2}$, $P_{CO_2}$ and pH, and wet/dry ratio of the lung of group A rabbits during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$), and of group B rabbits during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$)

| | | $P_{aO_2}$ mmHg | $P_{aCO_2}$ mmHg | $pH_a$ | wet/dry |
|---|---|---|---|---|---|
| Group A | | | | | |
| | $PEEP_1$ | 85±7 | 38.5±3.5 | 7.34±0.04 | |
| | $PEEP_2$ | 94±11 | 37.8±4.5 | 7.30±0.07 | 4.59±0.07 |
| Group B | | | | | |
| | $PEEP_1$ | 95±7 | 35.5±4.1 | 7.33±0.09 | |
| | $PEEP_2$ | 96±15 | 34.0±5.5 | 7.30±0.07 | 4.72±0.10 |

Table 2. Mean values (±SE) of constants in equation $V_o \cdot (1-e^{-k \cdot Pst})$ used to fit the lung inflation volume-pressure curve and of end-expiratory volume above resting volume (ΔEELV) during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h ($PEEP_2$) of ventilation on ZEEP (group A), and during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h ($PEEP_2$) of ventilation on PEEP (group B)

| | | $V_o$ ml | k $cmH_2O^{-1}$ | ΔEELV ml |
|---|---|---|---|---|
| Group A | | | | |
| | $PEEP_1$ | 75.7±2.4 | 0.180±0.005 | 24.2±1.0 |
| | $PEEP_2$ | 75.7±2.4 | 0.176±0.004 | 24.3±1.2 |
| Group B | | | | |
| | $PEEP_1$ | 78.3±4.0 | 0.187±0.012 | 25.0±1.4 |
| | $PEEP_2$ | 80.5±5.3 | 0.184±0.011 | 25.5±1.3 |

Table 3. Mean values (±SE) of quasi-static pulmonary elastance (Est) and interrupter resistance (Rint) of group A rabbits during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$), and at the beginning ($ZEEP_1$) and end of the ventilation period on ZEEP ($ZEEP_2$), and of group B rabbits during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$)

| | Est | Rint | | Est | Rint |
|---|---|---|---|---|---|
| | $cmH_2O \cdot l^{-1}$ | $cmH_2O \cdot s \cdot l^{-1}$ | | $cmH_2O \cdot l^{-1}$ | $cmH_2O \cdot s \cdot l^{-1}$ |
| Group A | | | | | |
| $PEEP_1$ | 178±9 | 16.2±1.1 | $ZEEP_1$ | 219±9§ | 20.3±1.9 |
| $PEEP_2$ | 182±10 | 19.7±1.5* | $ZEEP_2$ | 242±9*§ | 27.7±3.1*§ |
| Group B | | | | | |
| $PEEP_1$ | 166±10 | 14.8±1.0 | | | |
| $PEEP_2$ | 166±10 | 14.2±0.7 | | | |

* significantly different from values on $PEEP_1$ ($P<0.01$); § significantly different from corresponding values on PEEP ($P<0.01$).

Table 4. Mean values (±SE) of viscoelastic resistance (Rvisc) and time constant (τvisc) computed according to Eq.1 of ten open-chest rabbits (group A) during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$) and at the beginning ($ZEEP_1$) and end of the ventilation period on ZEEP ($ZEEP_2$), and of five open-chest rabbits (group B) during ventilation with PEEP of 2.3 $cmH_2O$ before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$)

| | Rvisc | τvisc | | Rvisc | τvisc |
|---|---|---|---|---|---|
| | $cmH_2O \cdot s \cdot l^{-1}$ | s | | $cmH_2O \cdot s \cdot l^{-1}$ | s |
| Group A | | | | | |
| $PEEP_1$ | 77.5±8.7 | 0.97±0.11 | $ZEEP_1$ | 91.3±7.3§ | 1.07±0.08 |
| $PEEP_2$ | 80.7±7.7 | 0.96±0.09 | $ZEEP_2$ | 100.0±6.9*§ | 1.06±0.10 |
| Group B | | | | | |
| $PEEP_1$ | 75.4±12 | 1.18±0.05 | | | |
| $PEEP_2$ | 69.4±10 | 1.08±0.08 | | | |

* significantly different from values on $PEEP_1$ ($P<0.01$); § significantly different from corresponding values on PEEP ($P<0.01$).

Table 5. Mean linear interecept (Lm), respiratory (RIS), membranous (MIS) and total bronchiole injury score (TIS) from lungs subjected to 3-4 h of ventilation on ZEEP (group A) or PEEP (group B)

| | N | Lm, μ | RIS% | MIS, % | TIS, % |
|---|---|---|---|---|---|
| Group A | 7 | 377 (286-403) | 18* (0-33) | 14.5* (9-22) | 12* (6-16) |
| Group B | 5 | 319 (294-414) | 0 | 2.7 (1-16) | 2.2 (1-14) |

values are medians with range in parentheses. *P<0.05, group A *vs* B.

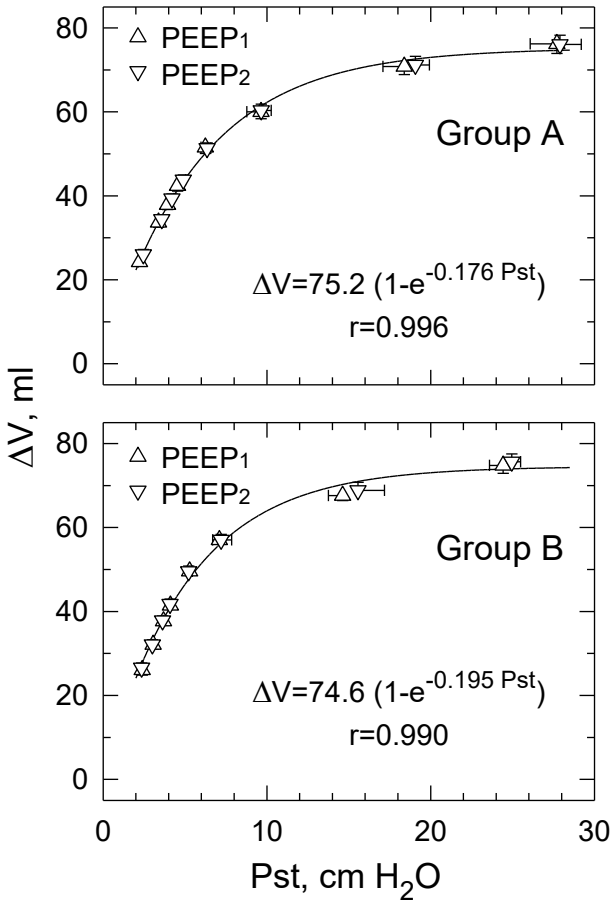
PEEP1
PEEP2
Group A
ΔV=75.2 (1-e-0.176 Pst)
r=0.996
Group B
ΔV=74.6 (1-e-0.195 Pst)
r=0.990
ΔV, ml
Pst, cm H2O
0
20
40
60
80
0
10
20
30

Figure 1

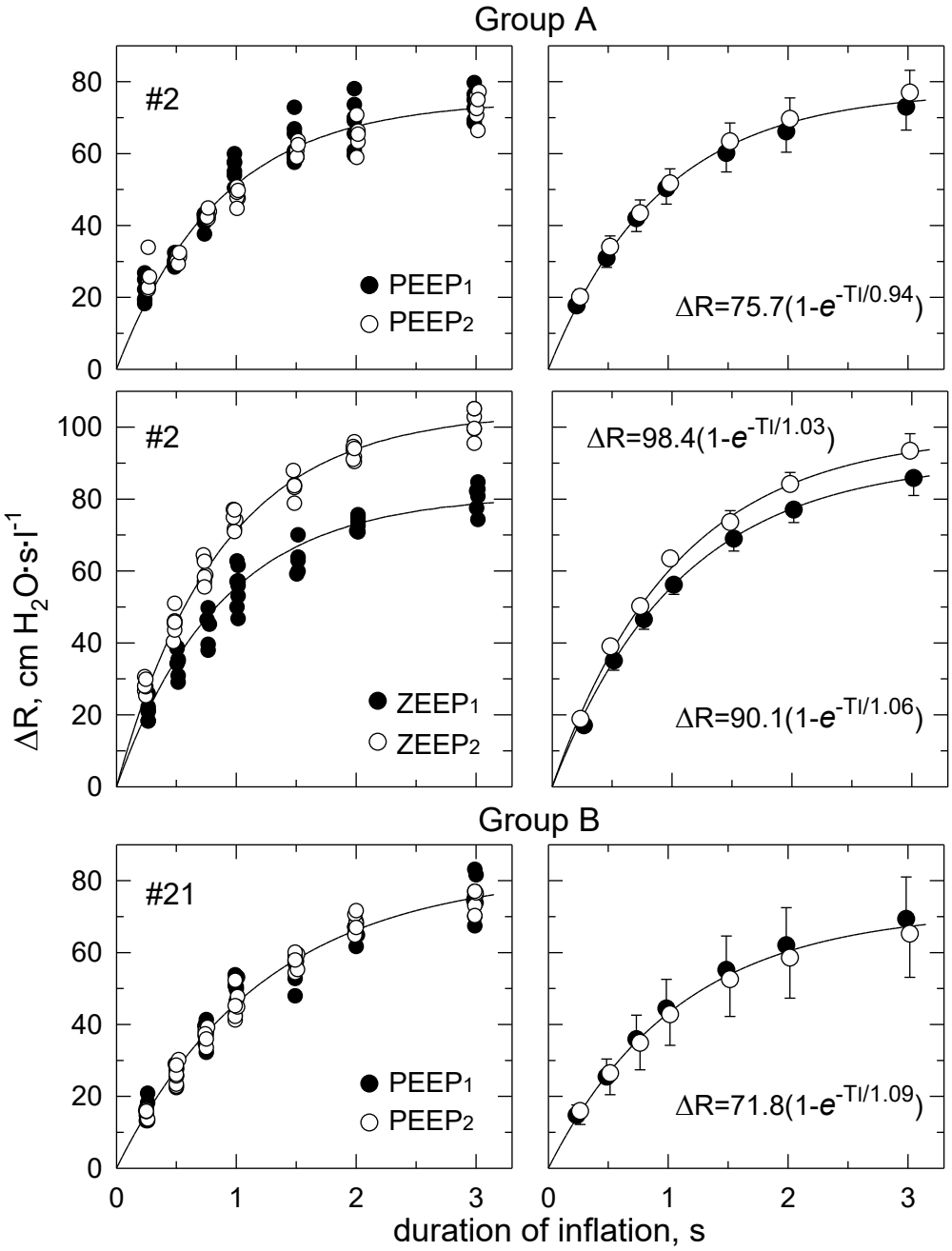
Group A
#2
PEEP1
PEEP2
ΔR=75.7(1-e-TI/0.94)
#2
ZEEP1
ZEEP2
ΔR=98.4(1-e-TI/1.03)
ΔR=90.1(1-e-TI/1.06)
Group B
#21
PEEP1
PEEP2
ΔR=71.8(1-e-TI/1.09)
ΔR, cm H2O·s·l-1
duration of inflation, s

Figure 2

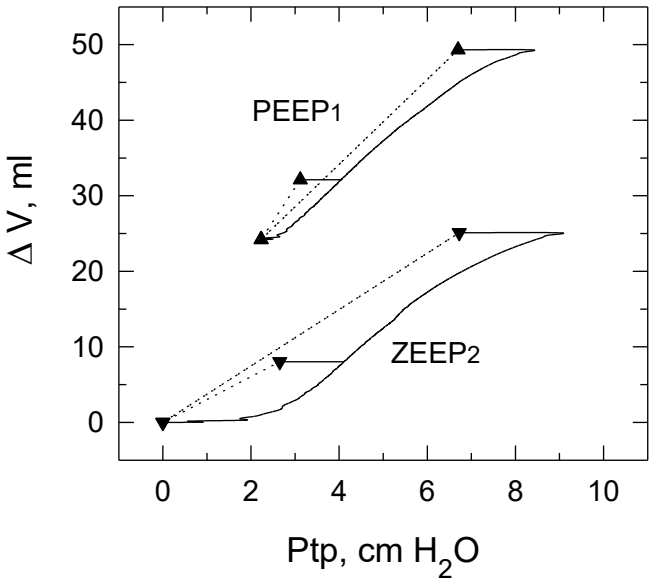


Figure 3

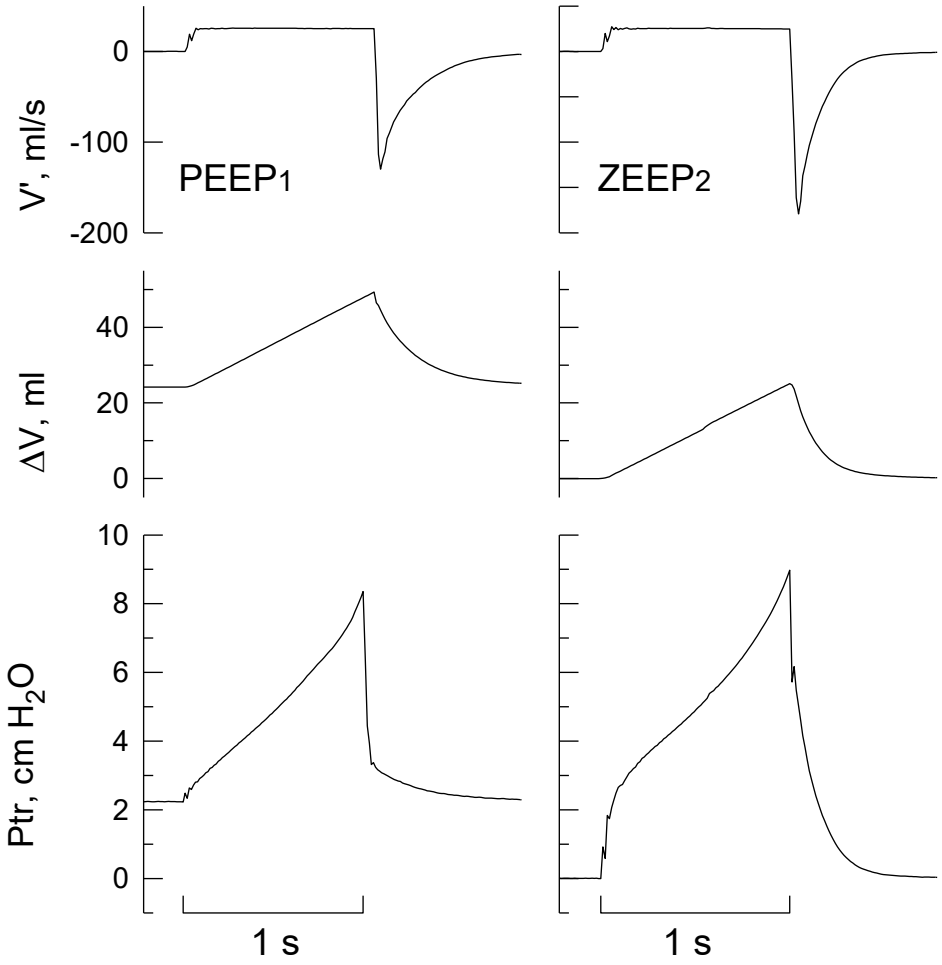


Figure 4

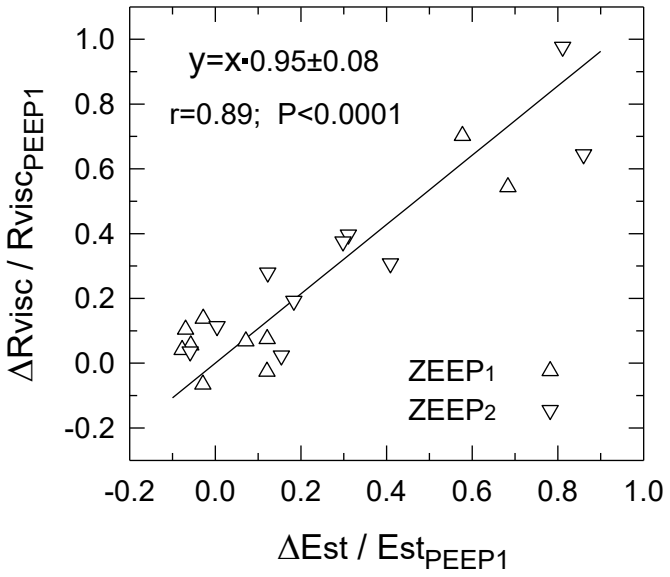
y=x·0.95±0.08
r=0.89; P<0.0001
ZEEP1
ZEEP2
ΔRvisc / RviscPEEP1
ΔEst / EstPEEP1
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.2
0.0
0.2
0.4
0.6
0.8
1.0

Figure 5