IMPACT OF MICROVASCULAR CIRCULATION ON PERIPHERAL LUNG STABILITY

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ABSTRACT

The involvement of the pulmonary circulation in the mechanical properties was studied in isolated rat lungs. Pulmonary input impedance (ZL) was measured at a mean transpulmonary pressure (Ptp) of 2 cmH₂O before and after physiological perfusion with either blood or albumin. In these lungs and in a group of unperfused lungs, ZL was also measured at Ptp values between 1 and 8 cmH₂O. Airway resistance (Raw) and parenchymal damping (G) and elastance (H) were estimated from ZL. End-expiratory lung volume (EELV) was measured by immersion before and after blood perfusion. The orientation of the elastin fibers relative to the basal membrane was assessed in additional unperfused and blood-perfused lungs. Pressurization of the pulmonary capillaries significantly decreased H, by 31.5±3.7% and 18.7±2.7% for blood and albumin, respectively. Perfusion had no effect on Raw, but markedly altered the Ptp dependences of G and H below 4 cmH₂O, with significantly lower values than in the unperfused lungs. At a Ptp of 2 cmH₂O, EELV increased by 31±11%, (p = 0.01) following pressurization of the capillaries, and the elastin fibers became more parallel to the basal membrane. Since the organization of elastin fibers results in smaller H values of the individual alveoli, the higher H in the unperfused lungs is probably due to a partial alveolar collapse leading to a loss in lung volume. We conclude that the physiological pressure in the pulmonary capillaries is an important mechanical factor in the maintenance of the stability of the alveolar architecture.

Key words: Forced oscillations, Pulmonary perfusion, Alveolar wall, Elastin, End-expiratory lung volume
INTRODUCTION

The mechanical properties of the lungs are significantly influenced by changes in the pulmonary hemodynamic conditions (3, 7, 9, 11, 20, 21, 27, 30, 35-37, 40). Numerous clinical (3, 7, 9, 11, 20, 27) and experimental studies (30, 36, 37) have demonstrated that elevation of the pulmonary blood flow (7, 20, 27) and/or pressure (3, 11, 30, 36, 37) leads to a deterioration of the lung function via a decrease in FRC (7) and/or stiffening of the alveolar wall (40). Although the qualitative examinations performed by von Basch in 1889 suggested that not only congestion, but also pulmonary hypoperfusion can alter the lung configuration (4), few data are available concerning the changes in the mechanical conditions of the lungs during hypoperfusion or the complete absence of pulmonary perfusion (21, 25, 30, 35). Mitzner et al. observed a transient increase in the respiratory resistance and a decrease in the compliance after occluding a pulmonary artery in mice, but the explanation of this finding remained unclarified (25). Furthermore, we recently demonstrated that low pulmonary venous pressures cause an impairment in lung mechanics, manifested in increases in the parenchymal resistive and elastic parameters, while the airway properties remain unaffected (30). However, the mechanisms responsible for the compromised lung mechanics at low vascular pressure or when there is no perfusion are still unclear. In the present study, we hypothesize that the pressurized pulmonary capillary network exerts a mechanical tethering effect that contributes to the maintenance of a normal lung function. In an attempt to test this hypothesis, we set out to investigate how the absence or presence of physiological perfusion affects the airway and parenchymal mechanical parameters and lung volume at different transpulmonary pressures in isolated rat lungs. We also examined whether or not the changes in lung function after the onset of pulmonary perfusion are similar for blood and albumin perfusates.
METHODS

Preparation of lungs

The procedure for the preparation of the isolated lungs was identical to that described in detail previously (30). After approval by the Animal Care Committee of the Canton of Geneva and by the Institutional Ethics Committee, 21 adult male Sprague-Dawley rats weighing 360-390 g were anesthetized with isoflurane (3% induction, 1.4% maintenance dose), then tracheotomized with a polyethylene cannula (14-gage, Braun, Melsungen, Germany) and mechanically ventilated (model 683, Harvard Apparatus Inc., South Natick, MA, USA) with a tidal volume of 7 ml/kg and a respiratory rate of 70-80/min while a positive end-expiratory pressure (PEEP) of 2.5 cmH₂O was maintained. Airway opening pressure was monitored continuously (DP 45 transducer and 2D15 carrier demodulator, Validyne, Northridge, CA, USA). The femoral artery was cannulated with a 28-gage catheter (Portex, Hythe, GB) for monitoring of the systemic blood pressure (model 156 PCE 06-GW2, Honeywell, Zürich, Switzerland). The femoral vein was also cannulated for drug delivery. Heparin (1.5 IU/g) was then administered iv for complete anticoagulation of the blood. Thirty-five ml of arterial blood was next gently withdrawn, while the collected blood was continuously replaced by the iv infusion of colloid solution (hydroxyethyl-starch 6%). This maneuver maintained a constant intravascular volume and a mean systemic blood pressure above 50 mmHg, and thus minimized the risk of ischemic lesions in the lungs. The collected diluted blood was centrifuged (4000 rpm for 10 min) and 17 ml of plasma was extracted. The resulting concentrated blood with a hematocrit level of ~35% served as priming perfusate.

The chest was widely retracted following a midline sternotomy, and a polyethylene catheter (14-
gage, Braun, Melsungen, Germany) was placed into the main pulmonary artery via the right ventricular outflow track, advanced until it was immediately proximal to the bifurcation, and next connected to medical grade silicone tubing (1.47 mm ID, Ulrich, St. Gallen, Switzerland). The animals were then completely exsanguinated by widely opening the left ventricle and the left atrium. To minimize the warm ischemic time period until reperfusion, the lungs were immediately flushed via the pulmonary artery cannula with 30 ml of cold (10 °C) hydroxyethyl-starch 6% solution from a height of 30 cm. Through the left ventriculotomy, another catheter was placed into the left ventricle, into which a Combifix Adapter (Braun, Melsungen, Germany) was tightly fixed and connected to medical grade silicone tubing. Finally, a third catheter (polyethylene tubing, ID 0.88 mm, Portex, Hythe, GB) was introduced directly into the left atrium for measurement of the left atrial pressure (Pla). The lungs and the heart were excised in a single block, dissected free of adjacent tissue and weighed. The heart-lung block was suspended from an isometric force displacement transducer (Grass FT03, Quincy, MA, USA) in a thermostabilized, humidified Plexiglas chamber. The lungs were ventilated with air mixed with 5% CO₂, and a respiratory rate of 50/min, a tidal volume of 7 ml/kg and a PEEP of 2.5 cmH₂O were maintained. A series of hyperinflations (peak pressure of 25-30 cmH₂O) were applied by occluding the expiratory port of the ventilator until the atelectatic areas were completely abolished.

Study groups

The lungs were randomly assigned to one or other of the following three protocol groups, each containing 7 lungs. In the first group, the lungs remained unperfused throughout the experimental protocol. In the other two groups, pulmonary perfusion was performed with either autologous blood or albumin perfusate (8%, Baxter AG, Vienna, Austria). The lungs were perfused from a
reservoir positioned so as to maintain a constant pulmonary artery perfusion pressure (Ppa) of 15 mmHg. The distal end of the left ventricle outflow cannula was placed at a sufficient height to furnish a constant Pla of 7.5±2 mmHg, which produces West zone 3 conditions (Ppa > Pla > alveolar pressure). The perfusate dripping from this cannula was collected in a 5-ml cylinder, and was aspirated from this reservoir via polyethylene tubing passing through a roller pump (Ismatec Pump, Glattburg, Zürich, Switzerland). A transit-time flowmeter (T-201 CDS, Transonic Systems Inc., Ithaca, NY, USA) was placed between the perfusion reservoir and the catheter cannulating the main pulmonary artery, for continuous monitoring of the pulmonary blood flow (Qp). After the start of the perfusion, a period of 20-30 min was allowed for the establishment of steady-state respiratory and hemodynamic conditions and for the preparation to become isogravimetric. If, as a consequence of technical problems in the surgical preparation, isogravimetric conditions were not reached within 30 min from the start of perfusion, or if Qp was lower than 5 ml/min at normal Ppa and Pla values, the lungs were excluded (this occurred in <10% of the experiments). The oncotic and osmotic pressures for the blood and albumine perfusates were similar, as evidenced by the finding that the lungs remained isogravimetric throughout the study. In the perfused lungs, the mean Ppa and Pla were recorded continuously with calibrated pressure transducers (Honeywell, model 156-PC 06-GW2, Zürich, Switzerland). Signals of lung weight, Ppa, Pla and Qp were sampled at a rate of 50 Hz by an analog-to-digital converter and were displayed by the data acquisition software (Biopac, Santa Barbara, CA, USA). Pulmonary vascular resistance (Rv) was calculated as \( Rv = \frac{Ppa - Pla}{Qp} \).

Measurement of airway and parenchymal mechanics

The forced oscillatory impedance of the isolated lungs (ZL) was measured to characterize the airway and tissue mechanics, as described in detail previously (29, 30). Briefly, the tracheal
cannula was connected from the respirator to a loudspeaker-in-box system at end-expiration. The loudspeaker generated a small-amplitude pseudorandom signal with frequency components between 0.5 and 21 Hz through a polyethylene wave-tube. Two identical pressure transducers (model 33NA002D, ICSensors, Milpitas, CA, USA) were used for measurement of the lateral pressures at the loudspeaker and at the tracheal end of the wave-tube. ZL was calculated as the load impedance of the wave-tube (38). In order to separate the airway and tissue parameters, a model was fitted to the ZL spectra by minimizing the relative differences between the measured and modeled impedance values (12). The model contained a frequency-independent airway resistance (Raw) and inertance (Iaw) in series with a constant-phase tissue compartment characterized by the coefficients of parenchymal damping (or tissue viscance; G) and elastance (H) (12). Parenchymal hysteresivity was calculated as \( \eta = G/H \) (8). The impedance of the tracheal cannula and the connecting tubing was also determined, and the Raw and Iaw values were corrected by subtracting the instrumental resistance and inertance values from them.

**Study protocol**

Ten-fifteen min after the onset of ventilation, a sigh was given to standardize the volume history. The PEEP level was then decreased to 2 cmH\(_2\)O, and 4-to-6 ZL recordings were collected in all lungs at end-expiration while a mean transpulmonary pressure (Pt\(_{\text{p,mean}}\)) of 2 cmH\(_2\)O was maintained. After the start of perfusion in the lungs receiving either blood or 8% albumin, a period of about 3-5 min was allowed for stabilization of the physiological pulmonary hemodynamics. A sigh was next given, and another set of ZL measurements was started 1 min later at the same Pt\(_{\text{p,mean}}\) level. These measurements allowed an estimation of the effects of the onset of perfusion in the normal range of breathing. To investigate how the perfusion affects the pressure dependence of the pulmonary mechanics, ZL was measured successively at Pt\(_{\text{p,mean}}\)
levels of 1, 2, 4, 6, 8, 6, 4, 2 and 1 cmH₂O. These pressure levels were set by adjusting the PEEP during ventilation to the corresponding Ptp\textsubscript{mean} level, and ZL was recorded at end-expiration. The pressure in the loudspeaker chambers was also adjusted to the pressure level at which the oscillatory measurements were made. A 2-min period was kept between the changes in PEEP and the oscillatory measurements.

*Measurement of end-expiratory lung volume*

In 6 additional isolated lung preparations, the changes in the end-expiratory lung volume (EELV) between the unperfused and perfused conditions were studied. The unperfused lungs were ventilated until a steady-state condition had been established; the tracheal tube and the perfusion cannulas were then clamped at a Ptp\textsubscript{mean} level of 2 cmH₂O, and the preparation was immersed in a glass cylinder containing 37° C saline, in order to read its total volume. The heart-lung preparation was returned to the Plexiglas box, steady-state perfusion with blood was established, and the immersion procedure was repeated. The values of EELV were calculated by subtracting the volumes of the connecting tubing and of the heart-lung tissue from the total saline displacement. The EELV of the perfused lungs was corrected for the elevations in vascular volume as follows. The weight of the heart-lung blocks was measured immediately following the immersions, and the increase in vascular volume due to the filling of the pulmonary capillaries with blood was calculated by dividing the increases in weight by the density of the blood.

*Histological preparations*

To assess the potential structural changes responsible for the altered lung mechanics, histological studies were performed on 8 additional isolated lungs, which were prepared in the same manner as described above. Four of them remained unperfused; the others were perfused with blood
while physiological perfusion pressures were maintained (Ppa = 17.5 mmHg, PLA = 7.5 mmHg).

In each group, 2 lungs were ventilated during maintenance of a PEEP of 2 cmH$_2$O, while the PEEP of 8 cmH$_2$O was kept in the other 2 lungs. The lungs were then immersed in buffered 4% formaldehyde solution for 12 h while a Ptp$_{mean}$ corresponding to the level of PEEP was maintained. The perfused lung was kept under normal hemodynamic conditions for 1 h after immersion in order to ensure adequate lung fixation while the pulmonary capillary network was filled with blood. Lung specimens (one from each side) were embedded in paraffin. Two 5-µm sections were prepared in each lung specimen and were stained with the Miller elastic van Gieson stain to label elastin fiber in the alveolar walls. The orientation of the elastin fibers was characterized by using an image analysis system (Q550-iw Quantimet, Leica) connected to a DMRBE microscope (Leica) via a video camera (Sony DXC 930p triccd). Image acquisition was performed by using a 63x dry objective (NPL-Fluothar 63/0.9) and a 10x lens. Four-to-six gray images were randomly selected from each histological preparation and the acute angles (<90°) of all elastin fibers relative to the local orientation of the basal membrane were recorded by using the interactive measurements facilities of the QWin software. A total of 5-15 angles were obtained on each image, resulting in 250-350 angle readings in each condition (PEEP 2 and 8 cmH$_2$O in unperfused and blood-perfused lungs). We occasionally noticed folds and buckles in the basement membrane, particularly in the unperfused lungs. Since these folds were generally smaller than the length of the elastin fibers, we were able to estimate the angle between the basement membrane and the elastin fibers even in this condition.

**Statistical analysis**

Scatters in the parameters are expressed in SE values. The paired t-test was utilized to estimate the effects of the onset of perfusion on the mechanical parameters. Two-way repeated measures
analysis of variance (ANOVA) was used with the perfusate as the first variable and the \( P_{\text{tp mean}} \) level as the second variable in order to establish the effects of perfusion on the pulmonary mechanical parameters at different values of \( P_{\text{tp mean}} \). One-way ANOVA was used to compare the protocol groups involved in the histological studies as concerns the angle of the elastin fibers. The Student-Newman-Keuls multiple comparison procedure was employed to compare the lung mechanical parameters under different conditions. In each test, a significance level of \( p<0.05 \) was applied.

RESULTS

The animals involved in the three protocol groups were comparable with regard to body weight and baseline lung mechanical parameters (Table 1).

Lung mechanical measurements

Figure 1 shows the airway and tissue parameters before and after perfusion with blood or albumin. The changes in \( R_{aw} \) and \( I_{aw} \) that occurred after the onset of perfusion with blood (7.5±9.1% and 6.6±4.3%, respectively) or 8% albumin (6.1±4.9% and 10.1±7.4 %, respectively) were not significant. However, perfusion with blood and with 8% albumin each induced significant decreases in \( G \) (-31.8±5.0% and -29.2±2.1%) and \( H \) (-31.5±3.7% and -18.7±2.7%). A mild, but statistically significant decrease in \( \eta \) was observed in the lungs perfused with 8% albumin (-12.7±3.0%), whereas \( \eta \) fell only slightly on blood perfusion (-0.8±3.8%).

Figure 2 illustrates the \( R_{aw} \) values in the unperfused and the perfused lungs at different \( P_{\text{tp mean}} \) levels. In the unperfused lungs, an increase of \( P_{\text{tp mean}} \) from 1 to 8 cmH\(_2\)O induced significant
stepwise decreases in Raw, whereas the return of Ptp\textsubscript{mean} to 1 cmH\textsubscript{2}O resulted in similar increases in Raw. The dependence of Raw on Ptp\textsubscript{mean} was comparable in the perfused lungs, and there was no significant difference between the values of Raw in the 3 groups at any Ptp\textsubscript{mean} level. The inertance values were not affected by either Ptp\textsubscript{mean} or the perfusion (data not shown).

The parenchymal resistive and elastic parameters are plotted against Ptp\textsubscript{mean} in Figs 3 and 4, respectively. In the unperfused lungs, the increase of Ptp\textsubscript{mean} induced gradual decreases in G and H until Ptp\textsubscript{mean} reached 4 cmH\textsubscript{2}O, which was followed by marked and statistically significant increases in both parameters between 6 and 8 cmH\textsubscript{2}O. The decrease of Ptp\textsubscript{mean} from 8 to 2 cmH\textsubscript{2}O resulted in profound drops in G and H, the values at 4 and 2 cmH\textsubscript{2}O being significantly lower than those obtained at the corresponding pressure levels during the increasing phase of Ptp\textsubscript{mean}. A further decrease of Ptp\textsubscript{mean} from 2 to 1 cmH\textsubscript{2}O caused substantial increases in G and H, which approached their corresponding initial values. In the lungs perfused with either blood or 8% albumin, the changes in G and H were much less in the Ptp\textsubscript{mean} range between 1 and 4 cmH\textsubscript{2}O. The differences between the G and H values in the unperfused and the perfused lungs were statistically significant at the Ptp\textsubscript{mean} levels of 1, 2 and 4 cmH\textsubscript{2}O, but no differences were observed at all at higher pressures. The nature of the fluid perfused had no effect on G and H at any stage of the experiment. In all groups, the parallel changes in G and H resulted in fairly constant $\eta$ values when Ptp\textsubscript{mean} was lower than 6 cmH\textsubscript{2}O. Slight, but statistically significant decreases in $\eta$ were observed at higher Ptp\textsubscript{mean} levels in both the unperfused lungs (-26.7±3.1% between the Ptp\textsubscript{mean} levels of 2 and 8 cmH\textsubscript{2}O) and those perfused with either blood (-24.0±1.9%) or 8% albumin (-11.2±4.1%). The $\eta$ values in the protocol groups were not different at any level of Ptp\textsubscript{mean}. 
Changes in lung volume

Perfusion with blood led to an increase in EELV from 3.5±0.6 ml to 5.0±0.2 ml (p<0.05) at the Ptp\textsubscript{mean} of 2 cmH\textsubscript{2}O. The changes in EELV (1.53±0.50 ml) correlated well with decreases in G (r\textsuperscript{2} = 0.76) and H (r\textsuperscript{2} = 0.64). Furthermore, the relative increase in FRC following the onset of perfusion (31±11%, p = 0.01) was similar in magnitude to the decreases in G (32±6%) and H (29±4%).

Lung histology

Figure 5 illustrates the lung histology in an unperfused isolated lung (A) and in a lung perfused with autologous blood (B). In the unperfused lung, the shape of the alveolar wall is distorted and convoluted, whereas in the perfused lung the alveolar structure appears smooth and regular. There is a near radial arrangement of the elastin fibers (arrows) across the alveolar wall in the unperfused lung, which is in contrast with the linear distribution of the fibers around the alveolar septa including perfused capillaries. Figure 6 summarizes the angles of the elastin fibers in the unperfused and perfused lungs fixed at normal and high Ptp\textsubscript{mean} levels. The angle of the elastin fibers relative to the local orientation of the basal membrane was significantly greater in the unperfused lungs when a Ptp\textsubscript{mean} of 2 cmH\textsubscript{2}O was maintained, suggesting a radial distribution of the fibers in the alveolar septa. The significantly smaller angles in the other three groups of lungs demonstrated that the elastin fibers were distributed more tangentially to the basal membrane around the alveolar septa.

Perfusion parameters

Figure 8 depicts the changes in weight, Rv and Qp with altered Ptp\textsubscript{mean} in the perfused lungs. The
stability of lung weight throughout the study protocol indicates the absence of edema in our experiments. Rv was generally lower and Qp was systematically higher in the lungs perfused with albumin. Increases of Ptp\textsubscript{mean} did not have statistically significant effects on the weight, Rv or Qp in the lungs perfused with either blood or albumin.

DISCUSSION

The present study was designed to investigate the combined effects of pulmonary perfusion and inflation pressure on the mechanical properties of the pulmonary system in isolated rat lungs. We demonstrated that 1) the re-establishment of pulmonary perfusion significantly decreased the viscous resistance and the elastance of the parenchyma, whereas it had negligible effects on airway mechanics, 2) the decreases in the parenchymal mechanical parameters were associated with increases in lung volume, 3) the filling of the pulmonary vasculature markedly affected the pressure dependence of the pulmonary mechanical properties, 4) the effects of perfusion did not depend on the hemoglobin content of the perfusate, and 5) the orientation of the elastin fibers in the alveolar septa was more radial in the unperfused lungs at a Ptp\textsubscript{mean} level of 2 cmH\textsubscript{2}O than for those with an intact pulmonary microcirculation, and a higher Ptp\textsubscript{mean} led to a significant reorganization of the elastin fiber network with near circumferential orientation.

Methodological considerations

The isolated rat lung model offers ideal conditions for comparison of the mechanical behavior of unperfused lungs with that of lungs perfused under the physiologic hemodynamic conditions encountered in vivo. In this experimental setting, the interactions between pulmonary perfusion
and lung mechanics can be determined in the absence of confounding systemic hormonal and neurogenic influences (39). Furthermore, the in vitro model applied in the present investigation allows control of the intraalveolar CO₂ level through maintenance of a constant inspired and expired fraction of CO₂. Thus, the potential biasing effects of hypocapnia on the lung mechanics (28, 34) were also avoided.

To decide whether the physiological transport of metabolites maintained by the blood plays a part in the distinct difference in parenchymal mechanics between unperfused and perfused lungs, we further applied 8% albumin as a perfusate. This solution does not contain hemoglobin, which plays an important role in the transport abilities of the perfusate and additionally modulates the pulmonary vascular tone (32). Since identical mechanical behavior of the lungs was observed with these two perfusates, it can be suggested that the mechanical effects of the perfusates on the alveoli played the major role in our findings.

Another feature of the blood and albumin perfusions is that they resulted in significantly different Qp and Rv levels while the same arterial-venous pressure gradient was maintained in the lungs. Although the lower Rv and the higher Qp for albumin can be attributed to its lower viscosity, the differences in Rv and Qp (~1.5 times) are smaller than the difference in the viscosities of blood with 35% hematocrit and plasma in the pulmonary circulation (~1.9 times) (1). This discrepancy could be explained by the lack of hemoglobin in the albumin perfusate, which may have led to a moderate vasoconstriction by inhibiting the release of NO from the pulmonary epithelium due to a decreased shear stress (32). Nevertheless, neither the different Qp nor the possible vascular contraction during albumin perfusion was reflected in the lung mechanical parameters, which indicates the primary importance of the intravascular pressure in the pulmonary circulation in our findings.
The pulmonary mechanical parameters obtained in the present study are in good agreement with earlier data on isolated perfused rat lungs (30). Although the values of EELV in the perfused lungs are ~30% higher than those estimated \textit{in vivo} by the nitrogen closed-circuit method (16) and body plethysmography (18), this can be explained by the different lung configuration and transpulmonary pressure prevailing in the closed-chest supine animals.

\textit{Effects of perfusion on lung mechanics}

We found that the onset of blood or 8% albumin perfusion caused an immediate and significant improvement in the lung parenchymal function, characterized by simultaneous decreases in the magnitudes of the tissue elastic (H) and viscous (G) properties (Fig. 1). Interestingly, the airway properties were not affected by perfusion. Two distinct mechanisms can produce decreases in G and H. The improvement can be attributed either to changes in the intrinsic mechanical properties of the parenchyma (40), or to an increase in the lung volume available for gas exchange, as a result of the reopening of airspaces (3).

The intrinsic viscoelastic properties of the parenchyma are determined by a number of important structural components, such as the connective tissue network, the interstitial cells and the surface lining layer (41). Indirect effects of the altered airway properties on the parenchymal mechanics (24) can be excluded, as no difference in airway properties was observed between the protocol groups. Changes in the surface-active forces were also unlikely to contribute to our findings for the following reasons. Surface film properties contribute significantly to the dynamic lung stiffness only when the lung volume is cycled through large excursions (31), which was not the case in our measurements. Furthermore, since the half-life for the clearance of surfactant is far longer than the length of the experiment (15) and the absence of pulmonary perfusion leads to an
increase in surface tension (6), there is no reason to assume a diminished surfactant function in the unperfused lungs. It is also possible that the higher lung inflation in the perfused lungs stimulates epithelial type II cells to produce surface active material (13); however, this is not likely to play a role, since an excess of surfactant compared to normal physiological levels makes little difference in lung mechanics. The proportional changes in G and H, indicating the lack of ventilation heterogeneities (2, 26) also confirm the absence of a surfactant dysfunction, which would have led to heterogeneous closures of peripheral airways at low lung inflation pressures (2, 26). It is also unlikely that the interstitial cells played a major role in the changes following the onset of perfusion, since perfusion with either 8% albumin or blood resulted in a similar lung mechanical response, whilst only blood is expected to provide full physiological nutritive support for these cells. It is therefore plausible to suggest that configurational changes in the connective tissue network were primarily responsible for the prompt improvement in the intrinsic parenchymal properties.

Collagen and elastin fibers are the principal constituents of the connective tissue network, and the interactions between these components contribute significantly to the viscoelastic properties of the parenchyma (23, 41). It has been demonstrated that the elastin and collagen fibers are connected in parallel mechanically (5) and their distributions, orientation and interaction dominate in determining the mechanical behavior of the lung parenchyma (41). Our results can be interpreted in the context of these findings as follows. The histological findings in Fig. 5 suggest that, in the absence of capillary pressurization, the contours of the alveoli are convoluted and there is a wide distribution of elastin fiber orientation, with some fibers running perpendicular to the direction of the basal membranes (Fig. 6). Following the onset of capillary
pressurization, however, the capillaries become turgid, which in turn eliminates the convoluted nature of the alveolar wall, and results in a significant reorganization of the elastin fibers (Figs 5 and 6). Re-establishment of the physiological pressures in the pulmonary capillaries therefore helps the alveoli to regain their optimal geometry.

To estimate how such reorganization of the elastic fibers affects the alveolar wall mechanics, we derived equations for the Young’s modulus of a fibrous material as a function of the uniaxial stretch ratio, the elasticity of the fibers, the Young’s modulus of the matrix and the distribution of the angles of the fibers with respect to the direction of the deformation (see Eqs 9 and 10 in Appendix A). Assuming that the contribution of the matrix is small, on use of the measured distributions of angles in Fig. 6 it is found that the Young’s modulus of the nonperfused tissue decreases from 75% of the Young’s modulus of the tissue with filled capillaries to 65% when the stretch ratio increases from 5% to 50%. This can readily be understood: when an alveolus is inflated in the perfused lung, the elastin fibers running parallel to the wall experience stretching, and hence contribute to the elastic resistance of the alveolus, to a degree depending on the extent to which the elastin contributes to the stiffness. In contrast, when the unperfused alveolus is stretched, the fibers at a large angle with respect to the wall do not contribute significantly to the elastic resistance of the alveolus.

The above calculations demonstrated that the Young’s modulus of the alveolar wall is larger in the lung with pressurized capillaries. The elastance of the alveolus also depends on the prestress or tension in the wall tissue and the surface tension at the air-liquid interface. The prestress was controlled and was the same in the two conditions. Based on the discussion above, surfactant is not likely to contribute here and hence the elastance of the perfused lung should be larger than that of the unperfused lung. However, our data in Fig. 1 suggest just the opposite behavior at the
organ level, since both G and H dropped significantly on fill-up of the pulmonary capillaries. To resolve this apparent contradiction, we hypothesize that the decreased elasticity of the alveoli in the unperfused lung causes the alveolar ducts to lose their elasticity, and at transpulmonary pressures lower than 4 or 6 cmH₂O, they are prone to closure. If a significant number of alveoli are collapsed, the lung elastance as measured by H increases. When perfusion starts at physiological vascular pressures, the convoluted walls of the alveoli become smooth, their elasticity increases and they reopen, allowing the communication of many more alveoli with the trachea. The reopening of alveolar units, manifested in increases in EELV, results in a significant decrease in the total lung elastance or H. The presence of alveolar derecruitment and recruitment in the unperfused lungs with changes in Ptp mean can also be substantiated from the hysteresis observed in G and H (see top left panels in Figs 3 and 4). The decreases in G and H on increase of Ptp mean from 1 to 4 cmH₂O are most likely due to alveolar recruitment. A Ptp mean of 6 cmH₂O is sufficient to recruit the entire unperfused lung, while decrease of Ptp mean to below 4 cmH₂O leads to alveolar decrecruitment, indicated by the steep increases in G and H. In Appendix B, we derive equations for Raw and lung elastance as a function of the number of alveoli communicating with the trachea when only the last-generation airways are closed in a tree structure. The simulation results illustrated in Fig. 7 indicate that the sensitivity of lung elastance to closure of the last-generation airways is much larger than that of Raw, which provides an explanation for the finding that G and H decrease, whereas Raw remains practically constant following the pulmonary capillary filling. Both the increases in EELV and the return of the alveolar architecture to normal physiological structure, as revealed by the histology, suggest that the pressurized pulmonary capillaries contribute to maintenance of the normal physiological alveolar geometry.
Effects of perfusion on pulmonary mechanics at different transpulmonary pressures

The concept that vascular engorgement leads to an increased lung volume and lung stiffening at low lung volumes was first proposed by von Basch in 1889 (4). This qualitative observation was confirmed by Hogg et al., who demonstrated a tendency to a positive correlation between blood flow and lung expansion (14). Subsequent studies involving lung function measurements have provided further evidence that pulmonary vascular congestion leads to an increase in lung size and/or stiffness at low lung volume by overstretching the capillary network in the alveolar wall (10, 30, 36, 37). It seems plausible to adapt this mechanism to explain the marked differences in the parenchymal parameters between the perfused and unperfused lungs at low lung volumes, on the basis of the stabilizing role of the filled pulmonary capillaries in the maintenance of the physiological alveolar architecture. The contribution of the pressurized capillary network is essential at lung volumes around or below FRC (which corresponds to $P_{\text{tp\_mean}}$ 2 cmH$_2$O in the rat), where the distending pressure alone is apparently unable to prevent the collapse of alveoli and the subsequent increase in tissue impedance, as demonstrated in Figs 3 and 4. During the decrease of $P_{\text{tp\_mean}}$, a critical pressure level is reached at ~2 cmH$_2$O, where G and H in the unperfused lungs suddenly start to increase. At higher lung inflation pressures ($P_{\text{tp\_mean}} > 6$ cmH$_2$O), this mechanism loses its importance, and the tissue impedance seems to be determined solely by the transpulmonary pressure.

Physiological implications

The mechanism of the circulatory-respiratory mechanical interaction discussed above may be of relevance in decreasing the ventilation/perfusion mismatch in the lungs. Assuming that our findings in whole lungs are also valid for the regional lung mechanics in vivo, lung areas with
decreased or diminished pulmonary capillary pressures would impose a higher parenchymal
impedance against the distension of the airspaces, which in turn would contribute to a redirection
of the airflow to the more compliant parts of the lungs, i.e. to perfused alveoli with physiological
capillary pressures. This adaptation mechanism may be of importance in situations where
impairment in pulmonary capillary perfusion may occur (e.g. in hypovolemia or embolism). This
phenomenon may also be involved in the airflow redistribution caused by hypocapnia-induced
airway narrowing (28, 34) that occurs during hypoperfusion.
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FIGURE LEGENDS

Figure 1. Airway and parenchymal parameters before and after perfusion with either blood or 8% albumin. Raw: airway resistance, law: airway inertance, G: parenchymal damping, H: parenchymal elastance, η: parenchymal hysteresivity. *: significant change between unperfused and perfused conditions.

Figure 2. Changes in airway resistance (Raw) with altered mean transpulmonary pressure (Ptpmean) in unperfused lungs and in lungs perfused with blood or 8% albumin. Arrows indicate the sequence of change in Ptpmean. *: significant intragroup changes compared to the initial value obtained at Ptpmean = 2 cmH2O.

Figure 3. Changes in parenchymal damping (G) with altered mean transpulmonary pressure (Ptpmean) in unperfused lungs and in lungs perfused with blood or 8% albumin. Arrows indicate the sequence of change in Ptpmean. *: significant intragroup changes compared to the initial value obtained at Ptpmean = 2 cmH2O. #: significant intergroup differences from the corresponding values obtained during blood perfusion.

Figure 4. Changes in parenchymal elastance (H) with altered mean transpulmonary pressure (Ptpmean) in unperfused lungs and in lungs perfused with blood or 8% albumin. Arrows indicate the sequence of change in Ptpmean. *: significant intragroup changes compared to the initial value obtained at Ptpmean = 2 cmH2O. #: significant intergroup differences from the corresponding values obtained during blood perfusion.

Figure 5. Lung histology of unperfused (A) and blood-perfused lungs (B). Arrows show elastin fibers. Original magnification x 400.
**Figure 6.** Distribution of elastin fibers (median, 10%, 25%, 75% and 90% percentiles) in the alveolar septa in unperfused lungs and in lungs perfused with blood. Lungs were fixed at either 2 or 8 cmH$_2$O mean transpulmonary pressure levels. *: significant difference from the unperfused lungs at P$_{tpmean}$ of 2 cmH$_2$O.

**Figure 7.** Relative increases in airway resistance (Raw) and lung elastance (EL) as functions of open terminal units in the lungs. Note the logarithmic scale on the y axis. See appendix B for further details.

**Figure 8.** Changes in lung weight, pulmonary vascular resistance (Rv), and pulmonary blood flow (Qp) with altered mean transpulmonary pressure (P$_{tpmean}$).
Table 1. Airway resistance (Raw) and inertance (Iaw), tissue damping (G), elastance (H), hysteresivity (η), and body weight (BW) under baseline conditions before onset of perfusion.

<table>
<thead>
<tr>
<th></th>
<th>Raw (cmH\textsubscript{2}O.s/l)</th>
<th>Iaw (cmH\textsubscript{2}O.s\textsuperscript{2}/l)</th>
<th>G (cmH\textsubscript{2}O/l)</th>
<th>H (cmH\textsubscript{2}O/l)</th>
<th>η</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unperfused</strong></td>
<td>11.3±0.87</td>
<td>0.055±0.0052</td>
<td>215±13</td>
<td>1594±87</td>
<td>0.135±0.0061</td>
<td>331±19</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>12.3±0.68</td>
<td>0.057±0.0033</td>
<td>272±30</td>
<td>1855±164</td>
<td>0.145±0.0044</td>
<td>347±12</td>
</tr>
<tr>
<td>perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>10.8±0.53</td>
<td>0.065±0.0038</td>
<td>226±15</td>
<td>1534±108</td>
<td>0.148±0.0024</td>
<td>373±16</td>
</tr>
<tr>
<td>perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.33</td>
<td>0.23</td>
<td>0.15</td>
<td>0.18</td>
<td>0.14</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Appendix A: Uniaxial stress-strain relation of fibrous material

A general theory of the stress-strain relation of soft tissues with wavy fibers embedded in a fluid matrix was derived by Lanir (19) using micromechanical considerations and tensor calculus. Instead of considering a special case of this general theory, here we derive a stress-strain relation for fibrous material embedded in an elastic matrix. The calculations are based on elementary considerations which lead to a formulation suitable for easy calculation of the contribution of the fibers. We first consider a two-dimensional case, which is then generalized to three dimensions.

Figure 8. Deformation of a two-dimensional body and a fiber inside the body during uniaxial stretching along the x axis.

Let us assume that we have a two dimensional body of incompressible elastic material with length L and width b before deformation. The body contains linearly elastic fibers with arbitrary orientation (Θ), length (l) and spring constant (k). We attach a rectangular coordinate system to the body as shown in see Fig. 8. Without loss of generality, we assume that a fiber originates
from the center of the coordinate system and makes an angle $\Theta$ with the x axis. The length of the fiber is $l$ and its end point is $P$. When the body is stretched in the x direction its length becomes $L'$. Due to incompressibility, the volume $V=lb$ does not change after deformation ($lb=L'b'$) and hence the new width is $b'=bL/L'$. The point $P$ with coordinates $(x,y)$ will move to $P'$ with coordinates $(x',y')$ given by the following transformations:

$$x' = \frac{L'}{L} x = \frac{L'}{L} l \cos \Theta$$  \hspace{1cm} (1)

and

$$y' = \frac{L}{L'} y = \frac{L}{L'} l \sin \Theta.$$  \hspace{1cm} (2)

The new length $l'$ of the fiber is given by

$$l' = (x'^2 + y'^2)^{1/2} = \frac{L'}{L} lQ$$  \hspace{1cm} (3)

where

$$Q = \left(\cos^2 \Theta + \frac{L^4}{L'^4} \sin^2 \Theta\right)^{1/2}.$$  \hspace{1cm} (4)

The force $F$ on the fiber is the displacement $u=l'-l$ times the spring constant $k$:

$$F = k(l'-l) = kl\left(\frac{L'}{L} - 1\right)$$  \hspace{1cm} (5)
To calculate the true stress $\sigma_f$ in the direction of the strain, we first calculate the x component of the force on the fiber and normalize it with the cross sectional area $b''=bL/L'$ of the body after deformation. Since the orientation of the fiber following deformation is $\Theta'$, the stress contributed by a fiber with original length $l$ and angle $\Theta$ is

$$\sigma_f(l, \Theta) = \frac{kl'L'}{bL'} (Q \frac{L'}{L} - 1) \cos \Theta'.$$

(6)

Eq. 6 still contains the unknown $\cos \Theta'$ which, from Fig. 1, can be written as $x'/l'$. Using Eqs. 1 and 3, the $\cos \Theta'$ can be written as $\cos \Theta/Q$ and Eq. 6 becomes

$$\sigma_f(l, \Theta) = \frac{kl'L'}{bL} (Q \frac{L'}{L} - 1) \cos \Theta.$$

(7)

It is interesting to consider the special cases when the fiber is parallel ($\Theta=0$) or perpendicular ($\Theta=\pi/2$) to the direction of the macroscopic strain. In the former case, $Q=1$ and Eq. 7 reduces to Eq. 5 normalized by the cross sectional area whereas in the latter case, the stress contributed by the fibers is zero.

Let us now consider the case when there are many fibers with a distribution of their orientation and length. Let the number of fibers with length between $l$ and $l+dl$ and angle between $\Theta$ and $\Theta+d\Theta$ per unit volume be $n(l, \Theta)dl d\Theta$ in the undeformed body. The total stress due to all fibers and the matrix can be expressed as

$$\sigma = \frac{klL'}{bL} \int_0^{\pi/2} \int_0^l \frac{1}{Q (Q \frac{L'}{L} - 1) \cos \Theta} n(l, \Theta) dl d\Theta + Y_m (\frac{L'}{L} - 1)$$

(8)
where $Y_m$ is the Young’s modulus of the matrix. For small deformations, $Q$ is nearly unity, and
the term $(QL'/L - 1)$ is essentially the uniaxial strain $\varepsilon = (L'/L - 1)$ which can be taken out of
the integral. Eq. 8 is then written in the form of a Hook law or linear stress-strain relation with an
equivalent Young’s modulus that depends on the elasticity of the matrix and the fibers as well as
the length and orientation distribution of the fibers:

$$Y = \frac{kL^4}{bL} \int_0^{x^2} \int_0^l \frac{l}{Q} \cos\Theta n(l, \Theta) dld\Theta + Y_m$$  

(9)

The above equations are readily extended to the case of a three-dimensional body. If we
denote the angle between the z axis and the fiber with $\Lambda$, then assuming that the dimension of the
body in the z direction is also $b$, Eq. 9 becomes

$$Y = \frac{kL^4}{b^3L^2} \int_0^{x^2} \int_0^{2\pi} \int_0^l \frac{l}{R} \cos\Theta \sin\Lambda n(l, \Theta, \Lambda) dld\Theta d\Lambda + Y_m$$  

(10)

where

$$R = \{\cos^2\Theta \sin^2\Lambda + \frac{L^3}{L'} \sin^2\Theta \sin^2\Lambda + \frac{L^3}{L'} \cos^2\Lambda \}^{1/2}$$

It is important to note that due to the gradual folding of the fibers into the direction of the
macroscopic strain, the equivalent Young’s modulus is no longer constant, but increases with
increasing stretch ratio $L'/L$. Finally, Eqs. 9 and 10 are now suitable to evaluate the relative
contribution of fiber orientation to the Young’s modulus of the alveolar wall.
Appendix B: Airway resistance and lung elastance as a function of terminal airway closure

We model the lung as a symmetric binary tree with elastic alveoli attached to the terminal airways. The diameter and length of the trachea are denoted by \( D_0 \) and \( L_0 \), respectively. We introduce a scaling relation as follows. The diameter and length of the airways at generation \( k+1 \) (\( D_{k+1} \) and \( L_{k+1} \), respectively) are scaled versions of the diameters and length of the parents \( D_k \) and \( L_k \):

\[
D_{k+1} = d D_k \quad \text{and} \quad L_{k+1} = l L_k
\]

where \( d < 1 \) and \( l < 1 \). Since we assume that \( d \) and \( l \) are constant scaling factors, airway dimensions can also be written as

\[
D_k = d^k D_0 \quad \text{and} \quad L_k = l^k L_0 .
\]

The resistance of airway segments are modeled by the Poiseuille flow resistance. Taking into account Eq.2, the resistance at generation \( k \) is given by

\[
R_k = C \frac{L_k}{D_k^4} = \frac{l^k}{d^{4k}} C \frac{L_0}{D_0^4} .
\]

where \( C \) is the product of a numerical factor and air viscosity. Introducing the scaling factor \( \beta = l/d^4 \) and realizing the \( CL_0/D_0^4 \) is the resistance \( R_0 \) of the trachea, we obtain

\[
R_k = \beta^k R_0 .
\]
We can now calculate the total resistance of the tree starting from the left bottom corner. If the maximum generation number is $N$, the parallel combination of two terminal segments is $R_{N/2}$ which is in series with a resistance of $R_{N-1}$ so that the resistance becomes $R_{N/2} + R_{N-1}$. We have two segments with resistance $R_{N-1}$ in parallel connected to $R_{N-2}$ in series. We can continue with this process and roll up the entire tree. Thus, the total resistance $R_{aw}$ is

$$R_{aw} = ((R_{N/2} / 2 + R_{N-1}) / 2 + R_{N-2}) / 2 + \cdots) / 2 + R_0. \quad (5)$$

Multiplying with the $\frac{1}{2}$ factors and rearranging Eq. 5 leads to

$$R_{aw} = R_0 + \frac{1}{2^1} R_1 + \frac{1}{2^2} R_2 + \cdots + \frac{1}{2^N} R_N. \quad (6)$$

By introducing $\delta = \beta/2$ and substituting Eq. 4 into Eq. 6, we obtain

$$R_{aw} = R_0(1 + \delta + \delta^2 + \cdots + \delta^N). \quad (7)$$

Eq. 7 is a geometric series which has the following closed form solution

$$R_{aw} = R_0 \frac{1 - \delta^{N+1}}{1 - \delta}. \quad (8)$$

Let us partition Eq. 7 into two terms. The first contains the geometric series up to $N-1$ and the second term is the $N^{th}$ term from the series. Adding up the series to $N-1$ and substituting $\delta = \beta/2$ into the $N^{th}$ term we obtain the following expression

$$R_{aw} = R_0 \frac{1 - \delta^N}{1 - \delta} + \frac{\beta R_0}{2^N} \quad (9)$$
For a given tree with fixed \( l, d \) and \( N \), the first term in Eq. 9 is constant. If we start closing the terminal airways one by one, then the denominator \( (2^N) \) in the second term becomes less and less while the nominator and the first term do not change. If \( p \) denotes the percent of the terminal airways at generation \( N \) that are open, then while \( p \) decreases from 1 to 0, the number of open terminal airways decreases from \( 2^N \) to 0. In the limit when \( p=0 \), Raw is infinite. For computational purposes, Eq. 9 can simply be written

\[
Raw = C_1 + \frac{C_2}{p}.
\]

(10)

With regard to the elastance of the model, the alveoli are modeled as elastic elements in parallel. Thus, the total elastance \( E \) of the model as a function of \( p \) is given by

\[
E = \frac{E_0}{p}
\]

(11)

where \( E_0 \) is the elastance of a single alveolus. In the numerical simulations, we chose \( N=16 \). The values of \( l=0.8 \) and \( d=0.8 \) were obtained based on the work of Kitaoka and Suki (17) which result in \( \delta=0.976 \).
Figure 1.
Figure 2.
Figure 3.

**G (cmH₂O/ml)**

- **unperfused**
- **perfusion with blood**
- **perfusion with albumin**

**Ptp_{mean} (cmH₂O)**
Figure 4.
Figure 6.
Figure 7.
Figure 8.