Mechanical properties of lung parenchyma during bronchoconstriction

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Okazawa, Mitsushi, Yulia D'Yachkova, and Peter D. Paré. Mechanical properties of lung parenchyma during bronchoconstriction. J. Appl. Physiol. 86(2): 496–502, 1999.—Interdependence between airways and the lung parenchyma is thought to be a major mechanism preventing excessive airway narrowing during bronchoconstriction. Because the elastance of the lung increases during bronchoconstriction, the lung’s tethering force could also increase, further attenuating bronchoconstriction. We hypothesized that the bulk (\(k\)) and shear moduli (\(\mu\)) of the lung increase similarly during bronchoconstriction. To test this hypothesis, we excised rabbit lungs and measured the lung volume, pulmonary elastance, \(k\), and \(\mu\) at transpulmonary pressures of 4, 6, 8, 12, and 16 cmH\(_2\)O using pressure-volume curves, slow oscillations of the lung, and an indentation test. Bronchoconstriction was induced by nebulizing carbachol by using small tidal-volume ventilation to prevent hyperinflation. The measurement of \(k\) and \(\mu\) was repeated after carbachol treatment. After carbachol treatment, the increase in \(k\) was significantly greater than in \(\mu\). The estimated value for \(\mu\) was \(\approx 0.5 \times \) transpulmonary pressure both before and after carbachol treatment. These data suggest that the tethering effect of the lung parenchyma, which serves to attenuate bronchoconstriction, is not significantly increased during carbachol administration unless there is hyperinflation.

MATERIALS AND METHODS

Animal preparation. Twenty-five New Zealand White rabbits were divided into five groups. The excised lungs of each group were inflated to a P\(_L\) of either 4, 6, 8, 12, or 16 cmH\(_2\)O (\(n = 5\) for each group). After deep anesthesia with \(\alpha\)-chloralose (100 mg/kg) and urethanol (1,000 mg/kg), the animals were intubated and the chest was opened. The rabbits were artificially ventilated by using 100% O\(_2\) (tidal volume of 7 ml/kg and respiratory rate of 40 breaths/min) for \(\approx 20\) min. The tracheal tube was then occluded at end expiration, and the lung was degassed by the uptake of O\(_2\) into pulmonary circulation. Then the animals were killed, and the lung and heart were carefully dissected en bloc from the thorax.

Pulmonary function. Airway pressure was measured through a side port of a tracheal tube by using a piezoresistive transducer (FPM-02PG, Fujikura, Tokyo, Japan) and was compared with atmospheric pressure to give P\(_L\). Airflow was measured by using a pneumotachometer (Fleisch no. 00) and a differential pressure transducer (±5 cmH\(_2\)O; model MP45, Validyne), and volume was calculated by integrating the flow signal. All pressure and airflow tracings were displayed continuously on a monitor and recorded by using a computer-supported data-acquisition system (Raytech, Vancouver, British Columbia). With the use of a device that allows inflation or deflation of the lung at a desired flow rate, the lungs were slowly inflated to total lung capacity (TLC), defined as the volume at a P\(_L\) of 25 cmH\(_2\)O. Two quasi-static pressure-volume curves were obtained at a P\(_L\) between 25 and 0 cmH\(_2\)O. The repeat curves were reproducible and not associated with an increase in gas trapping. The tracheal tube was then occluded at a P\(_L\) of 0 cmH\(_2\)O, and the weight of the heart and lung was measured. The tissue volume of the heart and lung was calculated by dividing tissue weight by specific tissue density (1.06 g/ml). Total heart and lung volume, including lung gas volume at a P\(_L\) of 0 cmH\(_2\)O, was measured by water displacement. The trapped gas volume at a P\(_L\) of 0 cmH\(_2\)O was obtained by subtracting tissue volume from water-displaced total volume. The lung gas volume at each P\(_L\) was calculated by adding the trapped gas volume at a P\(_L\) of 0 cmH\(_2\)O and the volume at that particular P\(_L\) obtained from the pressure-volume curves. After a slow TLC maneuver, the lung was deflated to one P\(_L\) level (4, 6, 8, 12, or 16 cmH\(_2\)O). Ten minutes were allowed for the lung volume to equilibrate, and then the lung was sinusoidally oscillated by using a small tidal volume (\(\approx 0.3\) ml) at a frequency of 6 Hz, keeping end-expiratory pressure constant at the selected P\(_L\) to obtain lung resistance (R\(_L\)) by using Mead and Whittenberger’s method (17). Slow and small pressure-volume loops (0.7 Hz

EXAGGERATED AIRWAY NARROWING in response to pharmacological agonists is a characteristic feature of patients who have asthma. The exact mechanism for the exaggerated airway narrowing is unknown. Because peripheral airways are surrounded by the lung parenchyma, the elastic load provided by lung elastic recoil can modulate the smooth-muscle shortening (8, 15, 16). Ding et al. (4) examined the effect of lung volume on bronchoconstriction induced by inhaled methacholine and suggested that an increase or decrease in lung volume alters the interdependence between the airways and lung parenchyma, attenuating or accentuating airway narrowing. Lai-Fook et al. (11) used canine lung lobes to measure shear modulus (\(\mu\)), which is an expression of a material’s ability to resist an isovolumic shape distortion. They reported that \(\mu\) increases linearly with transpulmonary pressure (P\(_L\)) and that the change in peribronchial pressure, which creates the airway-lung parenchymal interdependence, could be estimated. In calculating the peribronchial pressure change during bronchoconstriction, they used the value for \(\mu\) of the lung parenchyma that had not undergone bronchoconstriction. Because the stiffness of the lung increases during bronchoconstriction (2, 14, 18), the elastic properties expressed by \(\mu\) and bulk modulus (\(k\)) could increase and alter the airway-lung parenchymal interdependence. We hypothesized that \(k\) and \(\mu\) increase similarly during bronchoconstriction because of the stiffening of the lung parenchyma. To test this hypothesis, we measured lung volume, \(k\), and \(\mu\) using pressure-volume curves, slow oscillation of the lung, and an indentation test before and after carbachol nebulization in excised rabbit lungs.
and ~0.3 ml, respectively) were obtained at that P_l to measure lung elastance (E_L) by using a recursive least squares method to fit the equation of motion (13). Different frequencies were used to measure R_L and E_L because at low frequencies tissue resistance contributes significantly to R_L and we wanted to specifically examine the effects of carbachol on airway narrowing. At a frequency of 6 Hz, we can assume that R_L is a reasonable estimate of airway resistance.

Indentation test. The lung was kept inflated at the selected P_L and was supported by using gauze beneath the cardiac groove so that the flat surface of the right lung was horizontal. A plastic disk with a diameter of 9.5 mm and a thickness of 3 mm was attached to a force transducer (FT03, Grass Instruments) so that the surface of the disk was parallel to the lung surface. The force transducer was attached to a holding bar, which could be moved vertically along a metal rail by using a micrometer. After pulmonary function was measured at that P_L, the disk was lowered until the whole surface was completely in contact with the pleural surface. The disk was then lowered by using 0.25-mm steps until a total indentation of 2.25 mm was reached. The plateau force was recorded 1 min after each step. The indentation test was performed by using the upper and lower lobe of the right lung. The order of measurements was randomized for upper and lower lobes. In 13 animals, the upper lobe was measured first and the lower lobe second and vice versa in the other 12 animals.

Carbachol nebulization. A high concentration of carbachol (100 mg/ml) was nebulized through the trachea by using a Devilbiss nebulizer (model 646, Devilbiss) for 3 min. During nebulization, positive end-expiratory pressure (PEEP) was kept at the selected P_L by using a PEEP valve. Carbachol was administered by temporary occlusion of the exhalation port to give small tidal volumes, which resulted in P_l fluctuations of 3-5 cmH_2O above PEEP. After the nebulization, R_L and E_L were remeasured, the lung was kept inflated at that P_L, and the indentation test was repeated by using the same protocol as at baseline.

The lung volume after carbachol nebulization was estimated as follows: V_carb = αV_base. For this calculation, V_base and V_carb are lung volumes before and after carbachol nebulization, respectively, and α is the inflation factor. To calculate α, we made an assumption that lung volume changes uniformly by a factor equal to the change in the cube of the change in the line length on the pleural surface. Two pairs of small markers were arranged diagonally on the lung surface so that each pair was 1.5-3 cm apart and the lines connecting the two pairs crossed approximately at a right angle (1). The distance between two pairs of markers was measured before and after carbachol treatment by using an optical microscope. The coefficient α was calculated as α = (A/B)^3/2, where A and B are the "areas" formed by the markers after and before carbachol treatment, respectively.

Calibration for displacement of the force transducer. The displacement of the metal sensor in the force transducer during an indentation test was measured by pressing the disk onto an incompressible surface. The slope of the relationship between displacement of the metal sensor and measured force was very small (0.03 mm/g) and was linear between 0 and 10 g. The movement of the disk in the indentation test was corrected by using this relationship.

Calculation of κ and μ. The κ was calculated as

$$\kappa = V \times E_L$$

where V is the lung gas volume at the P_L calculated from the pressure-volume curve between 0 and 25 cmH_2O plus the trapped gas volume at 0 cmH_2O. E_L was obtained from the slow and small pressure-volume loops. From the theory of elasticity (24)

$$F/2wD = \mu/(1 - \nu)$$

$$\nu = (3\kappa - 2\mu)/(2(3\kappa + \mu))$$

where F is the applied force, w is the corresponding indentation of the lung, D is a diameter of the disk, and ν is Poisson's ratio of the lung. The μ was calculated by using Eqs. 2 and 3, and the data for κ were obtained from Eq. 1. The relationship between applied force (F) and displacement (w) was linear, both at baseline and after carbachol treatment (R^2 = 0.99 ± 0.01, P < 0.01 for both conditions).

Statistics. ANOVA and a multiple-comparison procedure with Bonferroni correction were used to compare baseline E_L, R_L, κ, and μ at different P_L. Analysis of covariance and a multiple-comparison procedure were used to compare E_L, R_L, κ, and μ after carbachol treatment at different P_L. Linear regression analysis was used for the relationships between P_L and μ before and after carbachol treatment. A paired t-test was used to compare μ between the first and the second measurements. Paired t-tests were used to compare E_L, R_L, κ, and μ before and after carbachol treatment. The changes in κ and μ after carbachol treatment were compared by using analysis of covariance. The Wilcoxon signed rank test was used to examine the difference in the relationships of μ and %TLC before and after carbachol treatment by using values of %TLC > 75%. Below 75% TLC, paired t-tests were used to compare μ between baseline and after carbachol treatments.

RESULTS

Figure 1 shows the average deflation lung pressure-volume relationship. The mean lung volume varied from 60 to 93% TLC at P_L between 4 and 16 cmH_2O. Inflation factors at a P_L of 4, 6, 8, 12, and 16 cmH_2O were 1.06 ± 0.04, 1.01 ± 0.02, 1.06 ± 0.02, 1.07 ± 0.05, and 1.01 ± 0.04, respectively. There was no significant association between P_L and inflation factor. These results show that the largest increase in lung volume after carbachol was 6.7 ± 4.7% at a P_L of 12 cmH_2O.

![Fig. 1. Average deflation pressure-volume relationship (baseline ± SD). This is not the average pressure-volume curve for all animals. Each animal was studied at only 1 transpulmonary pressure (P_L) level. Resulting volumes, expressed as %total lung capacity (%TLC), at that P_L were averaged (± SD) to construct this curve (e.g., 5 animals were studied at a P_L of 16 cmH_2O and data were averaged).](http://jap.physiology.org/Downloadedfrom)
Baseline $R_L$, which primarily represents airway resistance, because the lung was oscillated by using a small tidal volume and a frequency of 6 Hz, was not significantly different at different $P_L$, although $R_L$ tended to be lower at a $P_L$ of 8 and 16 cmH$_2$O than at the other $P_L$ values (Fig. 2A). $R_L$ was significantly increased after carbachol treatment at $P_L$ of 4, 6, 8, and 12 cmH$_2$O but not at 16 cmH$_2$O (Fig. 2B). The percent change in $R_L$ after carbachol treatment was greatest at a $P_L$ of 4 cmH$_2$O and decreased at higher $P_L$ values (Fig. 2B).

$E_L$ increased as $P_L$ increased both before and after carbachol treatment (Fig. 3A). $E_L$ values at $P_L$ of 8, 12, and 16 cmH$_2$O were significantly greater than those at $P_L$ of 4 and 6 cmH$_2$O both before and after carbachol treatment. There were no significant differences in $E_L$ at $P_L$ of 8, 12, and 16 cmH$_2$O before and after carbachol treatment. $E_L$ significantly increased at all $P_L$ after carbachol treatment (Fig. 3B). The change in $E_L$ was greatest at a $P_L$ of 4 cmH$_2$O and decreased at higher $P_L$ values (Fig. 3B).

The $k$ increased as $P_L$ increased (Fig. 4A) both before and after carbachol treatment. The $k$ values at $P_L$ of 6, 8, 12, and 16 cmH$_2$O were significantly greater than that at $P_L$ of 4 cmH$_2$O both before and after carbachol treatment. The $k$ values at $P_L$ of 8, 12, and 16 cmH$_2$O were significantly greater than those at $P_L$ of 4 and 6 cmH$_2$O both before and after carbachol treatment. The $k$ values at $P_L$ of 8, 12, and 16 cmH$_2$O were significantly greater than at $P_L$ of 4, 6, 8, and 12 cmH$_2$O both before and after carbachol treatment. $k$ significantly increased at all $P_L$ after carbachol treatment (Fig. 4B). The increase in $k$ was greatest at a $P_L$ of 4 cmH$_2$O and significantly decreased at higher $P_L$ values.

There were no significant differences in $\mu$ between the first and second measurements (Fig. 5). Therefore, the data were pooled for further analysis. The $\mu$ increased as $P_L$ increased at $P_L$ of 8, 12, and 16 cmH$_2$O both before and after carbachol treatment. $\mu$ at $P_L$ of 12 cmH$_2$O was significantly greater than $P_L$ of 4 cmH$_2$O both before and after carbachol treatment. The $\mu$ at $P_L$ of 16 cmH$_2$O was significantly greater than at $P_L$ of 4 cmH$_2$O both before and after carbachol treatment. The $\mu$ at $P_L$ of 16 cmH$_2$O was significantly greater than at $P_L$ of 4, 6, 8, and 12 cmH$_2$O both before and after carbachol treatment. $\mu$ at $P_L$ of 16 cmH$_2$O because $\mu$ at $P_L$ of 4 and 6 cmH$_2$O was greater than expected both before and after carbachol treatment (Fig. 5A). The estimated regression lines

![Figure 2](http://jap.physiology.org/)

![Figure 3](http://jap.physiology.org/)

![Figure 4](http://jap.physiology.org/)

![Figure 5](http://jap.physiology.org/)
The percent increase in $\kappa$ was significantly greater than the percent increase in $\mu$ after carbachol treatment (Fig. 6). There was no significant association between the percent increase in $\kappa$ and $\mu$.

**DISCUSSION**

Because peripheral airways are surrounded by lung parenchyma, the elastic load provided by lung elastic recoil could attenuate smooth-muscle shortening and prevent occlusion of peripheral airways during bronchoconstriction (4). In a previous study, we (20) calculated the elastic load on carbachol-stimulated canine airway smooth muscle (ASM) provided by lung parenchyma before and after carbachol treatment were $\mu = 0.53 \times P_L - 1.22$ ($R^2 = 0.69$, $P < 0.01$) and $\mu = 0.50 \times P_L - 0.47$ ($R^2 = 0.65$, $P < 0.01$), respectively. There were no significant differences in slope or intercept before and after carbachol treatment. There was a small but significant increase in $\mu$ after carbachol treatment at $P_L$ of 4, 6, 8, and 12 cmH$_2$O (Fig. 5B). The relationship between lung volume and $\mu$ was linear above 75% TLC both before and after carbachol treatment (Fig. 5C). The estimated slope and intercept of the regression line for the difference in $\mu$ and %TLC between baseline and after carbachol treatment were not significantly different from 0. The $\mu$ was significantly greater after than before carbachol treatment below 75% TLC (Fig. 5C).

The percent increase in $\kappa$ was significantly greater than the percent increase in $\mu$ after carbachol treatment (Fig. 6). There was no significant association between the percent increase in $\kappa$ and $\mu$.
using the equations developed by Lambert and Wilson (12) and Lai-Fook and associates (11). In that study, we assumed that the $\mu$ values for control and carbachol-treated lobes were identical. We compared the calculated load and the observed ASM shortening to that which would be predicted from the in vitro canine ASM length-stress relationship (21). The results showed that smooth-muscle shortening of peripheral airways is neither isotonic nor isometric but is elastic loaded. However, we also found that the calculated load was not sufficient to explain the attenuation of smooth-muscle shortening in situ (20). Although it is possible that ASM activation was not maximal, another possible explanation for this result would be an underestimation of the load provided by lung elastic recoil because we assumed the same $\mu$ for control and carbachol-treated lobes. Bates and Peslin (2) have reported that intravenous histamine administration at a fixed lung volume in dogs induces a greater increase in EL than the 10–20% of baseline predicted by Smith et al. (23) after slowing the airway tree. They suggested that the increase in EL is attributable to the activation of the contractile elements in the lung that have been demonstrated by histological studies (5, 7). Although heterogeneity of bronchoconstriction and resultant nonuniformity of ventilation distribution contribute to the changes in EL, a significant portion of the increase is due to actual changes in tissue properties (25). These studies suggest that the increased stiffness of the lung parenchyma during bronchoconstriction could enhance the interdependence between lung parenchyma and airway caliber. Evidence in support of this view has been reported by Romero and Ludwig (22), who showed that maximal stimulation of the rabbit tracheobronchial tree by methacholine resulted in a negative relationship between tissue resistance and airway resistance. They reasoned that the stiffened lung parenchyma during bronchoconstriction, which was demonstrated by elevated EL and tissue resistance, increased the load on ASM contraction and limited further airway narrowing.

The mechanical properties of the lung parenchyma can be described by three interrelated elastic moduli (Young’s modulus, $k$, and $\mu$). Because bronchi are surrounded by alveoli, which must be deformed during bronchoconstriction, $\mu$ is the most important modulus in creating the change in peribronchial pressure, which mediates parenchymal-airway interdependence (11). Therefore, to estimate the parenchymal load impeding ASM contraction, it is necessary to measure $\mu$ during bronchoconstriction. In the present study, we measured $k$ and $\mu$ during carbachol-induced bronchoconstriction. The $k$ and $\mu$ significantly increased during bronchoconstriction (Figs. 4 and 5); however, the increase in $k$ was significantly greater than that in $\mu$ (Fig. 6). One of the possible reasons for the discrepancies in the increase in $k$ and $\mu$ could be the sequence of the measurements. After carbachol nebulization, the measurement of $\mu$ always preceded the measurement of $k$, and it is conceivable that the degree of bronchoconstriction was diminishing with time. However, we used carbachol, which is a long-acting contractile agonist, and we did not see a difference in $\mu$ between the first and second measurements after carbachol treatment. In a previous study, we (20) showed stable increases in RL after nebulization of carbachol in excised dog lungs. Therefore, the results suggest that different mechanisms are contributing to the increases in $k$ and $\mu$. Because slow vital-capacity maneuvers and induced bronchoconstriction can cause gas trapping and $\mu$ increases as the lung volume increases (11, 24), we tried to minimize gas trapping by oscillating the lung and nebulizing carbachol using a small tidal volume. After slow vital-capacity maneuvers at baseline PL, $\mu$ was not different at PL of 4, 6, and 8 cmH2O (Fig. 5A). Lai-Fook et al. (11) reported similar results; in fact they found an increase in $\mu$ at lower lung volume. After several slow vital-capacity maneuvers, alveolar pressure in the area of trapped gas could become higher than airway pressure because the trapped alveoli cannot deflate. If we assume that the trapped area is homogeneously distributed in the lung, $\mu$ could be similar despite overall lung deflation, as was observed in our results (Fig. 5, A and C). Therefore, the regression line for PL vs. $\mu$ was constructed for values of $\mu$ at PL between 8 and 16 cmH2O, where the effect of gas trapping would be negligible (Fig. 5A). Although $\mu$ significantly increased after carbachol treatment at each PL except 16 cmH2O, there were no significant differences in the PL-$\mu$ relationship over this range of PL.

In the present study, EL, $k$, and $\mu$ increased significantly (Figs. 3–5) during bronchoconstriction. Although $\mu$ increased significantly after carbachol treatment at each PL except 16 cmH2O, the lung volume-$\mu$ relationship was not significantly different before and after carbachol treatment (Fig. 5C), suggesting that the small increase in $\mu$ after carbachol treatment was the
result of the small amount of hyperinflation that occurred during bronchoconstriction. There were no significant differences in the slopes or intercepts of the regression lines for the PL-\(\mu\) relationship before and after carbachol treatment, and the estimated \(\mu\) both before and after carbachol treatment was \(-0.5 \times PL\). These results indicate that less force is necessary to deform alveolar shape than to change volume both before and after carbachol treatment. Therefore, the load on the ASM contraction related to distortion of the alveoli during bronchoconstriction does not increase beyond that associated with the effect of hyperinflation.

Our results are consistent with previous studies. Using axially symmetrical force loading, Hoppin et al. (9) showed that asymmetric loading gave greater value for compliance than did symmetrical loading, suggesting that it was easier to change the shape of the lung parenchyma than to change its volume. Our results suggest that their analysis is also applicable to the constricted lung parenchyma. In a theoretical analysis, Budiansky and Kimmel (3) and Kimmel et al. (10) investigated the mechanical properties of the lung parenchyma using a dodecahedron model composed of elastic line members connected by pin joints, which have negligible bending moment. They compared elastic moduli calculated by this model, measured values obtained from in situ studies, and found that the model provided a good estimate of elastic properties of the lung parenchyma. They pointed out that normalized moduli [Young modulus-to-inflation pressure (P), \(\mu\)-to-P, and \(k\)-to-P ratios] respond differently to the change in stress. If the stiffness of the line members of the dodecahedron model increases and the pin joints still have negligible bending moment, one would predict a greater increase in \(k\) than in \(\mu\), as we observed.

We tried to avoid gas trapping during bronchoconstriction by nebulizing carbachol using a small tidal volume so that we could investigate the relationship between lung stiffening and \(\mu\). Because bronchoconstriction is usually associated with hyperinflation of the lung when a nebulized bronchoconstrictor is administered during tidal ventilation or a vital capacity maneuver, \(\mu\) will increase (11). Nagase et al. (19) have reported that patchy hyperinflation of the lung parenchyma is usually observed when severe bronchoconstriction is induced. It is possible that the load on the smooth muscle in the airways, which are surrounded by these hyperinflated areas of lung parenchyma, would be larger than in the normally inflated region because \(\mu\) would increase because of alveolar hyperinflation. However, because the change in \(\mu\) due to hyperinflation does not occur homogeneously, the effect of stiffening of the lung during bronchoconstriction may not contribute significantly to attenuate bronchoconstriction.

In summary, because the changes in PL before and after bronchoconstriction were significantly less at higher lung volume (Fig. 2B), the load on smooth muscle provided by parenchymal tethering is still important; however, the results suggest that changes in the elastic moduli of the lung parenchyma caused by nebulized carbachol do not significantly increase the interdependence effect in constricted lungs.

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