Locally measured shear moduli of pulmonary tissue and global lung mechanics in mechanically ventilated rats

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Locally measured shear moduli of pulmonary tissue and global lung mechanics in mechanically ventilated rats. J Appl Physiol 113: 273–280, 2012. First published May 24, 2012; doi:10.1152/japplphysiol.01620.2011.—This study was aimed at measuring shear moduli in vivo in mechanically ventilated rats and comparing them to global lung mechanics. Wistar rats (n = 28) were anesthetized, tracheally intubated, and mechanically ventilated in supine position. The animals were randomly assigned to the healthy control or the lung injury group where lung injury was induced by bronchoalveolar lavage. The respiratory system elastance Ers was analyzed based on the single compartment resistance/elastance lung model using multiple linear regression analysis. The shear modulus (G) of alveolar parenchyma was studied using a newly developed endoscopic system with adjustable pressure at the tip that was designed to induce local mechanostimulation. The data analysis was then carried out with an inverse finite element method. G was determined at continuous positive airway pressure (CPAP) levels of 15, 17, 20, and 30 mbar. The resulting shear moduli of lungs in healthy animals increased from 3.3 ± 1.4 kPa at 15 mbar CPAP to 5.8 ± 2.4 kPa at 30 mbar CPAP (P = 0.012), whereas G was −2.5 kPa at all CPAP levels for the lung-injured animals. Regression analysis showed a negative correlation between G and relative Ers in the control group (r = −0.73, P = 0.008 at CPAP = 20 mbar) and no significant correlation in the lung injury group. These results suggest that the locally measured G were inversely associated with the elastance of the respiratory system. Rejecting the study hypothesis the researchers concluded that low global respiratory system elastance is related to high local resistance against tissue deformation.

Knowledge about the relation between the local and global mechanics of lung tissue has recently come into focus of scientific interest (3–5, 7, 14, 16, 32). One of the reasons for this is that the material properties of lung tissue, for example its elastic moduli, play a crucial role in understanding the pathophysiology of several forms of respiratory injury (30).

Hence, there are multiple studies comparing lungs under different conditions with respect to the material properties of lung parenchyma, whereby material properties are numerically expressed by the shear modulus (parameter that describes the resistance against isovolumetric deformation) and the bulk modulus (parameter that describes the resistance against volumetric deformation) (17, 21, 24, 27, 29). Thereby, the dependence of the mechanical properties on the transpulmonary pressure was of special interest (10, 17, 21, 29, 33). However, there are few reports showing measurements performed in vivo, and to the authors’ knowledge there are no studies that determined the shear moduli of lung parenchyma in vivo under conditions of an intact thorax. Although the dependence of lung tissue shear moduli on surface tension was investigated before (27), no data are available about the difference between the shear moduli of healthy lungs and lungs injured by surfactant washout. However, this difference seems of particular interest because lung injuries, specifically in the washout injury model, lead to a high degree of heterogeneity: different regions of the lung are damaged to a different degree, which leads to maldistribution of ventilation being a primary factor of ventilator associated lung injury. Hence, heterogeneity of the injured lung’s parenchyma is also expected to influence the results, especially during measurements in vivo. Thereby, heterogeneity could lead to a high variation in the results of local mechanics measurements.

One of the main reasons for the lack of data regarding shear moduli of lung parenchyma in vivo and under conditions of an intact thorax and, hence, data about lung parenchyma of injured lungs might have been the absence of an adequate method for its measurement without the need of a thoracotomy (7, 26).

The authors have developed an endoscopic system for in vivo imaging of the lung. Therewith, the necessary invasive opening of the thorax is reduced to a minimum to avoid major side effects on the cardiovascular and respiratory systems (25). By using this endoscopic system the local transalveolar pressure is adjustable. This was utilized to implement a method for the in vivo identification of mechanical lung tissue properties using an inverse finite element (FE) analysis (26).

For the present study, the endoscopic technique was used to analyze shear moduli of lung tissue in vivo in an animal model of the mechanically ventilated rat with either healthy or injured lungs. Simultaneously, the dynamic respiratory system elastance was analyzed as a parameter that reflects the global mechanical state of the lung (31). It is hypothesized that the elastance of the respiratory system reflects local tissue properties in vivo in such a way that high elastance would correspond to a stiff lung parenchyma with reduced elasticity (positive correlation).

METHODS

Experimental protocol. All animal experiments were performed in accordance with the German animal protection law (TierSchG). The rats were housed and handled in accordance with good animal practice as defined by the Federation of Laboratory Animal Science Associations (FELASA) and the national animal welfare body (GV-SOLAS). The animal welfare committees of the University of Freiburg and the local authorities (Regierungspräsidium Freiburg; 35-9185.81/G-09/22) approved all animal experiments.
Anesthesia was induced inside a special cage into which 3–5% isoflurane in oxygen was delivered. Anesthesia was maintained by continuous infusions of S(+)ketamine (Ketanest S, Pfizer, Karlsruhe, Germany) at 100 mg·kg\(^{-1}\)·h\(^{-1}\) and midazolam (Dormicum, Roche, Grenzach-Wyhlen, Germany) at 4 mg·kg\(^{-1}\)·h\(^{-1}\), both administered in saline solution through a lateral tail vein. Mechanical ventilation was facilitated by muscle relaxation with intravenous pancuronium bromide (Pancuronium Organon, Teknika, Eppelheim, Germany) at 1 mg/kg. The trachea was intubated with an endotracheal tube (OD 2 mm) via tracheotomy. The lungs were ventilated volume controlled by a research small animal ventilator (FlexiVent, Scireq, Montreal, Canada) at an inspired oxygen concentration (FiO\(_2\)) of 100%, a respiratory rate of 70 breaths/min, a tidal volume of 10 ml/kg, and a PEEP of 2 mbar.

For continuous mean arterial blood pressure (MAP) and blood gas monitoring, the right common carotid artery was cannulated with polythene tubing (Portex Non Sterile Polythene Tubing, 0.58 mm ID, 0.96 mm OD, SIMS Portex, Kent, UK). The MAP was measured via a clinical monitoring system (Sirecust, Siemens, Erlangen, Germany). Blood gases were determined by the i-STAT portable clinical analyzer (Heska, Loveland, CO). Inspiratory and expiratory gas flows were measured by two separate flow-sensors (Fleisch 000, Dr. Fenyes and Gut, Hechingen, Germany), All analog output signals were fed into a custom-made data recording system that utilizes software and hardware for digitization of analog, time-dependent signals (NI PCI-6289, National Instruments, Austin, TX) at a sampling rate of 500 Hz.

Following hemodynamic and respiratory stabilization the rats were randomly assigned (by sealed envelope technique) to either the control or the lung injury group (\(n = 14\) each). Lung injury was induced by bronchoalveolar lavage with normal saline, which results in wash out of surfactant (19). The lavage was repeatedly performed with normal saline at 15 ml/kg until a Horowitz index (PaO\(_2\)/FiO\(_2\)) of \(<200\) mmHg was reached, which is a criterion for the diagnosis of acute respiratory distress syndrome (ARDS) (34).

With the animal in the prone position, the fifth intercostal space was surgically opened in the left midaxillary line for placement of a metal tube of 7.2 mm OD through which an endoscope was subsequently to be placed for microscopic examination of subpleural alveoli. The tube was fixed between the ribs by an extrathoracically applied screw nut. The animal was then placed in the supine position, and the endoscope was inserted through the tube until its tip came into contact with the visceral pleura (Figs. 1 and 2). The suction at the tip of the endoscope (\(p_{\text{Tip}}\)) was adjusted to keep the subpleural alveoli in focus during mechanical ventilation (\(p_{\text{Tip}} = -3\) mbar on average).

Following placement of the endoscope and mechanical ventilation with a PEEP of 7 mbar for 5 min, ventilatory maneuvers were performed and the blood gases were analyzed before, immediately after, and 30 min after the endoscope placement. To equalize the volume history and to increase the comparability, a recruitment maneuver (i.e., low-flow maneuver with a peak pressure of 40 mbar)
was performed first. This was followed by a PEEP-wave maneuver where PEEP was increased from 0 to 15 mbar by steps of 3 mbar and then reduced in the same fashion. Each PEEP level was maintained for 10 breaths. After the PEEP wave the ventilation was continued for 5 min at a PEEP of 7 mbar. Maneuvers for determining material properties were performed immediately after the second and third set of ventilatory maneuvers, respectively. At the end of the experiment the rats were killed by exsanguination.

Identifying mechanical properties of alveolar tissue. To determine mechanical material properties of alveolar tissue, continuous mechanical ventilation was interrupted and the ventilator was switched to continuous positive airway pressure (CPAP) mode, which was applied for time intervals of 10 s (Fig. 3, top). CPAP levels were presented in a fixed sequence (30, 15, 20, and 17 mbar). During CPAP, no airway pressure swings were observed, indicating the absence of ventilation.

Immediately after establishing a CPAP, a predefined course of negative pressure (pTip; ramped from −5 to −30 mbar; Fig. 3, bottom) was applied to a circular region of the tissue under the tip of the endoscopic system (diameter = 3 mm; Fig. 2). Adjusting pTip was done by controlling the amount of liquid that was flushed through the endoscopic system (Fig. 2). This negative pressure acts like an inverse punch indentation, inducing a three-dimensional deformation of tissue below the endoscope with an approximate diameter and height of 5 mm (considering only tissue that was deformed at least 10% of the maximum deformation; Fig. 2). The deformation of the surface was determined by tracking the optical size of ceramic beads (ceramic beads B205, EW Würth, Bad Friedrichshall, Germany) with an average diameter of 25 ± 12 μm that were placed on the tissue surface as a size reference. For this purpose, the beads were embedded in a plastic silicone (polydimethylsiloxane) membrane of ~22 μm thickness produced by a spin-coating process (2). A strip of the membrane was glued to the tip of the endoscopic system so that it touched the lung surface during the measurement maneuvers. The positions of the particles reflect the stretch of the observed tissue in the 2D plane, whereas measuring their optical sizes in the recorded video images reflects the movements of the particles in the third dimension (axial direction toward the endoscope).

The three dimensional position of the beads was then used to identify shear moduli (G) of the lung tissue. Therefore, iterative finite element modeling was carried out with finite element modeling software (COMSOL Multiphysics 3.5a, COMSOL Multiphysics, Göttigen, Germany). G was changed for every iteration, and the number of iterations was increased until the sum of the squared differences between model and measured data was minimized (scripted in Matlab R2007b, The MathWorks, Natick, MA). For further details about the method see (26).

Between two measurements at different CPAP levels, mechanical ventilation was continued until the arterial blood pressure recovered from the drop during the CPAP maneuver, which was usually achieved after 30–120 s.

Identifying respiratory system elastance. The elastance of the respiratory system during volume-controlled mechanical ventilation was identified assuming a one-compartment resistance/elastance lung model. Tracheal pressure (Ptrach) was continuously calculated as the difference between measured airway pressure and flow-dependent pressure drop across the tracheal cannula [ΔPc(V)]. To obtain ΔPc(V), the pressure-flow curve of the tracheal cannula (length 20 mm, inner diameter 1.6 mm) and the tubing system was experimentally determined separately for inspiratory and expiratory gas-flow ranging from −30 to 30 ml/s. The pressure-flow curve was mathematically approximated according to the following equation used for the description of pediatric endotracheal tubes (9):

\[ ΔP_{tc} = k_1 \cdot \dot{V} + k_2 \cdot \dot{V}^2 + I_{tc} \cdot \ddot{V} \]  

with k1 and k2 being the coefficients for linear and quadratic gas flow dependency, Itc being the inertance of the tracheal cannula, and \( \dot{V} \) being the time derivative of the gas flow.

Subsequently, gas flow and lung volume above FRC (V = \( \int \dot{V} \cdot dt \) with t being the time) were numerically fitted into the equation of motion of the resistance/elastance model (eq. 2) to obtain respiratory system elastance:

\[ P_{trach} = R_{eq} \cdot \dot{V} + E_{eq} \cdot \dot{V} + P_0 \]  

with \( R_{eq} \) and \( E_{eq} \) being the respiratory system resistance and elastance, respectively, and \( P_0 \) being the dynamic intrinsic PEEP (6).

The data of a complete breath were used for each fit (8), and values from 10 consecutive breaths were averaged. The data used for the fit were recorded after the ventilatory measurement maneuvers during volume-controlled ventilation with a PEEP of 7 mbar.

As a global measure that incorporates the individual condition of each rat, the value for \( E_{eq} \) identified after the insertion of the endoscope were divided by the values for \( E_{eq} \) measured before the insertion of the endoscope, thus reflecting the relative change in elastance (rel E).

Analysis of endoscopic videos recorded from subpleural alveoli. Additional data about the state of the alveolar parenchyma were extracted from the endoscopic videos. The amount of collapsed or atelectatic alveolar tissue (further on referred to as atelectatic tissue) was assessed manually.

For this purpose the free software ImageJ (ImageJ 1.42q, Wayne Rasband, National Institute of Health, Bethesda, MD) was used to mark the areas in the image where no structures were visible (Fig. 4).
The sum of these areas was then divided by the area of the endoscopic field of view that was analyzed (cf. Fig. 5). The result equals the part of area in the observed video frame that is characterized by showing no visible structures and is, because of that, associated with alveolar collapse and atelectasis (1, 12). Because the optical appearance of the recorded tissue only showed little variability during the CPAP maneuvers, a single representative image taken at an end-expiratory point during normal ventilation (PEEP = 7 mbar) in between the CPAP maneuvers was evaluated for each animal.

**Statistical methods.** All data are presented as mean ± SD unless indicated otherwise. The Matlab (Matlab R2007b, The MathWorks, Natick, MA) statistics toolbox was used for all statistical analyses. Differences between values from the control and the lung injury group were assessed by Student’s *t*-tests (for $E_{rs}$, rel $E$, $P_{aO2}$, MAP, and atelectatic tissue). The significance of the shear moduli’s dependence on the applied CPAP and on lung injury was determined using a two-factor ANOVA.

Pearson correlation coefficients were calculated to compare $E_{rs}$ and rel $E$, $E_{rs}$ and $G$, and rel $E$ and $G$ using the pooled data from all animals. Thereby, correlations with $G$ were performed separately for data from each CPAP level. The corresponding *P* values were calculated against the hypothesis that the correlation is not zero. According to the hypothesis of this study that high elastance corresponds to stiff tissue, the significances of the correlation of $E_{rs}$ and $G$ were also calculated against the hypothesis that the correlation is positive. Statistical significance was defined for *P* ≤ 0.05. Additionally, the magnitude of the interdependence between $E_{rs}$ and $G$ was calculated by determining the respective regression slope for each CPAP level, including pooled data from all animals.

For the case of the correlation of the same $E_{rs}$ and rel $E$ with shear moduli at four different levels of CPAP, the significance level was corrected to *P* < 0.0125 according to the Bonferroni correction.

**RESULTS**

Of the 28 examined rats, data from 4 animals had to be excluded because of death prior to the end of the experiment. Of the remaining animals, video data from 6 rats was not usable (e.g., attributable to air bubbles in the endoscope’s field of view). Thus data from 10 animals of the control group and 8 animals of the lung injury group were used for the analysis.

Elastance was calculated based on tracheal pressure. For the tracheal cannula used in the rats the determined coefficients for inspiration were $k_{11} = 0.026$ mbar·ml$^{-1}$·s$^{-1}$ and $k_{31} = 0.0012$ mbar·ml$^{-2}$·s$^{-2}$, and the coefficients for expiration were $k_{1E} = 0.024$ mbar·ml$^{-1}$·s$^{-1}$ and $k_{2E} = 0.001$ mbar·ml$^{-2}$·s$^{-2}$. The inertance was $I_{rc} = 32.10^{-6}$ mbar·ml·s$^{-2}$.

The animals of the lung injury group had higher respiratory system elastance, lower arterial oxygen pressure, and lower...
mean arterial pressure compared with the control group with healthy lungs (Table 1).

Evaluation of video frames (Fig. 5) from the endoscopic recordings showed that the area representing atelectatic alveoli (area free from alveolar structures) amounted to 2% (95% confidence interval CI: 1.56% to 5.5%) in the control group. In the lung injury group the atelectatic area amounted to 53% (CI: 20% to 87%; \( P = 0.0017 \)).

The determined shear moduli increased in the lung healthy control group from 3.3 ± 1.4 kPa at a CPAP level of 15 mbar to 5.8 ± 2.4 kPa at the highest CPAP level of 30 mbar (\( P = 0.012 \); Fig. 6, left), whereas the shear moduli for the lung-injured group were −2.5 kPa at all investigated CPAP levels (Fig. 6, right). The two-factor ANOVA showed significance in the difference among CPAP level (\( P = 0.021 \)) and group (\( P < 0.0001 \)).

The correlation analysis of shear moduli and relative elastance showed a negative correlation in the control group and no correlation in the lung-injury group (Table 2). The hypothesis of a positive correlation between shear moduli and the respiratory system elastance was rejected. The highest significant (after Bonferroni correction) correlation was found between shear moduli and relative elastance at a CPAP level of 20 mbar in the control group (\( r = -0.73 ; P = 0.008 \)). There were no significant correlations between shear moduli and relative elastance in the lung injury group.

**DISCUSSION**

By using a novel endoscopic system, the shear moduli of lung tissue were determined under in vivo conditions of an intact thorax in mechanically ventilated rats with healthy and injured lungs. Local mechanics of alveolar parenchyma (i.e., the shear modulus) were compared with the respiratory system elastance, and the visual appearance of the lung tissue was recorded by endoscopy. A local tissue deformation was induced extrapulmonarily by setting defined negative pressures at the tip of the endoscope. The tissue deformation was determined optically and used in an inverse finite element modeling analysis to determine shear moduli at four CPAP levels.

The main results of this study are (1) a negative correlation between the shear moduli and the relative elastance of the respiratory system in the healthy lung control group because of which the study hypothesis that high respiratory elastance corresponds to stiff pulmonary tissue and vice versa had to be rejected and (2) a significantly lower dependence of the lung injured parenchymas’ shear moduli on CPAP.

Under the assumption that the elastance of lungs is an indicator of the lungs’ functional state and that the relative elastance is an indicator of a specific state of lungs compared with its original state, the positive correlation between rel E and G implies either that lungs with higher shear moduli were more resistant against damage because of the surgical intervention or that, the other way around, the shear modulus of lung tissue was reduced in the case of damage because of the surgical intervention.

Furthermore, the shear moduli of the lung healthy group showed a significant dependence on the airway pressure at which they were measured. This indicates rising rigidity with increasing pressure and reflects the stabilizing effect of PEEP on pulmonary tissue (11).

However, results look different in the surfactant-deprived group. The measured shear moduli neither show a significant correlation with respiratory system elastance nor a significant dependence on airway pressure.

Although, as expected, high heterogeneity was observed in the injured lungs, which is reflected in the high variation of atelectatic area in video frames among the injured animals, the statistical analysis showed highly significant differences between groups regarding the visible atelectatic areas (10-fold increase in the injured group) and the measured shear moduli. Thereby, mean shear moduli are significantly lower in the lung-injured group (2.74 ± 1.43 kPa) compared with the control group (4.2 ± 2.4 kPa).

The apparent question arising from the results of this study is, why, contrary to our hypothesis, a higher global respiratory system elastance seems to be associated with higher local shear moduli on the alveolar level. Although our findings are in contradiction to what we expected intuitively, a plausible interpretation is possible. It seems reasonable to assume that a high resistance against isovolumetric deformation of the highly structured alveolar parenchyma is a prerequisite to preserve the lungs’ functional volumetric distensibility.

Thus, a relatively high shear modulus of pulmonary tissue would correlate with a “good” (relatively low) respiratory system elastance. This can be brought into accordance with existing knowledge that the mechanical interdependence of alveolar structures is a prerequisite for the basic requirements of global parenchyma stability (G > 0) (28, 30). By arguing that the shear modulus is reflecting the degree of mechanical interdependence of the parenchyma and assuming that the interdependence reduces the deformability, a relatively high

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**Table 1. Local and global mechanics and hemodynamics of control and lung-injured animals**

<table>
<thead>
<tr>
<th>Ers, mbar/ml</th>
<th>rel E</th>
<th>( \text{PaO}_2 ), mmHg</th>
<th>MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.33 ± 0.52</td>
<td>1.35 ± 0.16</td>
<td>531.2 ± 29.2</td>
</tr>
<tr>
<td>Lung injury</td>
<td>3.24 ± 1.05</td>
<td>1.87 ± 0.51</td>
<td>120.8 ± 62.1</td>
</tr>
</tbody>
</table>

\( P \) value | 0.026 | <0.0008 | 0 | 0.093 |

Data are given as mean ± SD and include respiratory system elastance (Ers), relative elastance (rel E), arterial oxygen partial pressure (\( \text{PaO}_2 \)), and mean arterial pressure (MAP).

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![Fig. 6. In vivo shear moduli as a function of the CPAP level at which they were determined (x-axis). Left: control group; right: lung-injury group. [Box plot whisker mark the range of values outside (maximally 1.5 times the interquartile range) of the box. Values outside of the maximal whiskers range (outliers) are marked as +.](image)
shear modulus can be interpreted as an indicator for inherent mechanical stability of the lung parenchyma.

As an analogy of how structural rigidity is a precondition for functional elasticity, the green leaf of plants can exist either in the turgescent status or, after plasmolysis attributable to water loss, in the limp status. In the turgescent condition, intracellular hydrostatic pressure can range up to more than 3,000 kPa (23), leading to considerable turgor-induced elastic cell wall strain. This turgor-induced rigidity is the prerequisite for structural stability of the plant and for its mechanical elasticity. On the other hand, after considerable water loss and hence plasmolysis, the limp leaf has lost its structural stability.

The questions arising from main result 2 is why the surfactant-deprived lung tissue shows no dependence on airway pressure and why the average shear modulus in the lung-injured group is lower than in the control group.

To answer these questions it is necessary to take into account the basic differences between the two groups. Thereby, the most evident difference is that the injured lungs are deprived of surfactant, leading to an increased surface tension inside the alveolar cavities and, because of that, to an increased amount of collapsed or atelectatic lung tissue.

On the basis of this premises, we suggest two mechanisms that would explain this difference between healthy and surfactant depleted lungs:

1) ALI/ARDS deeply affects interstitial fluid pressure and fluid absorption processes in lung parenchyma that leads to the formation of atelectatic or collapsed tissue regions. These regions are not participating in gas exchange, and hence their mechanical properties are less dependent on transpulmonary pressure. This possibility is supported by results from a study about the difference in shear moduli between air- and liquid-filled lung tissue (29), which suggest that liquid-filled pulmonary tissue (comparable to atelectatic tissue) has lower shear moduli.

2) The volume- or pressure-dependent changes of mechanical properties in healthy lung tissue are largely related to surfactant. Surfactant leads to a rise of surface tension as alveolar tissue is distended. This means that an increase in volumetric distension will lead to a higher resistance against isovolumetric deformation because it is associated with the shear moduli that we measured in the healthy lungs and that are in line with a study from Hajji et al. (10). In contrast, complete lack of surfactant and the presence of atelectasis means that there is constant, i.e., volume- or pressure-independent surface tension in the corresponding parts of the lung. Thereby, shear moduli under conditions of constant surface tension were shown to be almost constant at lung volumes from 40% to 80% of total lung capacity (27). Hence, constant surface tension could be a reason for a constant G in atelectatic and surfactant deprived parts of injured lungs.

The determined shear moduli (4.2 kPa for healthy lungs and 2.74 kPa for injured lungs) were found to be in accordance with values reported in literature (17, 21, 24, 29, 33) [e.g., G≈4.9 kPa at a stress of 20 mbar (13, 18); G = 1.68–2.84 kPa at a transpulmonary pressure of 20 mbar (10)].

Furthermore, the pressure dependence of the shear moduli calculated from median values of each CPAP is 0.14 kPa/mbar. This is higher than results obtained from pig lungs (~0.1 kPa/mbar) and horse lungs (~0.08 kPa/mbar) according to (10).

However, caution should be taken when comparing our results directly with published data because of experimental differences in the transpulmonary pressures at which shear moduli were determined, the experimental setup (e.g., in vivo vs. ex vivo or conditions of open vs. closed thorax), and the species that were investigated.

Method limitations. We analyzed respiratory system mechanics without differentiating between lung and chest wall elastance. However, because lung injury was induced by bronchoalveolar lavage, significant differences in chest wall mechanics between healthy and lung-injured animal groups were not to be expected.

It has to be noted that there was a small increase in respiratory system elastance in the healthy lungs attributable to the surgical intervention, and we assume that a comparable increase in elastance has been induced in the injured lungs. Therefore, we feel it justified to relate the significant increase in elastance observed in lung-injured animals to the lung injury. The endoscopic videos covered only a small area of the surface of the lung that was at the identical anatomical site in all animals. Especially in the case of the lung-injury group it has to be kept in mind that the alveolar damage attributable to the lavage and attributable to ventilator-associated lung injury is distributed heterogeneously and that the latter was shown do develop differently depending on the anatomical site (22). Gas redistribution between slow and fast lung compartments, i.e., the pendelluft phenomenon, is characteristic for the heterogeneously injured lung. Therefore it could be assumed that the analysis of global respiratory system elastance depends on whether the lung is under dynamic conditions of uninterrupted mechanical ventilation (as analyzed in this study) or under static conditions. To test this, the respiratory system elastance has also been analyzed under quasi-static conditions as realized during the low-flow maneuver performed prior to the determination of G. Correlation of “static” elastance with shear moduli (data not shown) led to nearly identical results as the correlation analysis including dynamic elastance.

Table 2. Comparison of control and lung-injured animals using correlation analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Correlation Ers/relE: r (P)</th>
<th>CPAP. mbar</th>
<th>Correlation Ers/G: r (P)</th>
<th>Correlation relE/G: r (P)</th>
<th>p (r &gt; 0) of corr (Ers, G)</th>
<th>Regr. slope G/E, kPa/mbar/ml</th>
<th>Regr. slope G/relE, kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.74 (0.015)</td>
<td>15</td>
<td>-0.53 (0.12)</td>
<td>-0.52 (0.06)</td>
<td>0.94</td>
<td>-1.4</td>
<td>-4.4</td>
</tr>
<tr>
<td>Lung injured</td>
<td>0.94 (0.0001)</td>
<td>15</td>
<td>-0.23 (0.55)</td>
<td>-0.1 (0.81)</td>
<td>0.73</td>
<td>-0.26</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

Table includes Pearson correlation coefficients between Ers, rel E, and shear modulus (G). It further shows the corresponding P values (in parentheses) and the resulting slopes from the regression lines.
Because of the small coverage of surface area, the recorded videos are not representative for the whole lung. However, we assume that the average overall local measurements is representative for the state of the lung as a whole organ.

The limitation of a very small coverage relative to the whole organ does, however, not apply to the local measurement of the shear modulus. According to results from finite element modeling in combination with knowledge about the alveolar geometry and the total number of alveoli in rat lungs (15), we estimate that ~190,000 individual alveoli are included in the determination of shear moduli.

Although accumulation of interstitial fluid in lung parenchyma is well known to parallel ALI/ARDS formation, we never observed significant fluid in the pleural space during experiments or in the lung excised after the experiments. All measurements were performed in rat models. However, their lung structure and dimensions on an alveolar level are similar to the human lung (20). Still, the absolute size difference is significant. Similarity and comparability of our results to other species are therefore not guaranteed.

Furthermore, we induced the lung injury by surfactant depletion. Therefore, results and conclusions of our study cannot directly be transferred to other types of lung damage.

In our study, the mechanical properties of alveolar tissue were determined at lung surfaces that were covered with pleura visceralis. This additional layer that is covering the lung tissue could have influenced the determined parameters. Especially in the control group this seems to be true. Although the absolute values of the determined shear moduli are reasonable, the pressure-dependent increase of shear moduli is higher than in studies where the pleura was not part of the measured tissue. This suggests an overestimation of shear moduli with increasing pressure. Such an overestimation could be caused by an increasing tension in the stretched pleura visceralis.

A limitation of the animal model was the requirement to apply a relatively high CPAP level for the measurement of the mechanical tissue properties. The minimum CPAP level had to be higher than the PEEP during ventilation to ensure a fresh gas supply during the 10-s intervals at which the CPAP level was held constant and thus to ensure the survival of the animal. The maximum CPAP level was limited to 30 mbar to prevent extensive barotrauma and cardiovascular problems (i.e., a reduced cardiac output and hence reduced blood pressure attributable to high pulmonary pressure). Furthermore, anesthesia might have influenced pulmonary and cardiovascular properties of the animals. Specifically ketamine is known to have a bronchodilatory effect. Hence, medication could have had an effect on mechanical tissue properties. Drug delivery was, however, uniform in all animals.

The visual evaluation of the lung tissue was done on a single video frame for every animal, and the marking of the collapsed areas was done manually. Hence, the resulting ratio of the collapsed areas should be interpreted in a qualitative rather than a quantitative way. However, the differences between the ratio of the collapsed areas for the control group and for the lung-injury group were highly significant.

### Conclusion

We found that for mechanically ventilated rats at constant airway pressures between 15 and 30 mbar the shear moduli (i.e., local resistance against isovolumetric deformation) of alveolar parenchyma are smaller in lung-injured animals compared with lung healthy control animals. In contrast to injured, surfactant-depleted lungs, shear moduli increased with lung pressure in healthy lungs. On the basis of our observations and on existing literature we interpret the differences in shear moduli between healthy and injured lungs as an effect caused by the increased surface tension in the injured lungs.

Our study hypothesis was that high elastance corresponds to stiff tissue. This had to be rejected considering our findings. Instead, our results indicate that in healthy lungs low global respiratory system elastance is related to high local tissue rigidity, whereas high global respiratory system elastance is related to soft alveolar tissue. Hence, a relatively high shear modulus seems to be associated with structural lung stability.

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### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

### AUTHOR CONTRIBUTIONS

Author contributions: D.S., H.R., J.H., and J.G. conception and design of research; D.S., H.R., and J.H. performed experiments; D.S. analyzed data; D.S., S.S., and J.G. interpreted results of experiments; D.S. prepared figures; D.S. drafted manuscript; D.S., S.S., and J.G. edited and revised manuscript; D.S., H.R., S.S., J.H., and J.G. approved final version of manuscript.

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